**Research, Validation and Commercialization of Technologies**

# Intact Nutrition (Intact Digest™ & Intact Endurance™) with Nutri-Mastic:

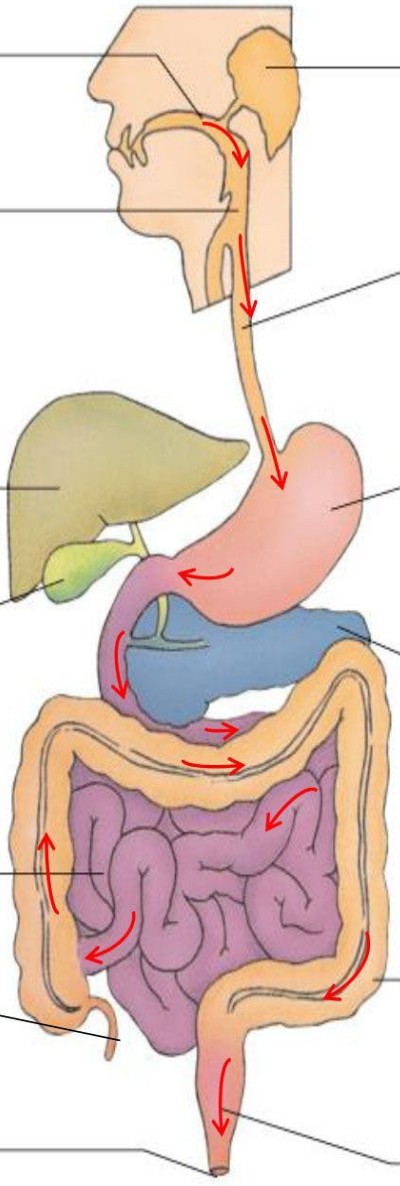
# Effect of Chios Mastic Gum (Pistacia lentiscus) combined with Ionic Minerals on Cardiovascular Function

## (Introduction and IFUS Point 1)

**Rev.1b-29May2026-ifus)**

**Introduction:** Consideration of the Human Digestive System and its Functions

## Digestive System



**Mouth**
Salivary enzymes begin carbohydrate digestion and break up food particles.

**Pharynx**
Directs food into the stomach to prevent choking.

**Liver**
The largest organ inside the body. Makes bile (fluid that helps break down fats and gets rid of wastes in the body); changes food into energy; and clears alcohol, some medicines, and poisons from the blood.
Stores vitamins and iron. Destroys old blood cells.

**Gallbladder**
Stores the bile made in the liver, then empties it into the small intestine to help digest fats. Stones can form within.

**Small intestine**
Digests protein, fats and carbohydrates. Bacterial metabolism plus nutrient and excess water absorption. Surface is covered in villi for greater absorption. Site of fructose malabsorption and Coeliac disease.

**Appendix**
A pouch attached to the first part of the large intestine. No one knows its function

**Anus**
The opening at the end of the digestive tract where bowel movements leave the body.

**Salivary glands**
Moistens and lubricates food. The enzyme amylase digests carbohydrates.

**Oesophagus**
Carries food down from the mouth to the stomach

**Stomach**
Secretes gastric juice and hydrochloric acid , and activates enzymes., continues to breakdown food and kill off any pathogens. Stores and churns food. Enzyme pepsin digests protein.

**Pancreas**
A gland that makes enzymes for digestion, and bicarbonate to neutralize stomach acid.
Also makes the hormone Insulin ,which helps the body turn food into energy ,and regulates blood sugar levels.

**Large intestine**
Also called the colon. It absorbs water and electrolytes from stool. It also contains beneficial bacteria which help produce certain vitamins. Forms and stores faeces.

**Rectum**
The lower end of the large intestine, leading to the anus. Stores and expels faeces.


**Reference**
1. Osiecki, H & Meeke, F, MD, 2005. The Digestive and Renal Systems – Volume 2. Bio Concepts Publishing, Eagle Farm QLD Australia.
2. http://teachers.concordiashanghai.org/lisahawkins/science-8-2/human-body-systems-2/digestive-system/

What we eat. How we eat it. When we eat it. All three pay a critical role in the digestion, and subsequent absorption…or the lack of digestion and absorption…of our intake, whether it be food or liquid.

From the moment a "substance" enters our mouths…and in some cases even before, as our sense of smell (or olfactory system) can trigger a reaction in the mouth that electrochemically ripples throughout the entire digestive system and even throughout our entire body…our Digestive System and its Functions are stimulated and prepared to go to work.

The question then becomes, do we optimize or sub-optimize the "Functionality" of each part of the "System" by what, how, and when we eat or drink. As a way of considering this, we explore "The Law of Requisite Variety", which suggests that in any system (whether human or machine), the element with the greatest "variability or choice" becomes the controlling element.

We take a step deeper into scientific thinking and explore the concept of "variety": "The term 'variety' was introduced by Dr. W. Ross Ashby, MD to extend his analysis of machines to their set of possible behaviors." [3]:121. As an aside the human body can be viewed as a very complex and integrated machine.

Dr. Ashby stated: "The word variety, in relation to a set of distinguishable elements, will be used to mean either (i) the number of distinct elements, or (ii) the logarithm to the base 2 of the number, the context indicating the sense used. [1]:126." For purposes of understanding, we can see the individual components of the Human Digestive System as "elements" (e.g., the Mouth, the Liver, the Large Intestine, etc.)

We find such information in treatises published by Dr. W.R. Ashby:

> Ref. 1: Ashby, W. R. 1956, An Introduction to Cybernetics, Chapman & Hall, 1956, ISBN 0-416-68300-2 (also available in electronic form as a PDF Archived 17 May 2023 at the Wayback Machine from Principia Cybernetica)
>
> Ref. 2: Ashby, W. R. 1958, Requisite Variety and its implications for the control of complex systems, Cybernetica (Namur) Vol. 1, No. 2, 1958.
>
> Ref. 3: Ashby, W. R. 1960, Design for a brain; the origin of adaptive behavior, 2nd ed. (Electronic versions on Internet Archive).

Dr. Ashby further suggested that. "Laws of nature constrain the variety of phenomena by disallowing certain behavior. ([1]:130). Ashby made two observations he considered laws of

nature, the law of experience and the law of requisite variety. The law of experience holds that machines under input tend to lose information about their original state, and the law of requisite variety states a necessary, though not sufficient, condition for a regulator to exert anticipatory control by responding to its current input (rather than the previous output as in error-controlled regulation)."

Practically speaking, the effects of friction and gravity alone on any machine (of for that fact living organism) causes normal wear and tear. Over time, the wear and tear become for the machine (or living organism) a "new normal" or sorts, with the memory of the original state fading as new memory is created, thus becoming the *status quo* of the current state. Hence, at some level, a healthy state of existence can be replaced with a dis-eased state of existence if the latter becomes the "new normal."

One could argue that at any given time from conception to death of a living organism, the current state of memory affects the health and well-being of that living organism. Hence, if the organism moves into a state of dis-ease, then an intervention of some sort is required to create a "new memory" that realigns with health and well-being. It is scientifically plausible that this can be achieved or at the least assisted by healthy nutrition.

Furthermore, it is of note that if an individual is placed into the dessert with all the food she or he can eat, but inadequate hydration, then that human will typically die within 2-4 days.

However, if that same human is placed into the dessert with no food, but proper hydration, she or he can survive for at least three weeks and in some cases longer (all other things being equal).

Once can conclude from this the importance of hydration, especially hydration with a mineral balance (not to forget vitamins).

Hydration with minerals and vitamins are critical to the survival of any living organism. Consider the diagram of the component parts (elements) and functions of the Digestive System required for proper digestion (viewed at a deeper level as another set of "elements"). One could continue by looking at a "function", then further dissect it into another group of "elements" (these being ionic minerals, vitamins, hormones, phytochemicals, etc.)

As Nature is filled with incredible "variety" of multiple elements at multiple levels, interrelated and interconnected Metabolic Pathways operate so as to manage "variety", and to create a particular or given outcome as illustrated below:

# Map of Metabolic Pathways

http://www.genome.jp/kegg/pathway/map/map01100.html

Generic Metabolic map
Organism specific maps

Functional Enzyme Nomenclature
Links to Sequence Databases

Genome =>Transcriptome => Proteome => Metabolome

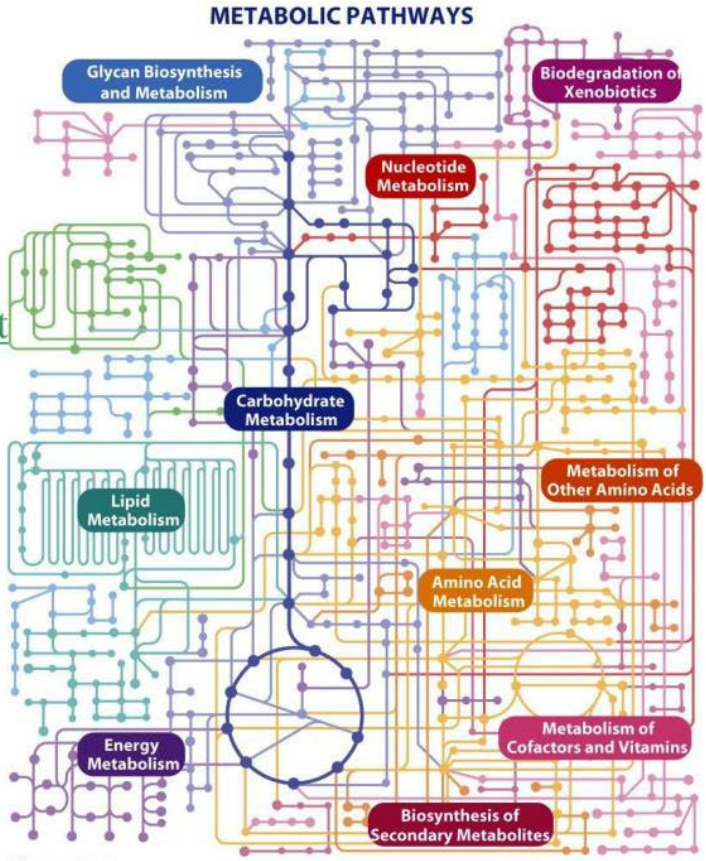



**Figure 15-1**
***Lehninger Principles of Biochemistry, Fifth Edition***


This complex array of chemical, physical, and electrical circuitry shown above is managed and regulated by any number of chemicals to include ionic minerals. For example, hormonal regulation (i.e., chemicals inside the body) along with said minerals act, interact, and relate to healthy tissue, cells, organs, and the like. If health is to be achieved, then these individual components and the systems that require their support MUST work as optimized individual entities, such that the respective actions, interactions, and relationships of each are also optimized. This results in the health and well-being of the human being in mind and body; i.e., an intact human being.

Consider for a moment just one small slice of the complex array of Metabolic Pathways, where Zinc, (Zn), Manganese (Mn), Iodine (I), Copper (Cu), Magnesium (Mg), and Phosphorus (P) in the form of Phosphate, work in cooperation to support Metabolic Function.

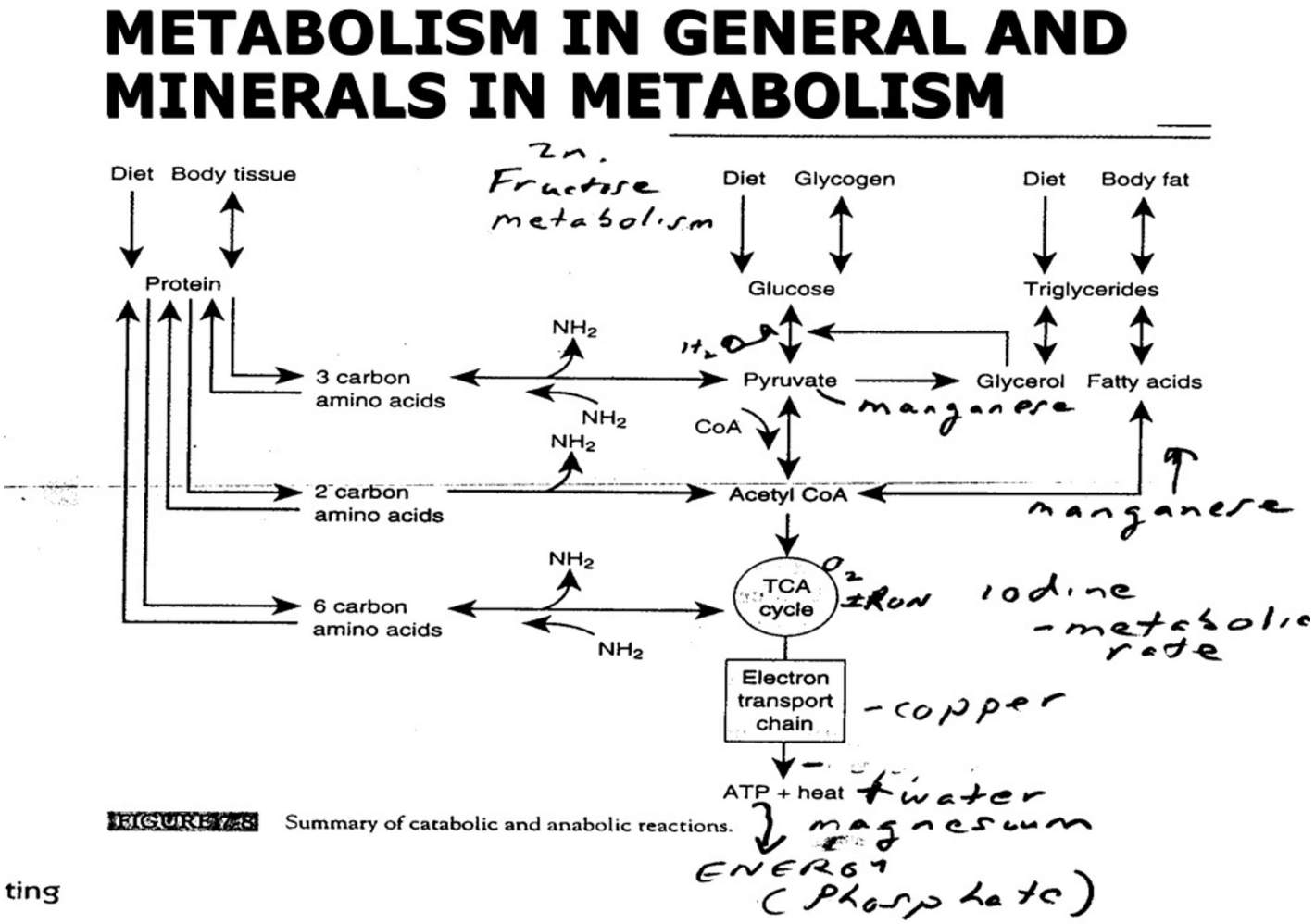



FIGURE 7.8 Summary of catabolic and anabolic reactions.

ting

Source: Mayra J Turner, Essential Water and Minerals in Human Health, Aug 09, 2024

If we flip the script, then the same can be said with "controlling elements" that are allowed to trigger, then operate in unhealthy ways; i.e., too much of a good thing, a group of bad things, the absence of something, and/or the imbalance of something. When this occurs, then a downward spiral of the human being can be found in both the mind and the body (i.e., a human being that is at dis-ease).

If we consider for a moment "The Law of Experience", science suggests that cells have a form

of memory. The questions become:

(1) Is cellular memory producing healthy outcomes for a human?

OR

(2) Has cellular memory been corrupted to produce outcomes filled with dis-ease for a human?

AND

(3) Can cells that have been programmed to be unhealthy be re-programmed through nutrition and supplementation to once more become healthy?

The latter question is of great interest as the Weizman Institute of Science tells us: "In the final tally, around 330 billion cells die and roughly the same number of new ones are born every day. By numbers, red and white blood cells – which live between one day and several months – are by far the largest portion, accounting for some 90%, of that turnover. Blood cells are quite light, so by mass, the daily total comes out to something a bit less than a hundred grams. Thus, roughly every 80 days our bodies produce a number of new cells roughly equal to the total number in the body." (Ref. 1 & 2)


Ref.1: "Mapping cellular turnover sheds light on the balance between renewal and stability in our bodies" / https://wis-wander.weizmann.ac.il/life-sciences/cell-replacement-numbers

Ref. 2: Sender, R., Milo, R. The distribution of cellular turnover in the human body. Nat Med 27, 45–48 (2021). https://doi.org/10.1038/s41591-020-01182-9


Hence, one could plausibly presuppose that enabling the body at the cellar level to reprogram itself into health would be at some level possible.

Additionally, in communities like that of Neuro-Linguistic Programming, we find concepts like that of "The Prime Directive of Human Survival." We find further supporting evidence of this Prime Directive in publications listed below (1,2,3)


Ref. 1: Piantadosi, Claude A, The Biology of Human Survival: Life and Death in Extreme Environments (New York, NY, 2003; online edn, Oxford Academic, 31 Oct. 2023), https://doi.org/10.1093/oso/9780195165012.001.0001, accessed 10 Feb. 2026.

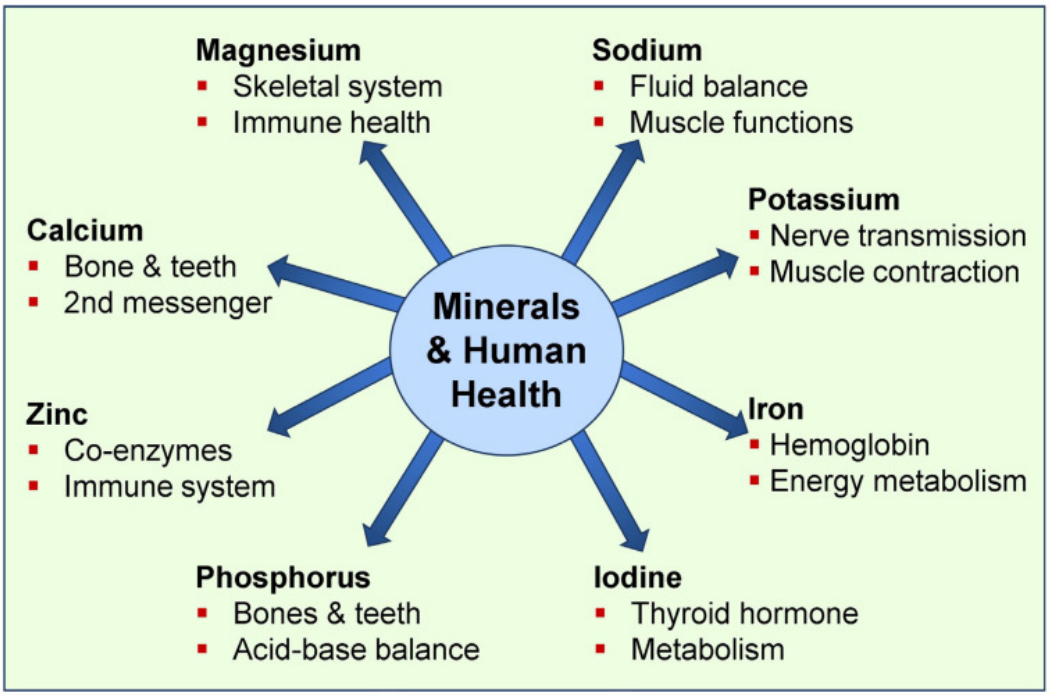
Ref. 2: Chatterjee D and Rai R (2021) Choosing Death Over Survival: A Need to Identify Evolutionary Mechanisms Underlying Human Suicide. Front. Psychol. 12:689022. doi: 10.3389/fpsyg.2021.689022

Ref. 3: Sharma, D.S.K. Creation, humanity, science and sustainability for human survival. Health Technol. 10, 1337–1341 (2020). https://doi.org/10.1007/s12553-020-00487-6

It is suggested the Prime Directive of Human Survival encompasses a range of principles that guide our actions and interactions," the most basic is said to be that of "Survival and Reproduction" so as to "ensure the continuation of the species. This includes seeking food and shelter, protecting oneself and others, and passing on genes to the next generation."

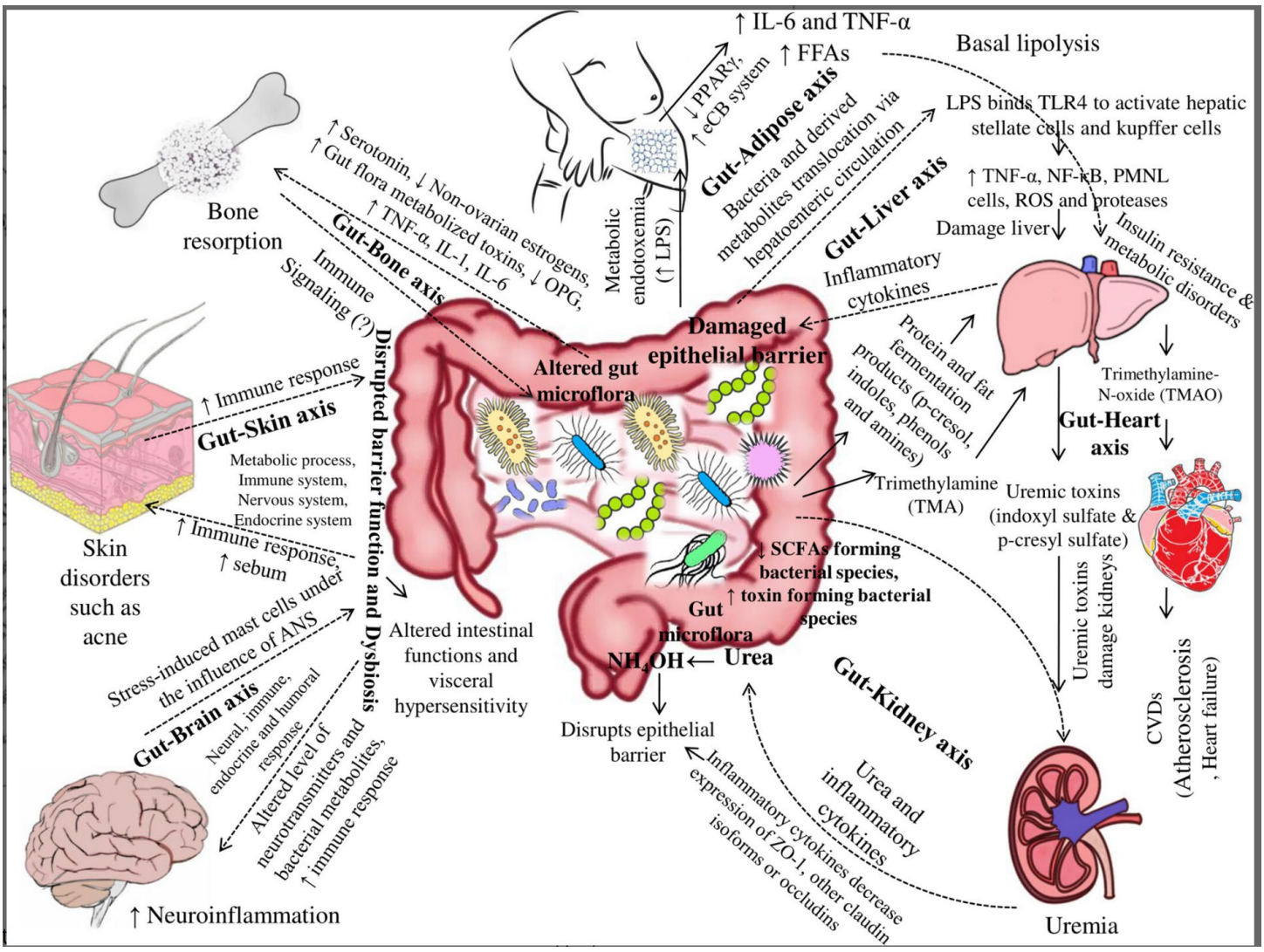
History would suggest to us that humanity has innate needs to first survive, and then thrive. Health in mind and body seem to be pre-requisites, with cellular rejuvenation being the mechanism that drives both. And, if cells are to rejuvenate, one could entertain that nutrition is at basis.

So, in a sense the age-old adage holds true: "You are what you eat." Hence, we are shown in the figure below by Razzaque MS, et.al., "Minerals and Human Health: From Deficiency to Toxicity." (Nutrients. 2025 Jan 26;17(3):454. doi: 10.3390/nu17030454. PMID: 39940312; PMCID: PMC11820417.)



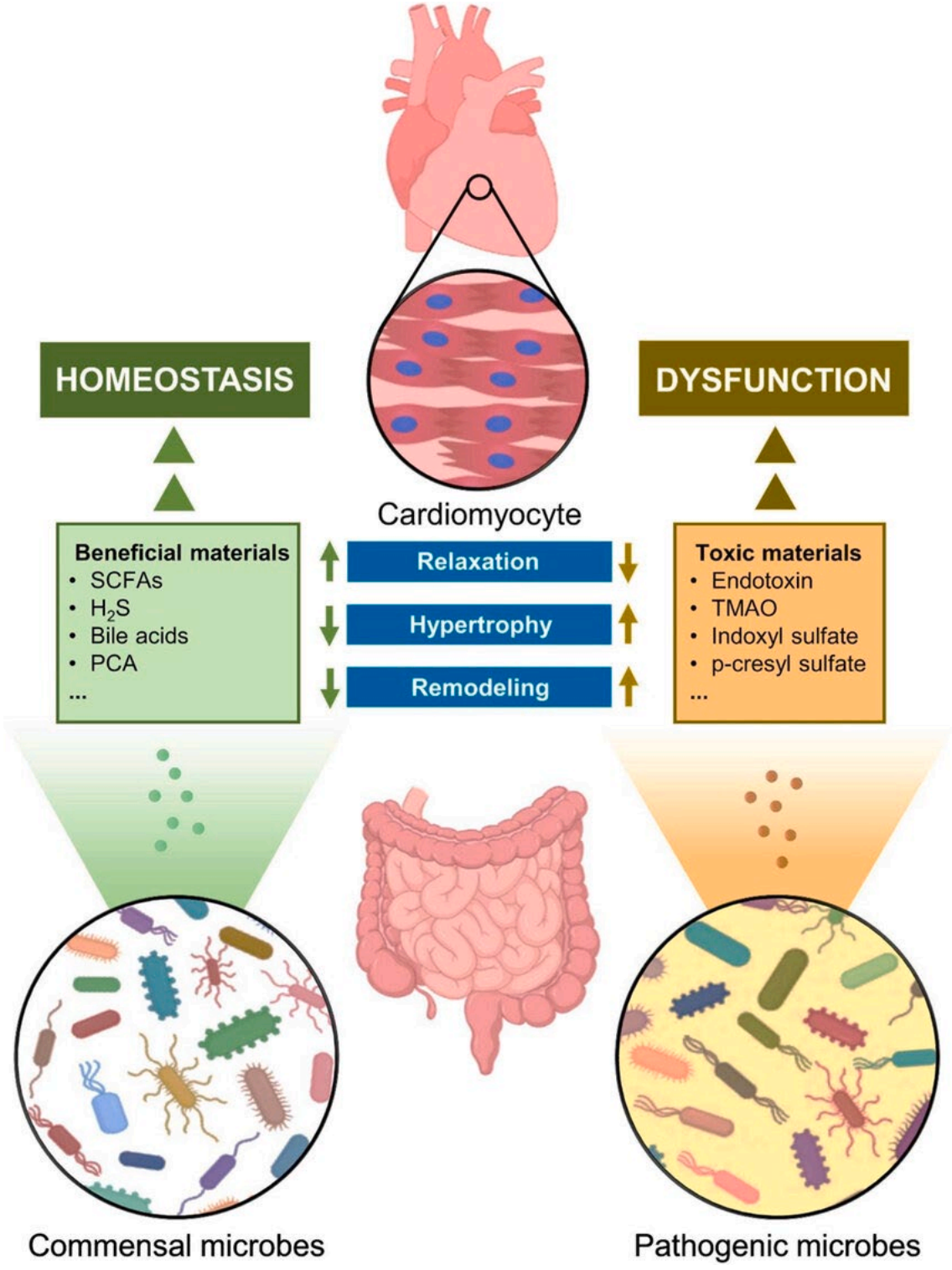
Many scientists believe that if you Fix the Gut… you Fix the human…through the multiple interconnections of various "axes" like that shown in Figure 1:

Figure 1: Representation of a bi- or multidirectional communication link or 'axis' between gut, associated microbiota and various organs. [Colour figure can be viewed at wileyonlinelibrary.com]




Ahlawat, S., Asha, & Sharma, K. K. (2021, June 1). Gut–organ axis: a microbial outreach and networking. Letters in Applied Microbiology. John Wiley and Sons Inc. https://doi.org/10.1111/lam.13333

Hence, we begin our journey in this White Paper (Part 1: Intact Nutrition™ (Intact Digest™ & Intact Endurance™) with Nutri-Mastic: Effect of Chios Mastic Gum (Pistacia lentiscus) combined with Ionic Minerals on Cardiovascular Function) with a focus on the Gut-Heart Axis:



From the illustration above, the authors tell us: "Gut-heart axis and cardiomyocyte homeostasis. Commensal and pathogenic microbes resident in the intestine can generate both beneficial and toxic substances and release them into the circulation. The beneficial substances sustain cardiomyocyte homeostasis, while the toxic agents promote cardiomyocyte dysfunction. H2S: hydrogen sulfide; PCA: protocatechuic acid; SCFAs: short-chain fatty acids; TMAO: trimethylamine N-oxide."

> Ref. 1: Chak Kwong Cheng, et. al, "The gut-cardiovascular connection: new era for cardiovascular therapy" October 2021Medical Review 1(1):000010151520210002 DOI:10.1515/mr-2021-0002

In this White Paper we will explore emerging science that is suggesting (and in many cases beginning to prove) the effect of phytochemically active substances (like Chios Mastic Gum and ionic minerals) on the general health and well-being of humans…in mind and body.

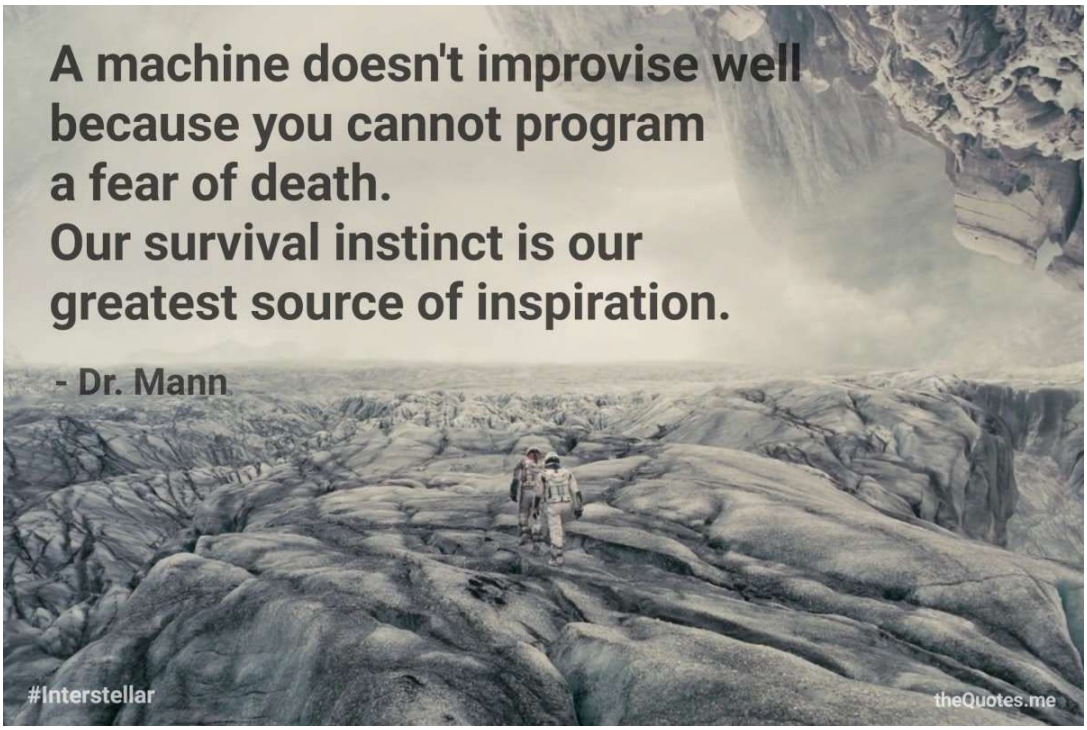
Studies like that shown above are focusing ever-more on the microbial biome of the human gut and body…and the influences these exsert on human health in mind and body. The is being linked to what we eat, when we eat it, and how we eat it…as well as supplementation to ensure our "intake" of fluid and liquid is as optimized as possible…and from natural sources.

Emerging science suggests that man-made manipulation of foods and liquids is beginning to be connected to sources of human dis-ease. Hence, the search for more natural alternatives is gaining focus…and that includes the soil, the plant, the animal, and the human.

Yet, for now we focus on the Human Cardiovascular System and a deeper exploration into the notion of Fix the Gut so we can Fix the Human.

As a learned colleague (who uses and supports others in the application of the Intact Digest™ line) recently shared with us, "So I will say that grandmas would tell the women in the family the way to a man's heart is his stomach. So, your science in the email provided is that ideology and thesis statement!" And, with that we add that maybe what Grandma meant was, "The way to a man's heart-health is through his gut!"

However, before we proceed, let us be reminded of a notion from a work of fiction (Interstellar-2014) that reinforces The Prime Directive of Human Survival:




## IFUS Table of Contents:

**Thoughts for Consideration:** A Contextual Perspective

We borrow notions that apply rules of well-founded jurisprudence. This “construct” begs consideration of the following:

- What we suspect.
- What we know.
- What we don’t know.
- What we don’t know, we don’t know.

In entertaining this thought-process, we hope to find evidence-based thinking and decision-making, upon which we find ourselves having our commentary governed by such.

In doing so, empirically accurate and precise “data” placed into proper context can then be transformed as evidence into reliable and correct “information”. When such “information” is applied with predictability, we find ourselves in the realm of “knowledge”. This “knowledge” can then be used as a basis for extrapolation and interpolation, so as to reach plausible conclusions and guidance. Without question, this is a “discipline” requiring in most cases patience, energy, and time.

Yet, patience, energy, and time, required to live within this “construct”, MUST be tempered with the practical realities confronting each of us individually and collectively. As an example, the practice of “Triage” is such a tool, applied to balance “idealism” with “pragmatic reality” that stops the patient from dying by focusing on that which immediately “threatens”, while strategically and tactically delaying that which can be managed later. And in some unfortunate cases, releasing to inevitable reality that which is beyond human capability to save.

Of this, we find ourselves governed by wisdom offered by individuals like Hippocrates of Kos, whose therapeutic approach is believed to be based on “*vis medicatrix naturae*” or as translated from the Latin, "the healing power of nature". And, with the maturation and evolution of his thinking, we find ourselves challenged to live what history suggests as Hippocrates final words to us:

“I swear by Apollo Healer, by Asclepius, by Hygieia, by Panacea, and by all the gods and goddesses, making them my witnesses, that I will carry out, according to my ability and

judgment, this oath and this indenture.

“To hold my teacher in this art equal to my own parents; to make him partner in my livelihood; when he is in need of money to share mine with him; to consider his family as my own brothers, and to teach them this art, if they want to learn it, without fee or indenture; to impart precept, oral instruction, and all other instruction to my own sons, the sons of my teacher, and to indentured pupils who have taken the Healer's oath, but to nobody else.

“I will use those dietary regimens which will benefit my patients according to my greatest ability and judgment, and I will do no harm or injustice to them. Neither will I administer a poison to anybody when asked to do so, nor will I suggest such a course. Similarly, I will not give to a woman a pessary to cause abortion. But I will keep pure and holy both my life and my art. I will not use the knife, not even, verily, on sufferers from stone, but I will give place to such as are craftsmen therein.

“Into whatsoever houses I enter, I will enter to help the sick, and I will abstain from all intentional wrong-doing and harm, especially from abusing the bodies of man or woman, bond or free. And whatsoever I shall see or hear in the course of my profession, as well as outside my profession in my intercourse with men, if it be what should not be published abroad, I will never divulge, holding such things to be holy secrets.

“Now if I carry out this oath, and break it not, may I gain for ever reputation among all men for my life and for my art; but if I break it and forswear myself, may the opposite befall me.”

Source: Translation from Greek provided by W.H.S. Jones

Hippocrates of Cos (1923). "The Oath". Loeb Classical Library. 147: 298–299. doi:10.4159/DLCL.hippocrates_cos-oath.1923. Retrieved 6 October 2015.

"Greek Medicine – The Hippocratic Oath". www.nlm.nih.gov. National Library of Medicine – NIH. Retrieved 29 July 2020.

Where aspects of this oath might well be challenged by contemporary thinking, what remains of substance for purposes of this White Paper, is that which is highlighted in yellow above: I will use those dietary regimens which will benefit my patients according to my greatest ability and judgment, and I will do no harm or injustice to them.

Hence, we begin our quest into the exploration of "The Gut" and the various "axes" that are affecting Human and Animal Health and Well-Being, while offering evolving thinking as to more natural ways in which to make these "axes" more "intact"…so as to improve the health and well-being of all living things (and doing so in an eco-friendly, cost-effective, and sustainable manner).

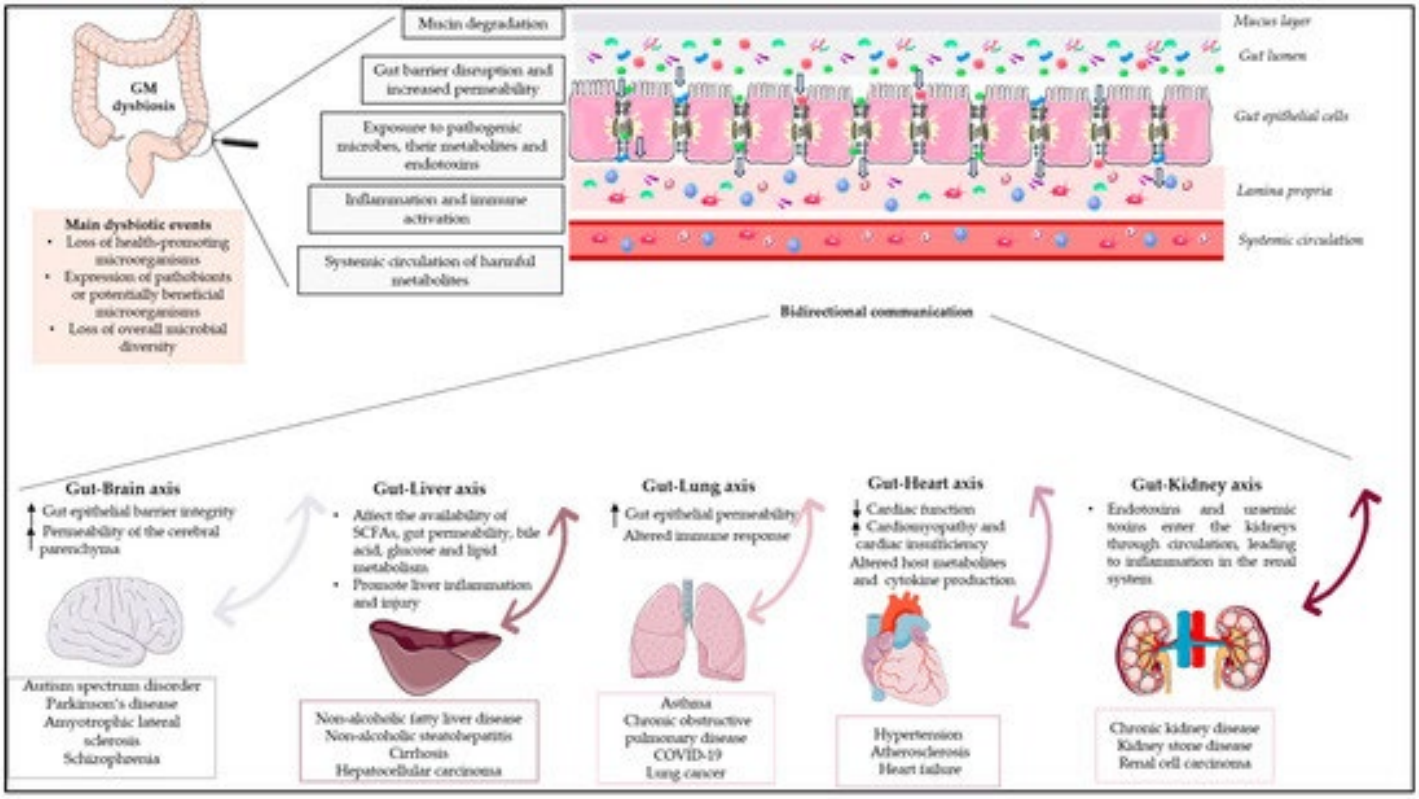



**Figure 1.** Schematic diagram depicting the influence of GM dysbiosis on the gut–organ axis. GM dysbiosis leads to the degradation of mucin, disrupts the gut's protective barrier, increases its permeability, and enables pathogenic microorganisms, along with their by-products and endotoxins, to infiltrate. This invasion results in the activation of immune cells and triggers systemic inflammation through the peripheral circulation. The impact of GM dysbiosis extends beyond the gastrointestinal tract. Recent research indicates two-way interactions between the GM and various organs, emphasizing the idea of a "gut–organ axis". This communication is facilitated through a range of signalling pathways and direct interactions between the host and the GM. Arrows indicate a bidirectional relationship between the gut and each organ. Parts of the figure were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (**https://creativecommons.org/licenses/by/3.0/** accessed on 25 August 2023).

Source: The Gut–Organ Axis within the Human Body: Gut Dysbiosis and the Role of Prebiotics by Georgia Saxami, et.al, Life 2023, 13(10), 2023; https://doi.org/10.3390/life13102023

In this context, it becomes worthy to consider the definition of "Dysbiosis". The Cleveland Clinic as well as Wikipedia offers this content:

**"Definition of Dysbiosis**

Dysbiosis is a **disruption or imbalance in the composition and function of the microbiome** — the community of microorganisms (bacteria, fungi, viruses, and other microbes) that live in and on the human body Cleveland Clinic+**1**. In a healthy microbiome, there is a diverse and balanced mix of microorganisms, each playing a role in digestion, immune regulation, and protection against harmful pathogens.

When dysbiosis occurs, **one or more types of microorganisms dominate, while others are reduced or absent**, leading to altered metabolic activities and reduced beneficial functions Cleveland Clinic+**1**. This imbalance can make the body more vulnerable to infections, impair digestion, and contribute to a range of health issues.

**Common Contexts**

- **Gut dysbiosis**: The most studied form, involving the imbalance of bacteria in the gastrointestinal tract. It can be linked to gastrointestinal diseases such as inflammatory bowel disease (IBD), small intestinal bacterial overgrowth (SIBO), and irritable bowel syndrome (IBS) Cleveland Clinic+**1**.
- **Other microbiomes**: Dysbiosis can also occur in the skin, mouth, vagina, and other body sites, affecting local health and potentially contributing to systemic conditions Wikipedia+**1**.

**Key Points**

- **Causes**: Antibiotics, dietary changes, stress, infections, radiation/chemotherapy, poor hygiene, and other health conditions can disrupt microbial balance Wikipedia+**1**.

- **Effects**: May range from mild digestive symptoms (diarrhea, constipation, bloating) to chronic diseases such as metabolic syndrome, mood disorders, and cardiovascular issues Cleveland Clinic+**1**.
- **Treatment**: Often addressed with dietary changes, probiotics, antibiotics (if infection is present), or fecal microbiota transplantation Wikipedia+**1**.

In summary, **dysbiosis is an imbalance in the body's microbial communities that can impair health and is a growing focus in medical research and clinical practice** Cleveland Clinic+**1**."

We find other studies in support of this content to include C. Sun, et.al., "Targeting the human gut microbiome: a comparative review of probiotics, prebiotics, synbiotics, and postbiotics, Journal of Advanced Research, 2025 ISSN 2090-1232, https://doi.org/10.1016/j.jare.2025.12.032. (https://www.sciencedirect.com/science/article/pii/S2090123225010227)

"Abstract: Background
The human gut microbiome plays a central role in regulating host health, serving as a core hub for systemic physiological interactions. Dysregulation of the gut microbiome is implicated in a wide spectrum of local and systemic diseases. Research has evolved from establishing associations to elucidating the mechanistic roles of gut microbes and developing targeted strategies for their modulation, with a growing emphasis on their bidirectional communication with other organ systems.

Aim of review
This review aims to synthesize current knowledge on the composition and function of the gut microbiome, its functional crosstalk with the host, and its integral role in both health and disease. A major focus is placed on critically evaluating the mechanisms, efficacy, and applications of key microbiome-directed interventions—probiotics, prebiotics, synbiotics, and postbiotics—in maintaining or restoring gut-centric ecological balance.

Key scientific concepts of review

The gut microbiome acts as a dynamic microbial organ essential for digestion, immune maturation, and metabolic homeostasis. Dysbiosis, characterized by a loss of beneficial microbes and an overgrowth of potential pathogens, is a critical factor in the pathogenesis of gastrointestinal disorders, metabolic diseases, and other systemic conditions. The gut microbiome engages in continuous bidirectional communication with distant organs, including the oral cavity, lungs, skin, and urinary tract, via specific axes (e.g., gut-oral, gut-lung, gut-skin), thereby exerting widespread influence on host physiology. Probiotics, prebiotics, synbiotics, and postbiotics represent complementary strategies to counteract dysbiosis and reestablish gut ecological integrity, ranging from introducing live beneficial bacteria to utilizing inactivated microbial cells and their bioactive metabolites. Enhancing the translational potential of these interventions requires deeper mechanistic insights and robust clinical validation."

Excerpts from the article published by C. Sun, et.al., include:

"The gut microbiome and its interactions with other microbiomes:

From birth onward, the human body is colonized by a diverse array of microorganisms, along with their genetic material and metabolic byproducts—collectively constituting the vertically transmissible human microbiome. This section spotlights several of the most functionally important microbial communities residing in the human body (Table 1) and provides a concise overview of the interactions between the gut and other organs.

Table 1. Primary microbiota colonising distinct anatomical sites and their associated disease links.

| **Microbiota** | **Microbiota count** | **PH** | **Predominant Microbes** | **Microbiota-associated Diseases** | **References** |
|---|---|---|---|---|---|
| Gut Microbiota | $10^7$–$10^{14}$ | 5.7–8.5 | Bacterial phyla: *Firmicutes* and *Bacteroides* Archaeal species: *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* | Inflammatory bowel disease, irritable bowel syndrome, celiac disease, and colorectal | [235], [236], [237] |

| Microbiota | Microbiota count | PH | Predominant Microbes | Microbiota-associated Diseases | References |
|---|---|---|---|---|---|
| | | | | cancer | |
| Oral Microbiota | $10^{11}$–$10^{12}$ | 6.7–7.5 | Bacterial phyla: *Actinobacteria,Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria*, and *Spirochaetes* Fungal genera: *Candida, Cladosporium*, *Saccharomycetales*, *Fusarium*, *Aspergillus*, and *Cryptococcus* | Dental caries, and periodontitis | [50], [57] |
| Respiratory Tract and Lung Microbiota | $10^{2}$–$10^{9}$ | 5.5–7.4 | Bacterial phyla: *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* Fungal species: *Candida albicans*, *Ceriporia lacerata*, *Saccharomycescerevisiae*, and *Penicillium brevicompactum* viruses: *Herpesviridae* | Asthma, and chronic obstructive pulmonary disease | [65], [73] |
| Skin Microbiota | $10^{11}$ | 5.0–5.5 | Bacterial phylum: *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* | Atopic dermatitis | [76], [77] |
| Urinary Tract Microbiota | $10^{3}$–$10^{4}$ | 5.5—6.5 | Bacterial phylum: *Firmicutes* | Urgency urinary incontinence, and urinary tract infection | [87], [88], [91] |

## Probiotics, prebiotics, synbiotics, and postbiotics as ‘actors’: active remodelling of gut microbiome functions.

Trillions of microbial inhabitants in the gut play a crucial role in regulating digestion, immune function, and even mental health; maintenance of this complex ecosystem is essential, as its disruption has been linked to metabolic disorders. This section describes four functional food agents—prebiotics, probiotics, synbiotics, and postbiotics—whose dietary intake has been shown to beneficially modulate the gut microbial community (Table 2).

Table 2. Health effects mediated by probiotics, prebiotics, synbiotics, and postbiotics: an overview.

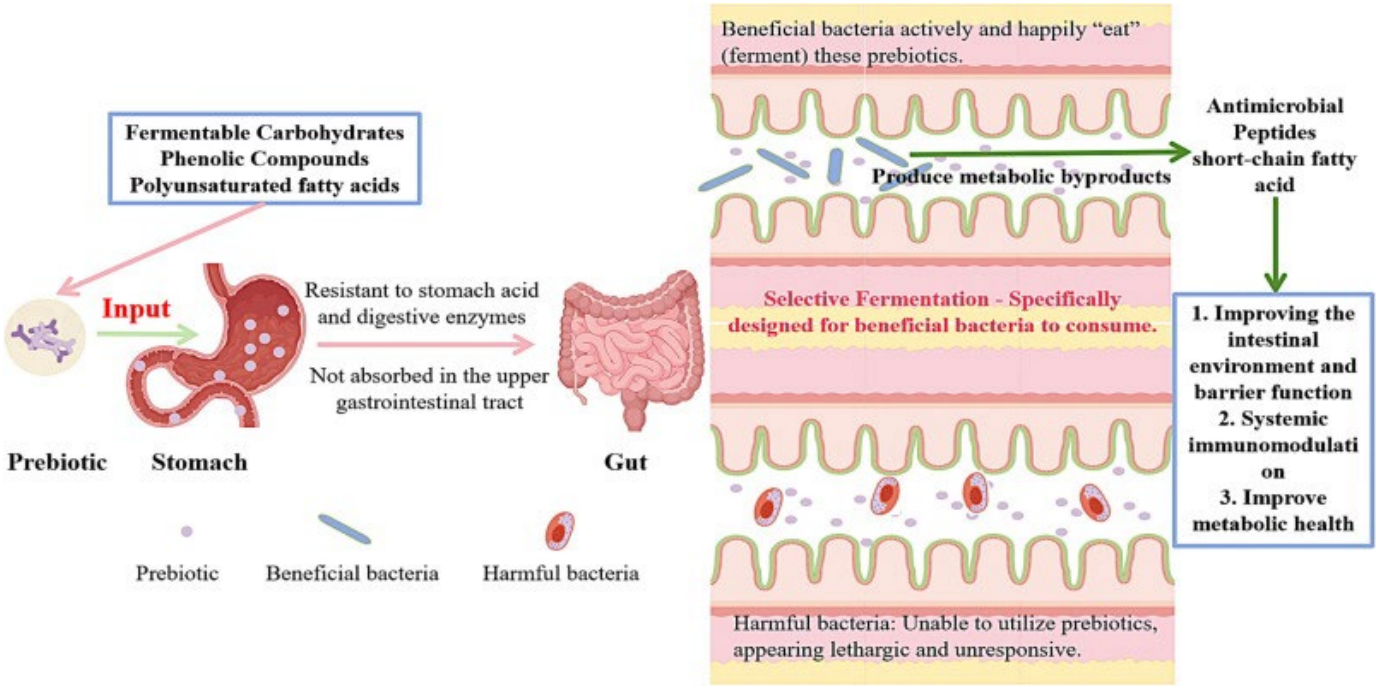
| Category | Definition | Exemplars | Mechanisms of Action | Evidence tier | References |
|---|---|---|---|---|---|

| Category | Definition | Exemplars | Mechanisms of Action | Evidence tier | References |
|---|---|---|---|---|---|
| Probiotic | Live microorganisms, when applied in sufficient quantities, can bring health benefits to the host | *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus acidophilus* | Enhance gut barrier function, produce antimicrobial substances, modulate immune responses | Human randomized controlled trial, and animal experimentation | [108], [110], [116], [219] |
| Prebiotic | Substrates selectively utilized by the host microbiota that bring health benefits to the host | Xylo-oligosaccharides, galacto-oligosaccharides, and fructo-oligosaccharides | Promote the growth of beneficial bacteria and alter gut microbiota composition | Human randomized controlled trial, and animal experimentation | [125], [130], [132], [140] |
| Synbiotic | Mixtures comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host | *Bifidobacterium lactis* HN019*,* *Lactobacillus rhamnosus* HN001*,* and Fructooligosaccharide *Lactobacillus suilingensis* AF91-01CMCA*,and* Inulin | Improve gut microbiota diversity, enhance the absorption of nutrients, synergistic effects between probiotics and prebiotics | Human randomized controlled trial, and animal experimentation | [15], [137], [139], [143] |
| Postbiotic | Probiotic microbial preparations and/or their components that confer health benefits to the host | Lipopolysaccharides,SCFA, and Trimethylamine Oxide | Influence gut microbiota composition, reduce intestinal inflammation, enhance antioxidant enzyme activity | Human randomized controlled trial, and animal experimentation | [159], [168], [174], [186] |

"Per the International Scientific Association for Probiotics and Prebiotics (ISAPP), a dietary prebiotic is defined as a selectively fermented substrate that brings about specific, beneficial

alterations in the composition and/or activity of the GI microbiota, thereby imparting health benefits [126]. To be classified as a prebiotic, a compound must fulfill three criteria: (1) resist gastric acidity and breakdown by mammalian enzymes, (2) remain unabsorbed within the upper GI tract, and (3) be selectively used by host microorganisms associated with health benefits. Chemically, prebiotics fall into four broad categories: fermentable carbohydrates, phenolic compounds, plant-derived phytochemicals (e.g., curcumin, naringenin, hesperetin), and polyunsaturated fatty acids (PUFAs) such as linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid. Among these, carbohydrate-based prebiotics—including short-chain oligosaccharides (e.g., inulin-type fructans, galacto-oligosaccharides (GOS), arabinoxylans, *xylo*-oligosaccharides (XOS), and human milk oligosaccharides) and longer polysaccharides (e.g., β-glucans, isomaltodextrin, and resistant starch)—remain the most extensively studied and widely applied, owing to their consistent efficacy in promoting beneficial gut bacteria [127]."

The aforementioned in demonstrated below in "Fig. 4. Prebiotics: Sources, Mechanisms and Health Benefits. Light blue arrows pointing upward indicate a promoting effect, while those pointing downward indicate an inhibitory effect."



Furthermore, the science suggests to us that Mastic Gum from Chios, Greece is viewed as a prebiotic and has been shown in studies to work cooperatively with probiotics. For, example:

> A. Terpou, et.al., Evaluation of Chios mastic gum as antimicrobial agent and matrix forming material targeting probiotic cell encapsulation for functional fermented milk production, LWT, Volume 97, 2018, Pages 109-116, ISSN 0023-6438, https://doi.org/10.1016/j.lwt.2018.06.045. (https://www.sciencedirect.com/science/article/pii/S0023643818305565)
>
> "Mastic gum has been proved to be safe and well tolerated by humans in addition to being able to contribute to the smooth operation of the gastrointestinal system (Kang, Wanibuchi, Salim, Kinoshita, & Fukushima, 2007; Morkhade, 2017; Paraschos et al., 2007) and lately has been monographed in the European Pharmacopeia (01/2008:1876, HMPC, 2015). Due to its unique shape and diverse efficacy, mastic gum has also been called as the tear drop of Christ. It is a hydrophobic and matrix forming material that can be fabricated in many shapes, having an excellent film forming ability (Mavrakis & Kiosseoglou, 2008). Mastic gum has been used in certain semi-solid products, like ice-cream and baked products, as a flavoring or antimicrobial agent as well as a texture modifier, while there have been reports of its use as a matrix forming material for sustainable drug release (Burešová et al., 2017; Morkhade, 2017; Saad & El-Zamkan, 2017). Likewise, reports of resins with similar characteristics to mastic have been used as natural food additives in which functional incidents can be encapsulated (Schoina et al., 2015, 2018)."

Hence, the question evolves as to the efficacy of Intact Digest™ and Intact Endurance™ in providing a prebiotic supplement that cannot only work synergistically with probiotics, but with other prebiotics, synbiotics, and postbiotics. This exploration is to be considered at a later time under separate cover to provide plausible science to elucidate this complex and evolving science.

Yet, it is important that we further establish the relevance of gut microbiota to the "gut-organ axes", such that **dysbiosis** is mitigated and/or ameliorated from the human body. Hence, we continue our exploration into another study published by Petersen, C. and Round, J.L. (2014),

How changes in microbiota structure influence health. Cell Microbiol, 16: 1024-1033. https://doi.org/10.1111/cmi.12308 and illustrated in Figure 1 below.

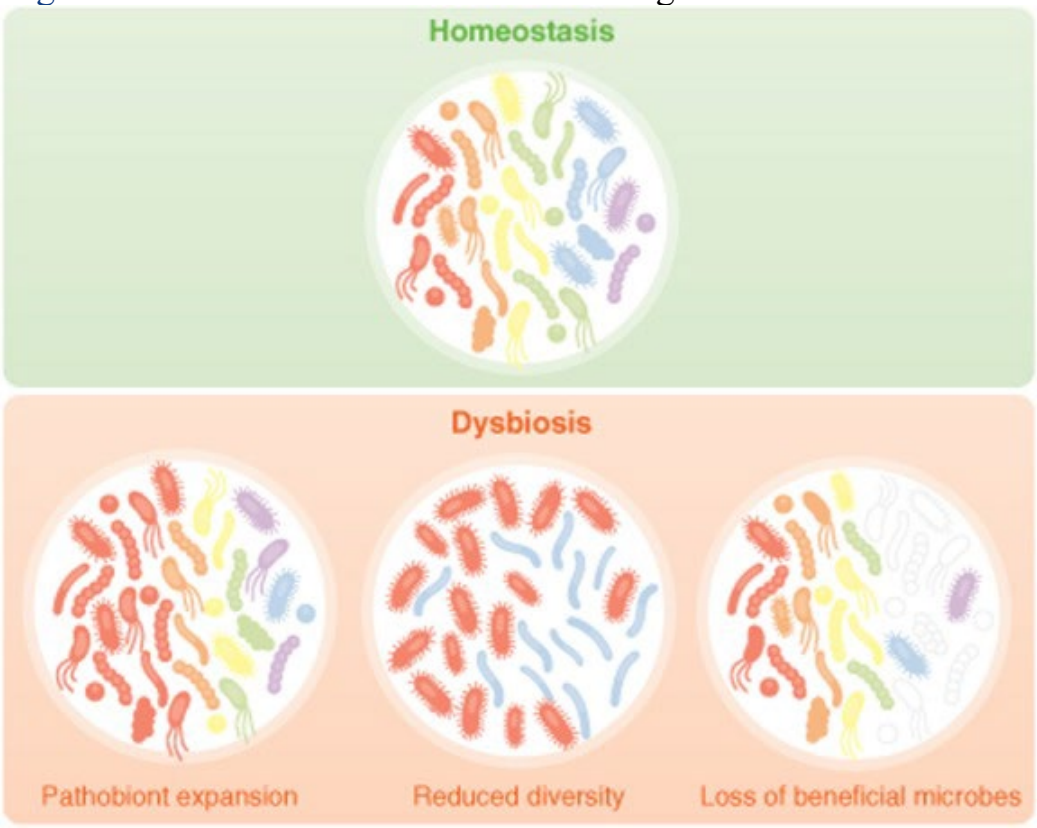


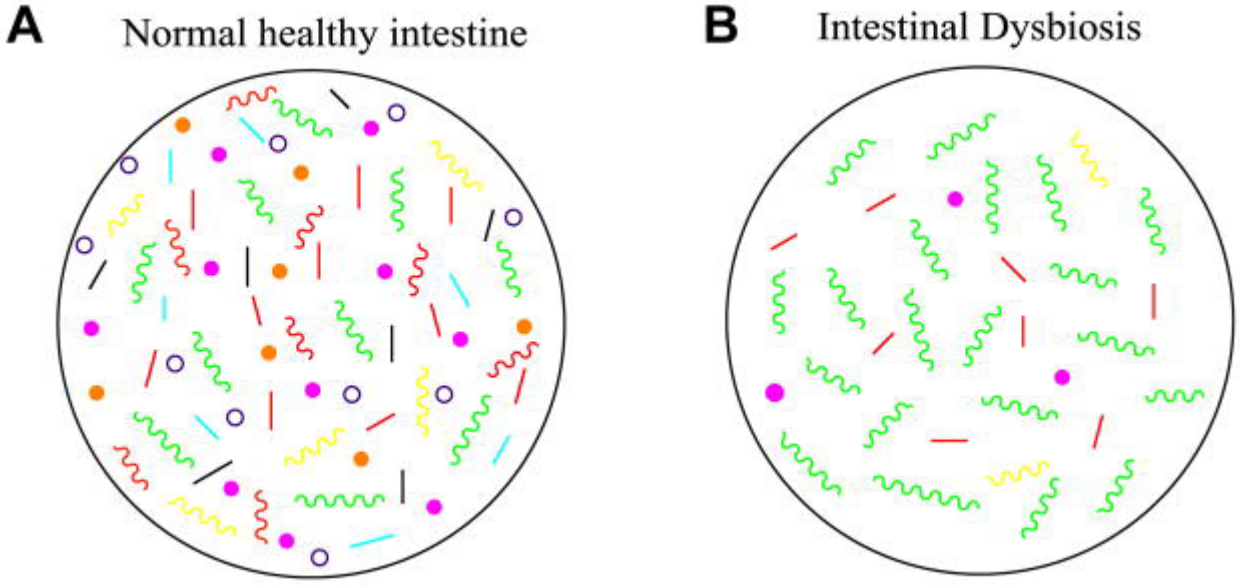

Figure 1 above illustrates "A loss of beneficial microbes, expansion of pathobionts, and loss of diversity are events that encompass dysbiosis. During healthy, homeostatic conditions the microbiota is composed of a diversity organisms that are known to benefit host development and health. However, environmental insults, such as antibiotic use or diet can lead to disruptions in the structure of the microbial community. These disruptions can lead to a loss of organisms that are beneficial to the host and a subsequent overgrowth of commensals that have the potential to cause harm, termed pathobionts. Domination of the microbiota by pathobionts can lead to inflammation and pathology. Additionally, multiple studies have described the diversity of contributions made by the various members of the microbiota. Oftentimes, these are non-redundant influences on host health, thus a total loss of diversity in the microbiota can also influence disease progression or severity and thus also represents a dysbiosis event."

Yet, another study published by A.K. DeGruttola, et.al., Current Understanding of Dysbiosis in Disease in Human and Animal Models. Inflamm Bowel Dis. 2016

May;22(5):1137-50. doi: 10.1097/MIB.0000000000000750. PMID: 27070911; PMCID: PMC4838534., further establishes a link between IBS/IBD and CVD.

"Abstract: Inflammatory bowel disease (IBD) is an intestinal inflammatory condition that affects over two million people in the United States. Although the etiology and pathogenesis of IBD are still largely unknown, dysregulated host/enteric microbial interactions are requisite for the development of IBD. So far, many researchers have tried to identify a precise relationship between IBD and an imbalance of the intestinal microbiota, termed "dysbiosis". In spite of the extensive efforts, it is still largely unknown about the interplay among microbes, their hosts, and their environments, and whether dysbiosis is a causal factor or an effect of IBD. Recently, deep-sequencing analyses of the microbiota in IBD patients have been instrumental in characterizing the strong association between dysbiosis and IBD development, although it is still unable to identify specific-associated species level changes in most cases. Based on many recent reports, dysbiosis of the commensal microbiota is implicated in the pathogenesis of several diseases, including IBD, obesity, and allergic disorders, in both human and animal models."

Figure 1. Normal and dysbiotic intestinal microbiota.




Open in a new tab

**A.** The healthy intestines of normal individuals are colonized by a wide range of bacteria of over 1000 species. In healthy individuals, these bacteria are in a homeostatic balance between commensal and potentially pathogenic bacteria, and the intestinal tract does not display overgrowth of pathogenic bacteria. The microflora provide the host with protection from foreign microbes, acting as a central line of

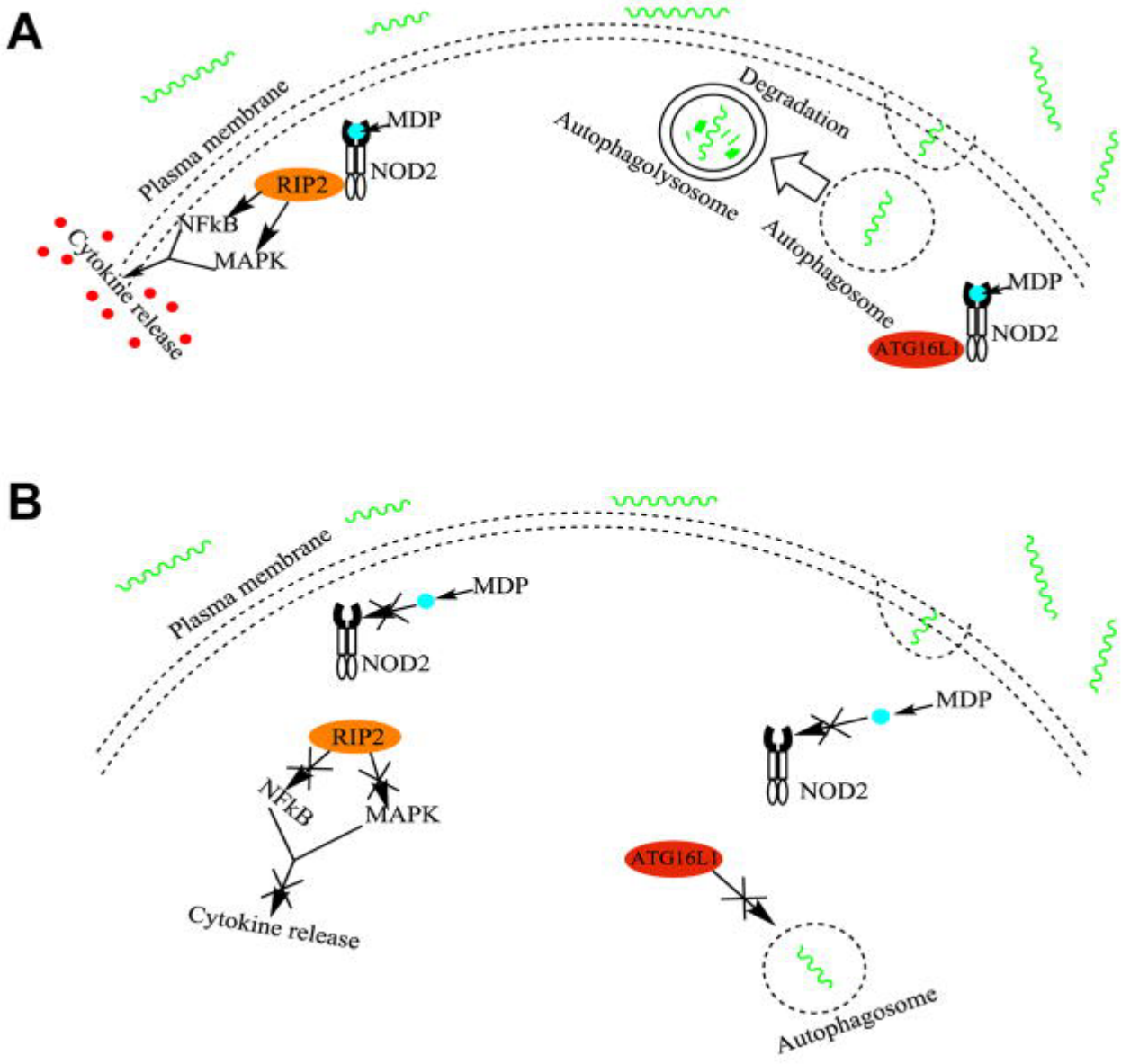
resistance to colonization by these exogenous bacteria. This protection is known as the “barrier effect”, or colonization resistance [1]. Through the mucosal surface of the intestine, the microbiota interacts with the host immune system, providing the host with immune regulatory functions, like priming the mucosal immune system [1,2]. The microbiota also possesses various metabolic functions, like breaking down complex carbohydrates and generating short-chain fatty acids, which the host benefits from [1, 3]. Surprisingly, the gut microbiota is also capable of interacting with distant organs, such as the brain, which has led to studies of the influence of the gut microbiota on mental disorders, like autism, and diseases such as Alzheimer’s [2].
**B.** When the intestinal bacterial homeostasis is disrupted, dysbiosis occurs. Dysbiosis is defined by an imbalance in bacterial composition, changes in bacterial metabolic activities, or changes in bacterial distribution within the gut. The three types of dysbiosis are: 1) Loss of beneficial bacteria, 2) Overgrowth of potentially pathogenic bacteria, and 3) Loss of overall bacterial diversity. In most cases, these types of dysbioses occur at the same time. Green colors representing pathogenic bacteria and each different color bacteria representing a different commensal species to show diversity or lack thereof in each case. Dysbiosis has been associated with diseases such as Inflammatory Bowel Disease (IBD), Obesity, Type 1 and Type 2 Diabetes, Autism, and certain gastrointestinal cancers.”

An excerpt from the study performed by A.K. DeGruttola, et.al. reveals to us through annotated additional references:

> “NOD2: The first identified CD susceptibility gene, nucleotide oligomerization domain 2 (NOD2), aka NOD2/CARD15 (CAspase Recruitment Domain-containing protein 15) is located on human chromosome 16q12 and synthesizes NOD2 protein, which acts as a type of pathogen-associated molecular pattern (PAMP) and specifically recognizes muramyl dipeptide (MDP), which is a component of the bacterial peptidoglycan cell wall present in both Gram-positive and Gram-negative bacteria. NOD2 is expressed in many types of cells including dendritic cells, macrophages, Paneth cells, as well as intestinal epithelial cells, and epithelial cells in the lungs and oral cavity [64]. NOD2 protein is important in innate immunity and microbial regulation, where it is involved in pathogen recognition and defense against these organisms [65]. MDP binds and activates NOD2, which then is localized to the plasma membrane and initiates signaling cascades by recruiting receptor-interacting protein 2 (RIP2), followed by activating the mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) cascades, resulting in the release of proinflammatory molecules to help kill the pathogenic bacteria [66].

NOD2 is also needed for expression of defensins by specialized epithelial cells called Paneth cells, which are located in small intestinal crypts [65]. Furthermore, NOD2 is important in inducing autophagy in epithelial cells by guiding ATG16L1 (autophagy-related 16-like 1) protein to the plasma membrane to induce autophagosome formation [66] [Figure 2]. Functioning NOD2 is essential for both Gram-positive and -negative bacterial regulation and helps to prevent pathogenic bacteria colonization and dysbiosis.

Figure 2. Normal and mutated NOD2 functions.



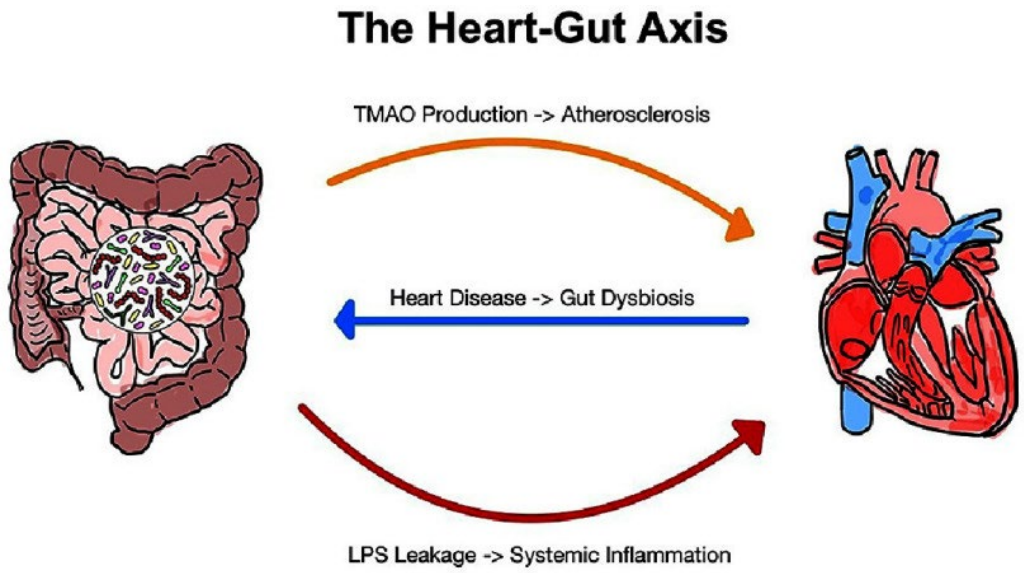
**A.** Normal functions of NOD2. The normal NOD2 variant is activated by muramyl dipeptide, which is a component of bacterial cell walls. Activated NOD2 recruits receptor-interacting protein 2 (PIP2), which then activates the mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NFκB) cascades. This results in the release of proinflammatory molecules to help kill pathogenic bacteria (**Left**). Activated NOD2 also guides autophagy-related 16-like 1 (ATG16L1) protein from the cytoplasm to the plasma membrane to initiate autophagosome formation (**Right**). **B.** Mutated variants of NOD2 cause impairments in sensing and recognizing MDP. Without this activation, RIP2 and the resulting cytokine release does not occur (**Left**). Without active NOD2, ATG16L1 is not guided to the plasma membrane and remains in the cytosol, which impairs autophagosome formation and results in impaired killing of invading bacteria (**Right**). NOD2 variants and their impaired cellular functions may lead to dysbiosis within the intestinal epithelium, and are associated with earlier onset of ileal Crohn's disease."

NF-κB will be discussed later in the White Paper series as it relates to the NLRP3 Inflammasome and the Cell-Adhesion Molecule (CAM1).

Furthermore, we will see later in this paper the links between Cardiovascular Disease, *H.pylori*, and leaky gut / gastrointestinal malfunctions, like that supported by the study: R. Abdulaal, et.al., The role of microbiome dysbiosis in cardiovascular disease: Mechanisms and therapeutic implications. Glob Cardiol Sci Pract. 2025 Feb 28;2025(1):e202503. doi: 10.21542/gcsp.2025.3. PMID: 40390988; PMCID: PMC12085923.

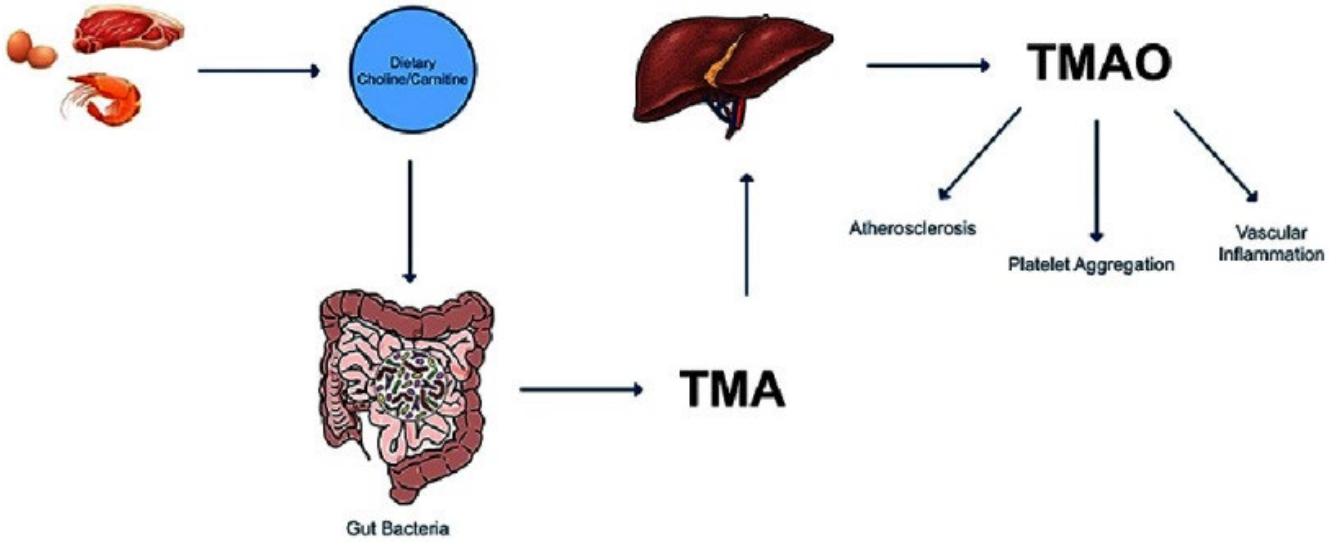
"Abstract: The gut microbiome plays a critical role in cardiovascular disease (CVD) pathogenesis through systemic inflammation, disrupted lipid metabolism, and proatherogenic metabolites like trimethylamine-N-oxide (TMAO). Dysbiosis contributes to increased intestinal permeability, platelet hyperreactivity, and reduced short-chain fatty acids (SCFAs), exacerbating cardiovascular risk. Emerging microbiome-targeted therapies, including probiotics, prebiotics, fecal microbiota transplantation (FMT), and dietary interventions, show promise in mitigating CVD. However, challenges remain in translating these findings into clinical practice due to strain-specific effects and interindividual variability. The gut-heart axis represents a transformative avenue for CVD prevention and management, warranting further research to optimize long-term efficacy and safety."

The aforementioned "Abstract" is supported through graphical illustration as shown through Figure 1. The heart-gut axis showing bidirectional interactions between the gut microbiome and cardiovascular health:"




R. Abdulaal, et.al., in "The role of microbiome dysbiosis in cardiovascular disease: Mechanisms and therapeutic implications" offer additional insight:

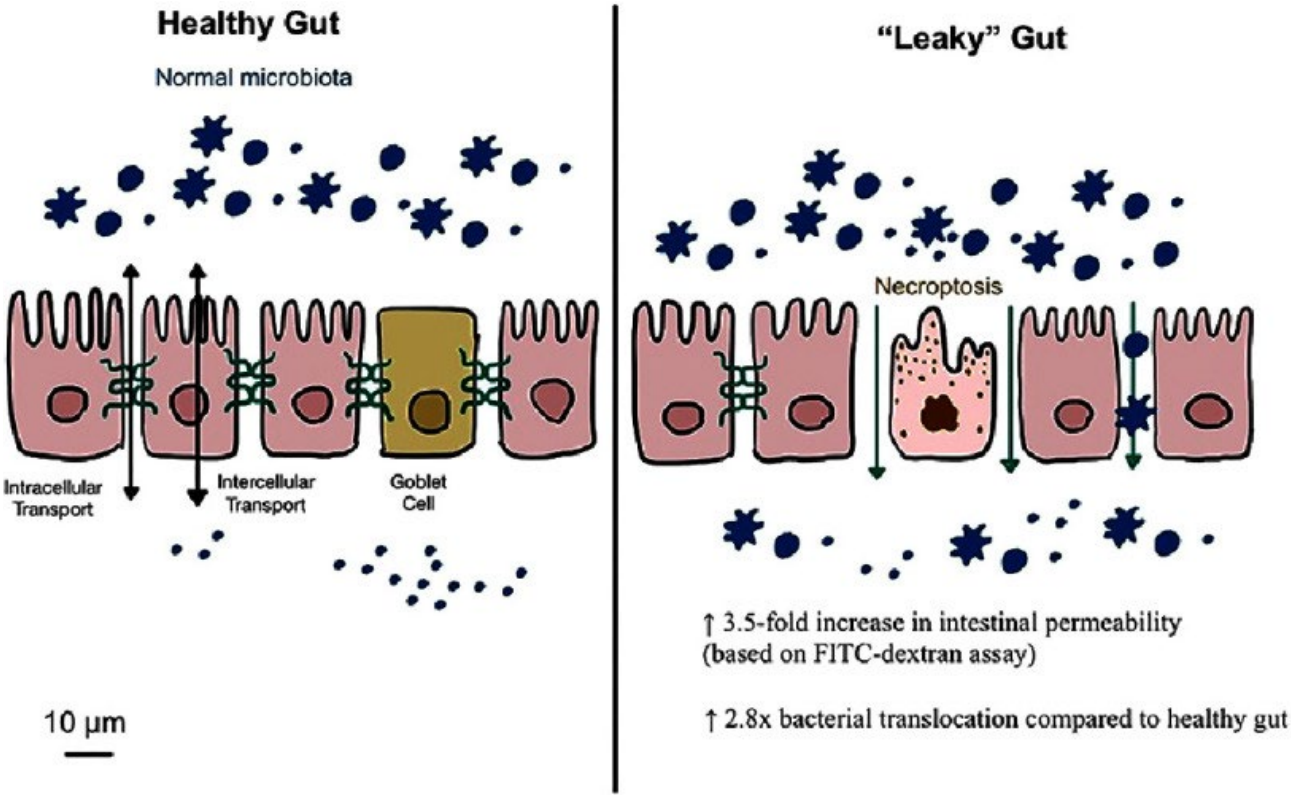
"Elevated TMAO levels have been shown to increase platelet responsiveness to agonists, thereby amplifying the risk of thrombosis, a major contributor to cardiovascular complications[18]. (Figure 2) summarizes the TMAO pathway. Figure 2. Pathway of TMAO production and its cardiovascular effects."




"Gut-derived inflammation and cardiovascular risk: The gut microbiota significantly influences systemic inflammation, a key driver of cardiovascular disease (CVD). Disruption of the intestinal microbiota, commonly referred to as dysbiosis, alters the gut barrier and facilitates the translocation of microbial products into systemic

circulation. One critical outcome of this disruption is increased intestinal permeability, often termed "leaky gut" (Figure 3), which allows endotoxins such as lipopolysaccharides (LPS) to escape into the bloodstream[20]. These endotoxins bind to Toll-like receptor 4 (TLR4) on immune cells, triggering pro-inflammatory cascades that contribute to systemic inflammation and endothelial dysfunction—key precursors of atherosclerosis and CVD[21]."

"Figure 3. Comparison of healthy and leaky gut barrier integrity.




"LPS-mediated endotoxemia is strongly associated with the progression of cardiovascular conditions. Elevated circulating LPS levels stimulate the production of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-$\alpha$), and interleukin-1 beta (IL-1$\beta$), which exacerbate endothelial injury and promote the recruitment of monocytes to vascular sites. These mechanisms establish a vicious cycle of chronic inflammation and vascular damage[22]. Experimental studies have demonstrated that animals exposed to high-fat diets, which induce dysbiosis and endotoxemia, exhibit accelerated atherosclerosis, highlighting the interplay between dietary habits, gut microbiota, and cardiovascular health[23].

The intestinal barrier itself is a dynamic and multifaceted structure comprising epithelial cells, tight junction proteins, and a protective mucus layer. Dysbiosis disrupts this barrier by degrading tight junction proteins, such as zonulin and occludin, thereby increasing permeability. Loss of barrier integrity facilitates not only the translocation of LPS but also other microbial metabolites, such as peptidoglycans and flagellins, further amplifying systemic inflammation[21]. Studies have indicated that interventions targeting gut barrier restoration, such as the use of dietary fibers or probiotics, can mitigate inflammation and reduce CVD risk[23]. Beyond LPS, other gut-derived factors, including microbial metabolites like short-chain fatty acids (SCFAs), influence cardiovascular health. SCFAs, produced through bacterial fermentation of dietary fibers, possess anti-inflammatory properties and help maintain the integrity of the gut barrier. However, dysbiosis often results in reduced SCFA production, depriving the host of their protective effects[22].

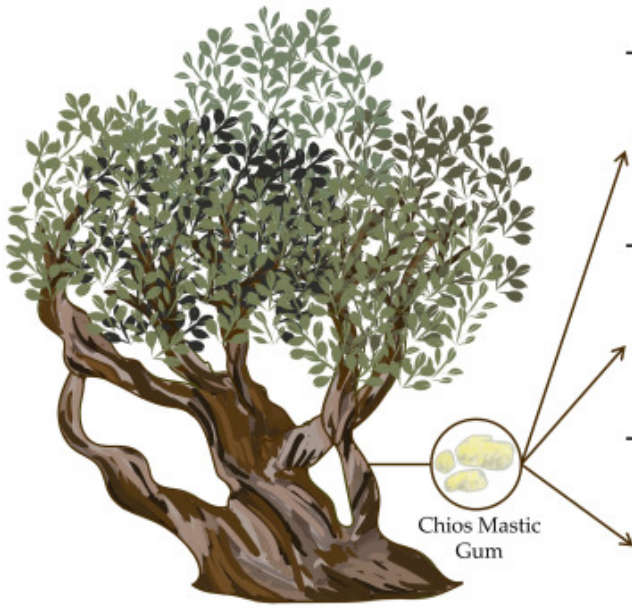
Restoration of SCFA levels has been proposed as a therapeutic strategy to modulate inflammation and improve cardiovascular outcomes.

In summary, gut-derived inflammation represents a critical link between intestinal health and cardiovascular risk. Dysbiosis, endotoxemia, and barrier dysfunction collectively exacerbate systemic inflammation and vascular damage, underscoring the need for strategies targeting gut health in the prevention and management of CVD[23,24].”

“Elevated circulating LPS levels stimulate the production of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β)…” will be also considered later in this series of White Papers, as several studies provide cumulative data on these individual biomarkers as well as links to the NLRP3 Inflammasome and CAM1. This scientific evidence is creating a link from gut health to the respective gut-organ axes to the specific impact created and now becoming elucidating by advanced Biochemistry, Molecular Biology, Molecular Physical Chemistry, Polymer Chemistry, and more. Furthermore, science (like that illustrated by the diagram below) is now illustrating links to Chios Masic Gum as not a simple “supplement”, but as a legitimate natural pharmacological agent.

IFUS Point 1L-2a: (Please note that this information will be more deeply explored later in this White Paper Series. However, for point of present clarity it is offered here for consideration). Figure 1: Impacts of Chios mastic gum on disease mechanisms and cardiometabolic outcomes. Abbreviations used: * adjunct metabolic syndrome treatments only, ** CMG-gene interactions only, 11β-HSD1 = 11-beta-hydroxysteroid

dehydrogenase, adipo = adiponectin, ALT = alanine aminotransferase, AMPKα = AMP-activated protein kinase alpha, Apo(B) = apolipoprotein B, AST = aspartate aminotransferase, BF = body fat, CD36 = cluster of differentiation 36, CRP = C-reactive protein, FG = fasting glucose, GGT = gamma-glutamyl transferase, Gpx = glutathione peroxidase, GR = glucocorticoid receptor, GSH = glutathione, HDL = high density lipoprotein, HOMA = HOMA-IR (homeostatic model assessment for insulin resistance), IL-10 = interleukin 10, IL-6 = interleukin 6, Ins = insulin, LDL = low density lipoprotein, LPC = lysophosphatidylcholine, LPE = lysophosphatidylethanolamine, Lp(a) = Llipoprotein(a), MAP = mean arterial pressure, Microbiota div. = microbiota diversity, NAFLD = non-alcoholic fatty liver disease, NF-κB = nuclear factor kappa B, NOX-2 = NADPH oxidase 2, NRF-2 = nuclear factor erythroid 2-related factor 2, oxLDL = oxidized low-density lipoprotein, p65 = p65 subunit of NF-κB, PEPCK = phosphoenolpyruvate carboxykinase, PPARα = peroxisome proliferator-activated receptor alpha, PPARγ = peroxisome proliferator-activated receptor gamma, pPP = peripheral pulse pressure, SBP = systolic blood pressure, TAS = total antioxidant status, TC = total cholesterol, TG = triglycerides, TNF-α = tumor necrosis factor alpha, VF = visceral fat, Wt = weight.




| | Inflammation & immunity | Oxidative stress & antioxidants | Cardiovascular & hepatic | Metabolic & microbiota |
|---|---|---|---|---|
| **Humans** | ↓ IL-6 + gene expression**<br>↓ TNF-a gene expression**<br>↓ IL-10** | ↓ oxLDL<br>↓ NOX-2<br>↑ TAS**<br>↓ or ↑ Gpx** | ↓ SBP<br>↓ ALT*, AST*<br>↓ GGT*<br>↓ pPP | ↓ TG, LDL, TC<br>↓ BF*, VF*, Wt*<br>↓ Ins, FG, HOMA<br>↑ HDL, adipo*<br>↑ Microbiota div.<br>↓ Lp(a), Apo(B)<br>↓ LPC, LPE<br>↓ Cholic acid<br>↓ Hemoglobin** |
| **Animals** | ↓ CRP<br>↓ IL-6 | | ↓ Hepatic steatosis, NAFLD, fibrosis, ALT<br>↓ SBP, DBP, MAP<br>↓ Renin<br>↓ Infarct size<br>Improved cardiac indices | ↓ Glucose<br>↓ TG, LDL, TC<br>↓ Total lipids<br>↑ HDL<br>↑ Microbiota div. |
| ***In vitro, In silico*** | ↓ Monocyte attachment<br>↓ Adhesion molecules<br>↓ NF-κB, p65<br>↓ Cell migration | ↓ oxLDL<br>↓ NRF-2<br>↓ CD36<br>↑ GSH | | ↓ PEPCK<br>↓ GR<br>↓ PPARα<br>↓ AMPKα<br>11β-HSD1 inhibition<br>PPARγ agonist |

Further evidence is presented later in this White Paper as to the efficacy of Chios Mastic Gum in the treatment of *H.pylori* with subsequent linkage to Cardio-Vascular Disease. For example, IFUS Point 1g-4, IFUS Point 1J-2, IFUS Point 1, and IFUS Point 1H-2 and H-3.

Lastly, we find tangential, yet supportive evidence of the effect of the microbial community on human health in a study published by Márcia Dinis, Nini C. Tran, Chapter 5 - Oral immune system and microbes, Editor(s): Tanima Bose, Microbes, Microbial Metabolism, and Mucosal Immunity, Academic Press, 2024, Pages 147-228, ISBN 9780323901444, https://doi.org/10.1016/B978-0-323-90144-4.00005-7.
(https://www.sciencedirect.com/science/article/pii/B9780323901444000057)

In all, one can plausibly conclude the gut-organ axis as expressed as only one of several gut-organ axes plays a crucial role in achieving both human and animal health. One could conjecture that some similar inter-relationship between microbiota is playing out in soil-plant axes. Hence, we now focus on the specific nature of the gut-heart axis as we explore how Chios Mastic Gum emulsified in Ionic Minerals and Water create what seems to be highly unique formulations of Intact Digest™ and Intact Endurance™…and how these two products, when consumed or applied as technologies, can contribute to human health and well-being.

In a report Marone et al. (2001) and Bona et al. (2001) assessed the antibacterial effect of mastic on the clinical isolates of H. pylori at concentrations of 2000 to 1.9 µg/ml. The MBCs, calculated by microdilution method showed that mastic exhibited remarkable bactericidal effect on 12 strains isolated from patients of H. pylori, killing 50% of the executives in concentration of 125 µg/ml and 90% at concentration of 500 µg/ml. Furthermore, the microscopic observation of the morphology of the bacteria by electron emission led to the conclusion that the resin induces the release of air bubbles, the challenge morphological anomalies and segmentation of cells of H. pylori. It was also attempted to place the bactericidal property of mastic on H. pylori in arabino-galactan proteins (AGPs) isolated from the resin (Kottakis et al., 2009). Specifically, the inhibition of growth of H. pylori in the presence of aqueous mastic extracts containing AGPs was studied. The results showed that the extracts of at least 1.4 g resin affect the viability of bacterium, preventing cell growth. There were no indications if AGPs cause abnormal morphology in H. pylori, as mentioned for total mastic (Bona et al., 2001).

IFUS STRONGLY recommends that every shareholder and stakeholder fully read the European Medicines Agency "Assessment report on Pistacia lentiscus L., resina (mastic)." The full report shown above can be found on the IFUS Website (ADD LINK). The report offers emerging pharmacological data on Chios Mastic Gum. More is to come as this is but the tip of the iceberg with what our IFUS Scientific Team is discovering of late.

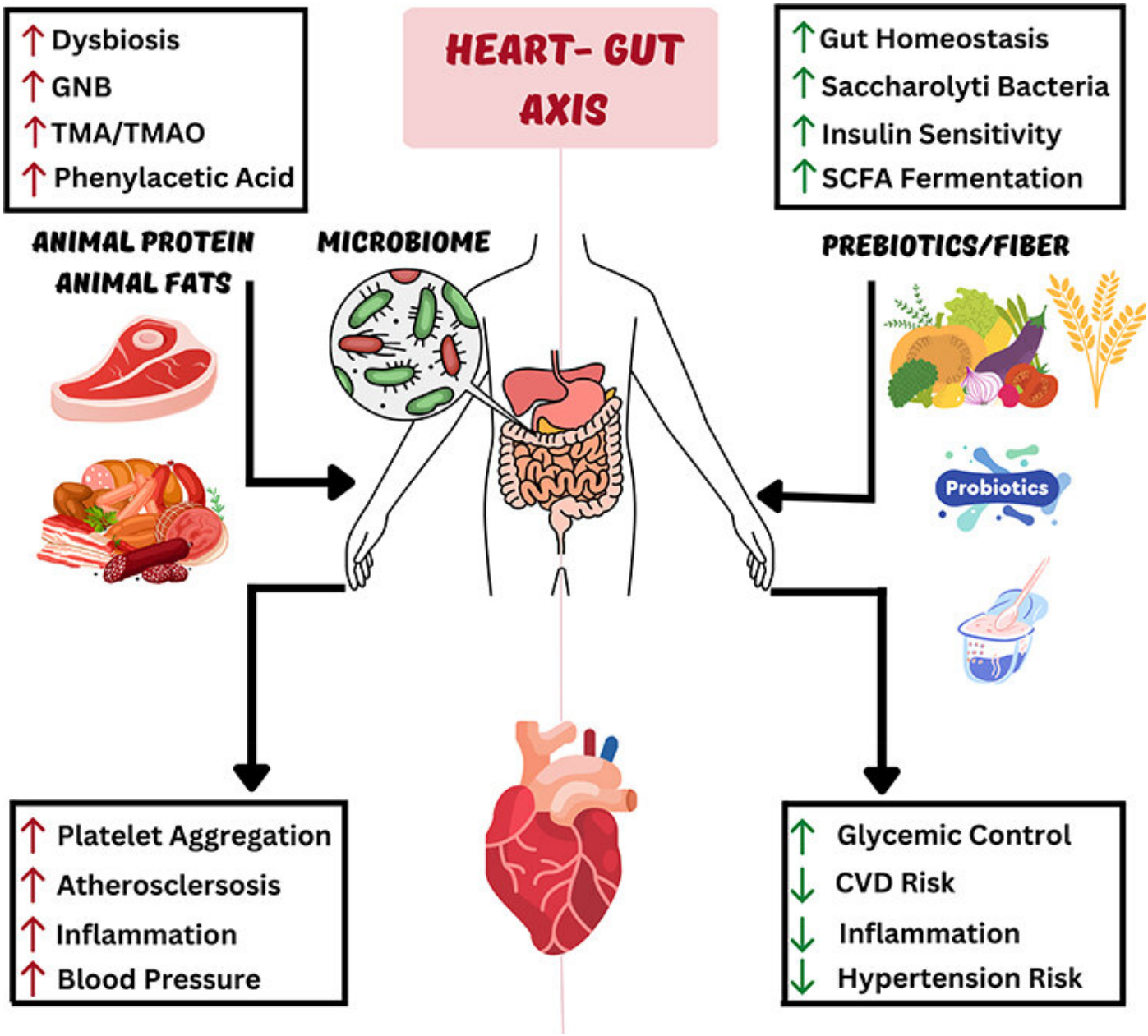
(As a caution, ANY supplement applied orally and/or topically should be used under the consultation of a physician to ensure safe application and minimize contraindications with

existing health issues and/or medications.)

Lastly, as research is evolving even as we write this content, the information contained within will be modified to reflect whatever science is discovered. Hence, the information contained within is considered a “Work-In-Progress” and an evolving part of the IFUS “Knowledge Base” dedicated to present scientific truth supported by studies published in juried journals.

## IFUS Point 1: Overview: The Interconnection between Gut and Heart Health

Where any number of "axes" would be an appropriate starting point, we begin with what and how we eat…as when we eat it…as a basis for a healthy cardiovascular system. The "Heart-Gut Axis" as illustrated below has an interconnection that drives both overall health and/or dis-ease within the human being.




Source: Alaa Diab, et.al., "A Heart-Healthy Diet for Cardiovascular Disease

Prevention: Where Are We Now?" (Vascular Health and Risk Management, April 202319:237-253 DOI:10.2147/VHRM.S379874))

Further evidence is provided by an "Abstract" that tells us: "The heart and the gut seem to be two organs that do not have much in common. However, there is an obvious and clinically relevant impact of gut functions on the absorption of drugs and oral therapies on the one hand. On the other hand, the gut determines the quantity of nutrient uptake and plays a central role in metabolic diseases. Patients with inflammatory bowel diseases appear to have a higher risk for coronary heart disease despite a lower prevalence of 'classical' risk factors, indicating additional links between the gut and the heart. However, they certainly have a 'leaky' intestinal barrier associated with increased permeability for bacterial wall products. An impaired intestinal barrier function will be followed by bacterial translocation and presence of bacterial products in the circulation, which can contribute to atherosclerosis and chronic heart failure (CHF) as recent data indicate. Impaired cardiac function in CHF vice versa impacts intestinal microcirculation leading to a barrier defect of the intestinal mucosa and increased bacterial translocation. These pathways and the most recent insights into the impact of the gut on acute and chronic heart disease will be discussed in this review."

Source: Rogler G, Rosano G. The heart and the gut. Eur Heart J. 2014 Feb;35(7):426-30. doi: 10.1093/eurheartj/eht271. Epub 2013 Jul 17. PMID: 23864132.

The dis-ease caused by malfunctions and managed through the interconnection of the "Heart-Gut Axis" is summarized and illustrated by Table 1 below.

**Table I Alterations of gastrointestinal function in patients with chronic heart failure (according to Sandek *et al.*[70])**

| |
|---|
| Increased small intestinal and large intestinal paracellular permeability in stable compensated chronic heart failure patients |
| Diminished carrier-mediated transport for D-xylose |
| Excessive enteric protein loss in infants with severe congenital heart disease |
| Decreased absorption of fat and protein |
| Thickened bowel wall of the terminal ileum and the colon |
| Elevated collagen content in small intestinal biopsies |
| Increased distance between the capillary wall and the basal membrane of the enterocyte |
| Increased bacterial biofilm on sigmoid biopsies |

Source: Sandek A, Anker SD, von Haehling S. The gut and intestinal bacteria in chronic heart failure. Curr Drug Metab. 2009 Jan;10(1):22-8. doi: 10.2174/138920009787048374. PMID: 19149510.

Any number of scientific studies or publications establish the importance interplay at work in the "Heart-Gut Axis." As seen below, we see deeper science in connecting "Cytokine receptors" to Atherosclerosis and Coronary Heart Disease being initiated by "intestinal microbiota."

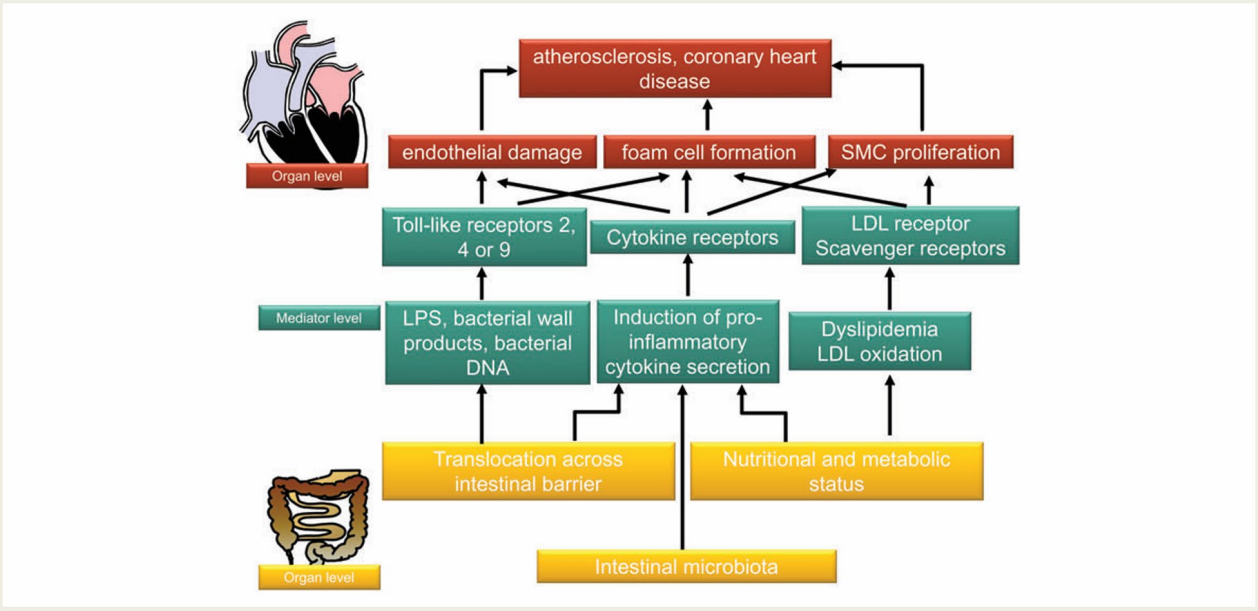



**Figure 1** Potential pathways of gut involvement in the pathogenesis of atherosclerosis and coronary heart disease. The intestinal microbiota has a profound influence on mucosa barrier functions and on the nutritional/metabolic status of its 'host'. Certain bacterial families such as *Firmicutes* contribute to a higher uptake, for example, of short-chain fatty acids. In addition, a leaky barrier or impaired intestinal epithelial barrier function allows bacterial products such as lipopolysaccharide, bacterial DNA (CpG motifs), or peptidoglycans to enter the circulation. Furthermore, the microbiota can directly influence the cytokine production of epithelial cells and innate immune cells. Those mediators also enter the circulation. Lipopolysaccharide itself but also the metabolic situation can induce the production of oxidized low-density lipoprotein. These mediators are recognized by specific receptors (such as Toll-like receptors for bacterial wall products, cytokines receptors, or scavenger receptors) on (or in) endothelial cells, macrophages, or smooth muscle cells (SMCs) of the arterial wall. They are able to induce endothelial damage, foam cell formation, and SMC proliferations, which are features of atherosclerosis and coronary heart disease.

The exploration into the "Heart-Gut Axis" continues with the illustration below, which links "Intestinal barrier defect" and "impaired intestinal absorption" to "Chronic heart failure."

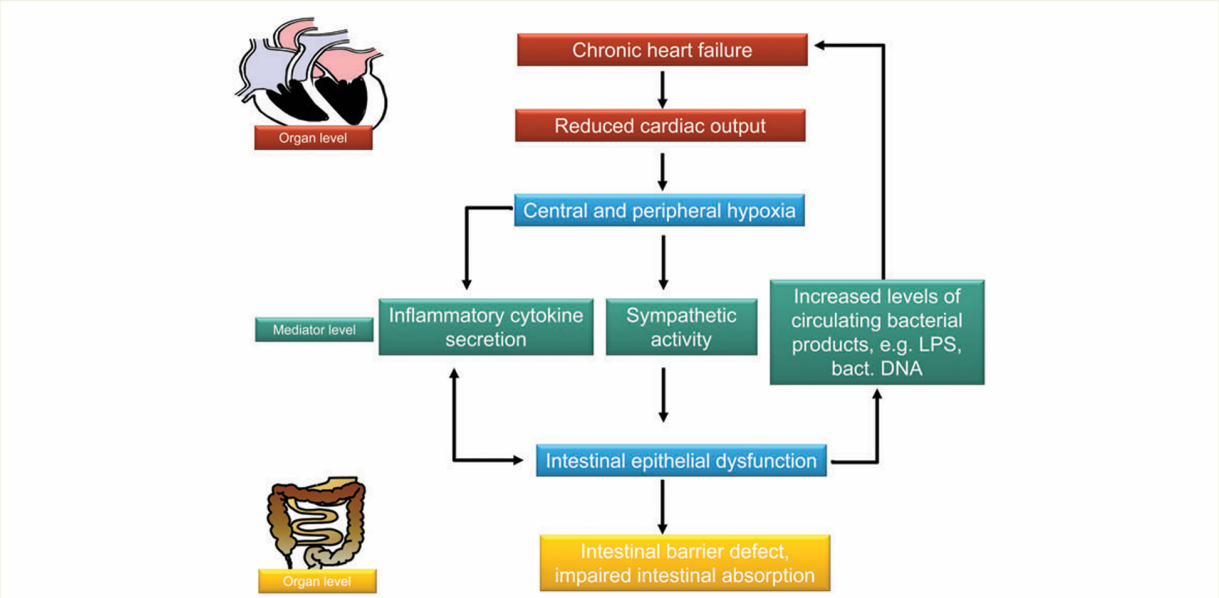
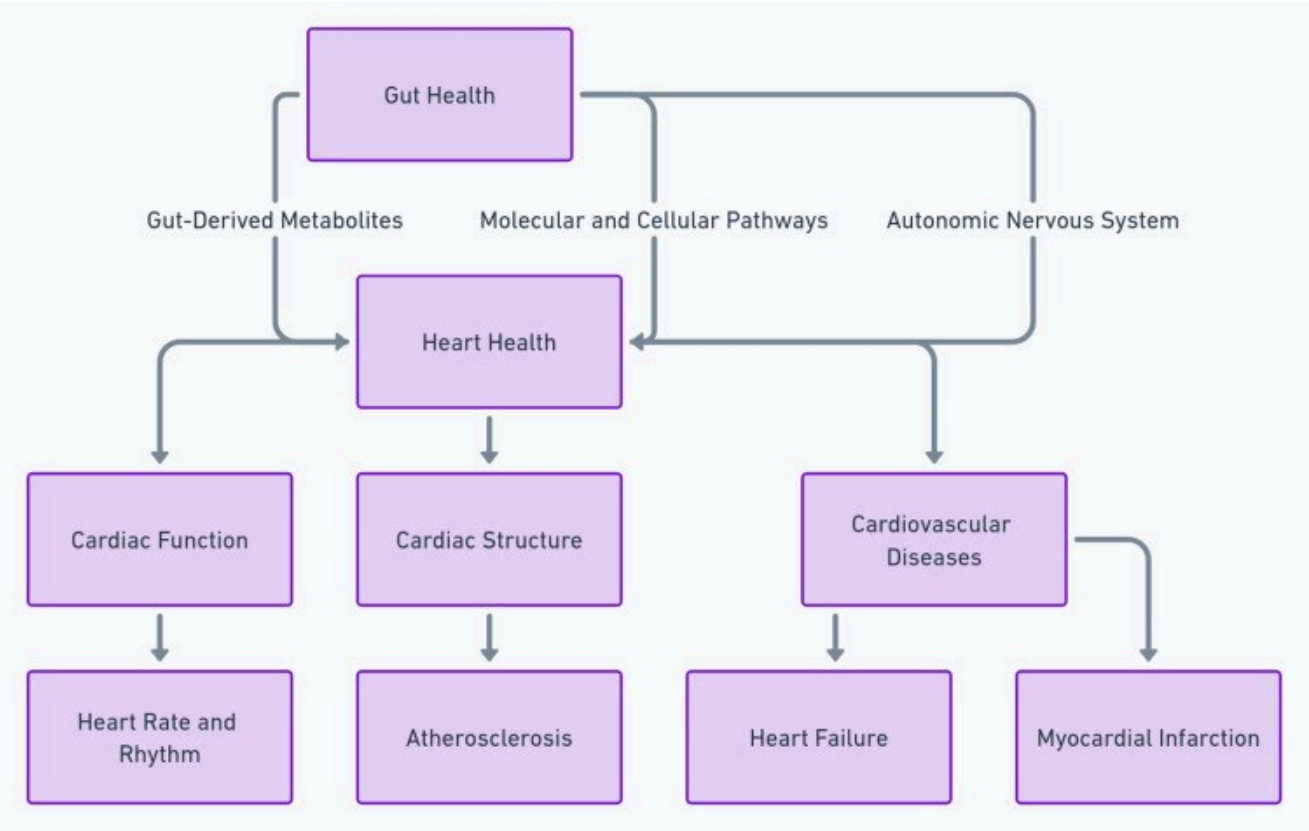



**Figure 2** The heart and the gut in the pathophysiology of chronic heart failure. Chronic heart failure will cause a reduction in cardiac output which in turn will cause central and peripheral hypoxia. Among the organs that are affected by peripheral hypoxia is the small and large intestine. Hypoxia will cause an increase in inflammatory cytokine production, sympathetic activity, and production of other mediators (such as leucotrienes, prostaglandins, and others that are not depicted in this graph). These mediators and the sympathetic activity may cause a malfunction of the gut. A further contributor will be a venous stasis increasing mucosal hypoxia. The mentioned factors have been shown to impair epithelial barrier function leading to a penetration of bacterial products across the intestinal barrier. Preliminary data indicate that the presence of those products in the circulation further aggravate chronic heart failure. Further studies with modern technologies such as mass spectroscopy and pyro sequencing of bacterial DNA will be necessary to confirm this. On the other hand, a dysfunction of the intestinal barrier will also cause impaired absorption negatively influencing the nutritional status of patients with end-stage heart disease.

Hence, it is plausible to conclude that the "Heart-Gut Axis" is critical to human health in mind and body, and certainly worthy of greater exploration. We continue with IFUS Point 1a.

IFUS Point 1a: Figure 1 below is provided to us in a recent study published by Akshay A, Gasim R, Ali TE, Kumar YS, Hassan A. "Unlocking the Gut-Cardiac Axis: A Paradigm Shift in Cardiovascular Health." Cureus. 2023 Dec 24;15(12):e51039. doi: 10.7759/cureus.51039. PMID: 38264397; PMCID: PMC10805229.

**Figure 1. Exploring the gut-cardiac axis: a visual guide to interconnected health.**



The image is generated by the authors.

In the aforementioned study continues with a substantive "Abstract", that states:

"The gut-cardiac axis represents an emerging area of research focusing on the relationship between gut health and cardiovascular function. This narrative review examines the Gut-Cardiac Axis, emphasizing its emerging role in cardiovascular health and disease management.

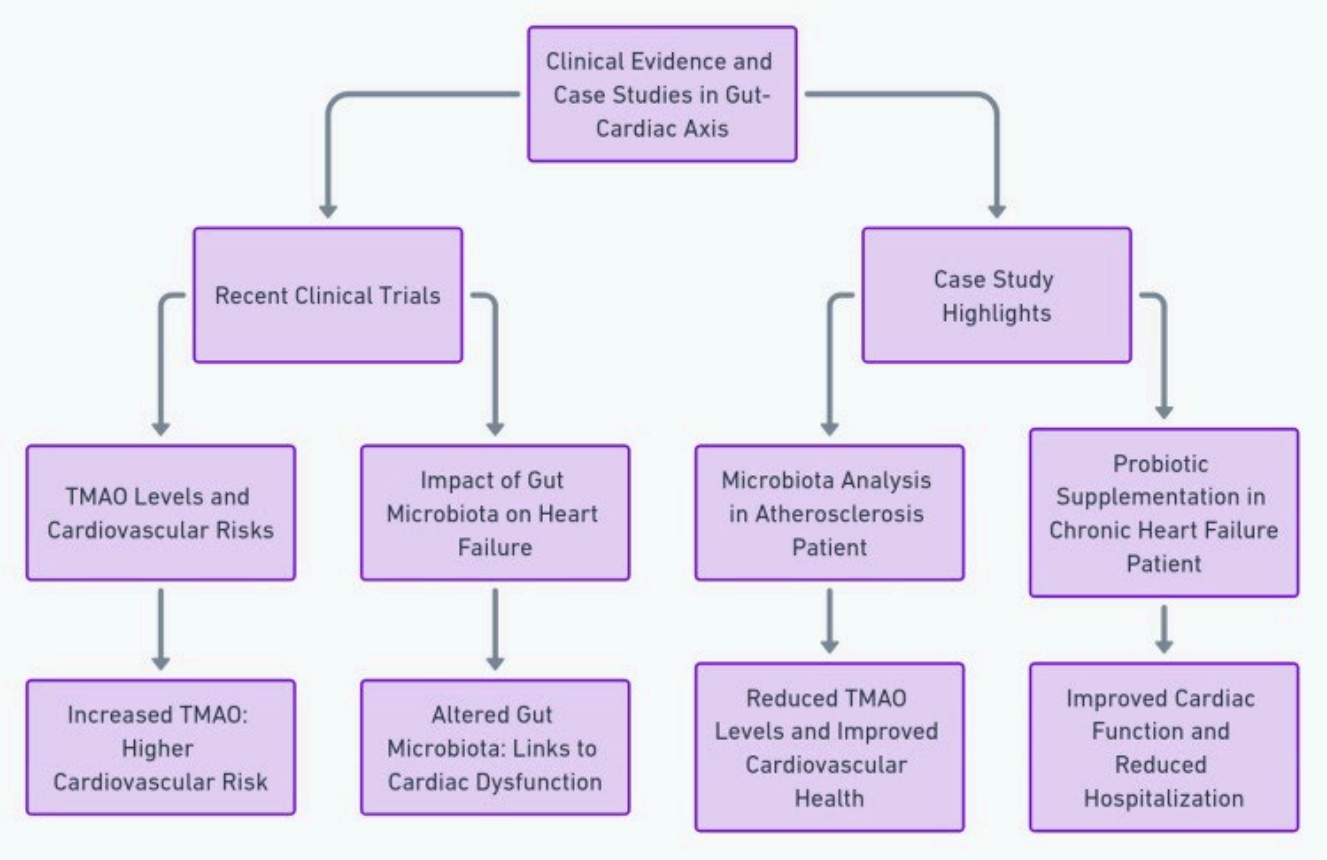
Traditionally viewed as a component of the digestive system, the gut is now recognized for its significant influence on cardiac health. The gut microbiota, its metabolites, and gut-related inflammation are key factors affecting heart structure and function. This review highlights how dietary and nutritional interventions can effectively modulate the gut-cardiac axis, leading to personalized strategies for optimizing cardiovascular health. We discuss the clinical relevance of the gut-cardiac axis, particularly its role in providing diagnostic and prognostic markers for cardiovascular diseases.

This exploration of the gut-cardiac axis marks a significant shift in cardiology, integrating gut health into cardiovascular risk assessment and treatment strategies. The review provides an in-depth overview of current research and its potential to impact cardiovascular medicine significantly. We emphasize the importance of this research in advancing patient

care and improving cardiac outcomes, underlining the potential of the gut-cardiac axis to transform cardiovascular health management."

We find in the experimental design of this study, validation of its statistical viability:"

Figure 2. This diagram provides a structured overview of pivotal clinical evidence and case studies that have significantly advanced our understanding of the gut-cardiac axis.




The authors generated the image."

As this article is so very content rich, we offer significant excerpts from it for your consideration.

"1. Establishing the connection:

1a. The Complex Interplay: Understanding the Gut-Cardiac Link

The intricate interplay between the gastrointestinal and cardiovascular systems defines the gut-cardiac axis. To fully understand this relationship, exploring the connections that link

these two systems is crucial. Central to this connection is the gut microbiome. Recent studies have shown that the gut microbiota, far from being just a digestive aid, significantly regulates various aspects of cardiac function. It communicates with the heart through diverse pathways, including immune signaling, microbial metabolites, and neural connections [16].

> Supported by Ref.16: The gut microbiota and host health: a new clinical frontier. Marchesi JR, Adams DH, Fava F, et al. Gut. 2016;65:330–339. doi: 10.1136/gutjnl-2015-309990. [DOI] [PMC free article] [PubMed]

1b. Communication Pathways Between the Gut and Heart

The communication pathways between the gut and the heart are complex and multifunctional. Research highlights the role of gut-derived metabolites like SCFAs in cardiac function regulation [16]. These metabolites act as messengers in the gut-cardiac dialogue, influencing processes such as inflammation and oxidative stress in the heart.

Moreover, the connection between the gut and the heart extends to the autonomic nervous system. The "gut-brain-cardiac axis" field focuses on how the nervous system mediates communication between these organs [17]. The vagus nerve, for example, is critical in transmitting signals between the gut and the heart, influencing heart rate, rhythm, and arrhythmia risk.

> Ref.16: The gut microbiota and host health: a new clinical frontier. Marchesi

> Ref. 17: The vagus nerve in appetite regulation, mood, and intestinal inflammation. Browning KN, Verheijden S, Boeckxstaens GE. Gastroenterology. 2017;152:730–744. doi: 10.1053/j.gastro.2016.10.046. [DOI] [PMC free article] [PubMed]

2. Gut microbiome and heart health:

2a. Gut Microbiota: A Novel Player in Cardiovascular Health

The gut microbiota's role in cardiovascular health represents a significant shift in medical understanding. Previously seen mainly as a digestive component, the gut microbiome is now acknowledged as a critical player in cardiovascular health. Recent research highlights how the composition and diversity of gut microbes substantially affect cardiovascular outcomes [6].

Ref. 6: Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Wang Z, Klipfell E, Bennett BJ, et al. Nature. 2011;472:57–63. doi: 10.1038/nature09922. [DOI] [PMC free article] [PubMed]

This paradigm shift is based on the understanding that gut bacteria produce metabolites like SCFAs and TMAO. These metabolites significantly impact systemic inflammation, lipid metabolism, and endothelial function. They act as critical messengers and modulators in the gut-cardiac interaction, influencing the risk of atherosclerosis, hypertension, and other cardiovascular conditions [18].

Ref. 18:Modulation of gut microbiota by foods and herbs to prevent cardiovascular diseases. Panyod S, Wu WK, Chen CC, Wu MS, Ho CT, Sheen LY. J Tradit Complement Med. 2023;13:107–118. doi: 10.1016/j.jtcme.2021.09.006. [DOI] [PMC free article] [PubMed]

2b. The Microbial Impact on Cardiac Function

The influence of the gut microbiota on cardiac health goes beyond systemic effects to directly impact cardiac function. Research indicates that the gut microbial community can directly alter the heart's structure and functionality. Microbial products and metabolites can induce chronic low-grade inflammation in the heart, contributing to conditions like myocardial infarction and heart failure [19].

Ref. 19: Heart failure is associated with depletion of core intestinal microbiota. Luedde M, Winkler T, Heinsen FA, et al. ESC Heart Fail. 2017;4:282–290. doi: 10.1002/ehf2.12155. [DOI] [PMC free article] [PubMed]

Recent studies have also explored the role of microbial regulation of the autonomic nervous system and its significant implications for cardiac health. This regulation affects heart rate and rhythm, potentially increasing the susceptibility to arrhythmias [9]. Dysbiosis in the gut microbiota, characterized by an imbalance in microbial communities, has been linked to changes in heart rate variability, a measure of the heart's adaptability. Such dysbiosis can disrupt the balance in the autonomic nervous system, affecting cardiac response to stressors and potentially leading to rhythm disturbances [5].

Ref. 9: Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. Tang WH, Wang Z, Levison BS, et al. N Engl J Med. 2013;368:1575–1584. doi: 10.1056/NEJMoa1109400. [DOI] [PMC free article] [PubMed]

Ref 5: Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. Singh R, Chandrashekharappa S, Bodduluri SR, et al. Nat Commun. 2019;10:89. doi: 10.1038/s41467-018-07859-7. [DOI] [PMC free article] [PubMed]

## 3. Inflammation, immunity, and cardiovascular disease

### 3a. Inflammation in the Gut and Its Influence on Cardiac Inflammation

Inflammation is crucial in the gut-cardiac axis, significantly impacting cardiovascular health. Traditionally, inflammation within the cardiovascular system has been linked to conditions like atherosclerosis and heart disease. Recent research, however, has highlighted a direct link between gut inflammation and cardiac inflammation. This connection is vital as it demonstrates how the gut can modulate systemic inflammation, subsequently influencing the development and progression of cardiovascular diseases [20].

Ref. 20: Anti-inflammatory therapy in chronic disease: challenges and opportunities. Tabas I, Glass CK. Science. 2013;339:166–172. doi: 10.1126/science.1230720. [DOI] [PMC free article] [PubMed]

A key element in this relationship is the gut barrier. When this barrier is compromised, microbial products can enter the bloodstream, initiating an inflammatory response [21]. This gut-derived inflammation contributes to endothelial dysfunction and the formation of atherosclerotic plaques. Understanding the influence of gut inflammation on cardiac inflammation is essential for developing interventions to reduce cardiovascular risks [22].

Ref. 21: Biofilms and Microbiomes. [ Oct; 2023 ]. 2023. https://www.nature.com/npjbiofilms/ https://www.nature.com/npjbiofilms/

Ref. 22: Dysbiosis of gut microbiota with reduced trimethylamine-N-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. Yin J, Liao SX, He Y, et al. J Am Heart Assoc. 2015;4:0. doi: 10.1161/JAHA.115.002699. [DOI] [PMC free article] [PubMed]

### 3b. Immune Responses Linking Gut Health to Cardiovascular Diseases

The immune system plays a central role in the gut-cardiac axis, bridging gut health and cardiovascular diseases. Studies have shown that an imbalance in gut microbiota, or dysbiosis, can activate the immune system, producing pro-inflammatory cytokines [3]. These cytokines can then travel to the cardiovascular system, promoting inflammation and

contributing to the onset of cardiovascular diseases.

> Ref. 3: Microbial modulation of cardiovascular disease. Brown JM, Hazen SL. Nat Rev Microbiol. 2018;16:171–181. doi: 10.1038/nrmicro.2017.149. [DOI] [PMC free article] [PubMed]

Furthermore, the impact of the gut on the immune system goes beyond systemic inflammation. It also affects the regulation and activity of immune cells like monocytes and macrophages, vital in developing atherosclerotic plaques. These immune responses directly link gut health and cardiovascular diseases, highlighting the importance of understanding the gut-cardiac axis in the context of immunity [23].

> Ref. 23: Immunity, atherosclerosis and cardiovascular disease. Frostegård J. BMC Med. 2013;11:117. doi: 10.1186/1741-7015-11-117. [DOI] [PMC free article] [PubMed]

We can identify potential therapeutic targets by exploring the complex relationship between gut inflammation, immune responses, and cardiovascular diseases. These targets could help prevent or mitigate the effects of inflammation on heart health, paving the way for more effective treatments in cardiovascular medicine.

4. Metabolism at the crossroads

4a. Metabolic Implications of the Gut-Cardiac Axis

The gut-cardiac axis represents a crucial intersection of metabolic processes significantly influencing cardiovascular health. Both systemic and microbial metabolism play vital roles in this complex relationship. Key metabolic aspects of the gut-cardiac axis include lipid metabolism, glucose homeostasis, and overall energy balance [24].

> Ref. 24: The failing heart—an engine out of fuel. Neubauer S. N Engl J Med. 2007;356:1140–1151. doi: 10.1056/NEJMra063052. [DOI] [PubMed

A central component of this interplay is the role of gut microbes in modulating metabolic processes. The gut microbiota is involved in transforming dietary components and endogenous molecules, leading to the production of various metabolites with significant effects on health. SCFAs, for instance, are metabolites produced by gut bacteria during the fermentation of dietary fibers. These SCFAs act as metabolic signaling molecules and are known to regulate lipid metabolism, inflammation, and energy homeostasis, all of which

directly influence cardiac health [25].

> Ref. 25: The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. J Lipid Res. 2013;54:2325–2340. doi: 10.1194/jlr.R036012. [DOI] [PMC free article] [PubMed]

4b. Gut-Produced Metabolites and Their Role in Heart Health

Gut-produced metabolites are pivotal in shaping the gut-cardiac axis. These compounds, acting as biochemical messengers, significantly affect cardiovascular health. TMAO, a metabolite resulting from the microbial metabolism of dietary choline and carnitine, is a prime example. TMAO has received considerable attention due to its involvement in atherosclerosis, thrombosis, and other cardiovascular conditions [6].

> Ref. 6: Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Wang Z, Klipfell E, Bennett BJ, et al. Nature. 2011;472:57–63. doi: 10.1038/nature09922. [DOI] [PMC free article] [PubMed]

The impact of these gut-derived metabolites on the cardiovascular system is diverse. They modulate inflammation, oxidative stress, blood pressure regulation, and endothelial function. Recognizing the role of these metabolites enhances our understanding of the metabolic communication between the gut and the heart.

5. Dietary influences and nutritional strategies

5a. The Power of Diet: Nourishing the Gut and Heart

Diet plays a critical role in shaping the gut-cardiac axis, offering a means to nourish the gut and the heart simultaneously. The efficacy of diet in this context lies in its ability to alter the composition and function of the gut microbiota, thereby impacting cardiovascular health [26]. Diets enriched with dietary fibers, prebiotics, and probiotics can promote diverse and balanced gut microbiota, enhancing gut health and positively influencing cardiac outcomes [27].

> Ref. 26: Diet rapidly and reproducibly alters the human gut microbiome. [ Oct; 2023 ]. 2013. https://www.nature.com/articles/nature12820. https://www.nature.com/articles/nature12820 [DOI] [PMC free article]

Ref. 27: What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GA, Gasbarrini A, Mele MC. Microorganisms. 2019;7:14. doi: 10.3390/microorganisms7010014. [DOI] [PMC free article] [PubMed]

Dietary choices also directly affect the production of gut-derived metabolites. For instance, foods high in choline and carnitine contribute to increased TMAO levels, a metabolite associated with heightened cardiovascular risk and atherosclerosis. Recognizing the dietary elements that either support or undermine the gut-cardiac axis is essential for developing effective heart-healthy nutritional strategies [28].

Ref. 28: Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: a randomized controlled trial. Cho CE, Taesuwan S, Malysheva OV, et al. Mol Nutr Food Res. 2017;61 doi: 10.1002/mnfr.201600324. [DOI] [PubMed

5b. Nutritional Approaches for Cardiovascular and Gut Well-Being

Nutritional strategies within the gut-cardiac axis framework present a promising route for bolstering cardiovascular and gut well-being. These strategies encompass dietary patterns to optimize gut health, reduce inflammation, and address metabolic risk factors linked to heart disease [29].

Ref. 29: Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. Schwingshackl L, Hoffmann G. Nutr Metab Cardiovasc Dis. 2014;24:929–939. doi: 10.1016/j.numecd.2014.03.003. [DOI] [PubMed ]

Key nutritional strategies include adopting diets abundant in antioxidants, polyphenols, and omega-3 fatty acids, known for their cardioprotective properties. Furthermore, precision nutrition, which involves tailoring dietary plans to an individual's specific gut microbiota profile, can enhance the gut-cardiac axis and aid in preventing cardiovascular diseases. Adjusting dietary fiber intake is also crucial, as fibers act as substrates for the gut microbiota, producing beneficial metabolites such as SCFAs [30].

Ref. 30: Dietary fiber and prebiotics and the gastrointestinal microbiota. Holscher HD. Gut Microbes. 2017;8:172–184. doi: 10.1080/19490976.2017.1290756. [DOI] [PMC free article] [PubMed]

This exploration into the role of diet underscores its significant influence on gut and heart health. It goes beyond standard dietary recommendations, highlighting the fundamental role of dietary choices in shaping the gut's microbial composition, producing bioactive metabolites, and modulating systemic inflammation. Embracing this holistic approach acknowledges the comprehensive impact of diet on our health, emphasizing its role as a mediator between the gut microbiota and the heart.

6. Conclusions

The exploration of the gut-cardiac axis marks a pivotal advancement in cardiovascular health, revealing the critical interplay between the gut and the heart. This connection, once underappreciated, is now recognized for its significant impact on cardiac well-being. Influences from gut microbiota, metabolites, and inflammation are now understood to directly affect heart structure and function. This shift in understanding has led to the development of personalized dietary and nutritional interventions, reshaping the landscape of cardiology risk assessment and treatment."

Hence, we find in the aforementioned study a basis by which the positive and plausible effects of substances like Chios Mastic Gum can be established. Later is the document we will explore the phytochemical components said to be contained in Chios Mastic Gum, along with links to both Gut and Cardiovascular health. Additionally, we will explore ionic mineral efficacy on the Heart-Gut Axis. But, first we will more deeply explore the microbial biome of the gut and the human body at large.

IFUS Point 1c:

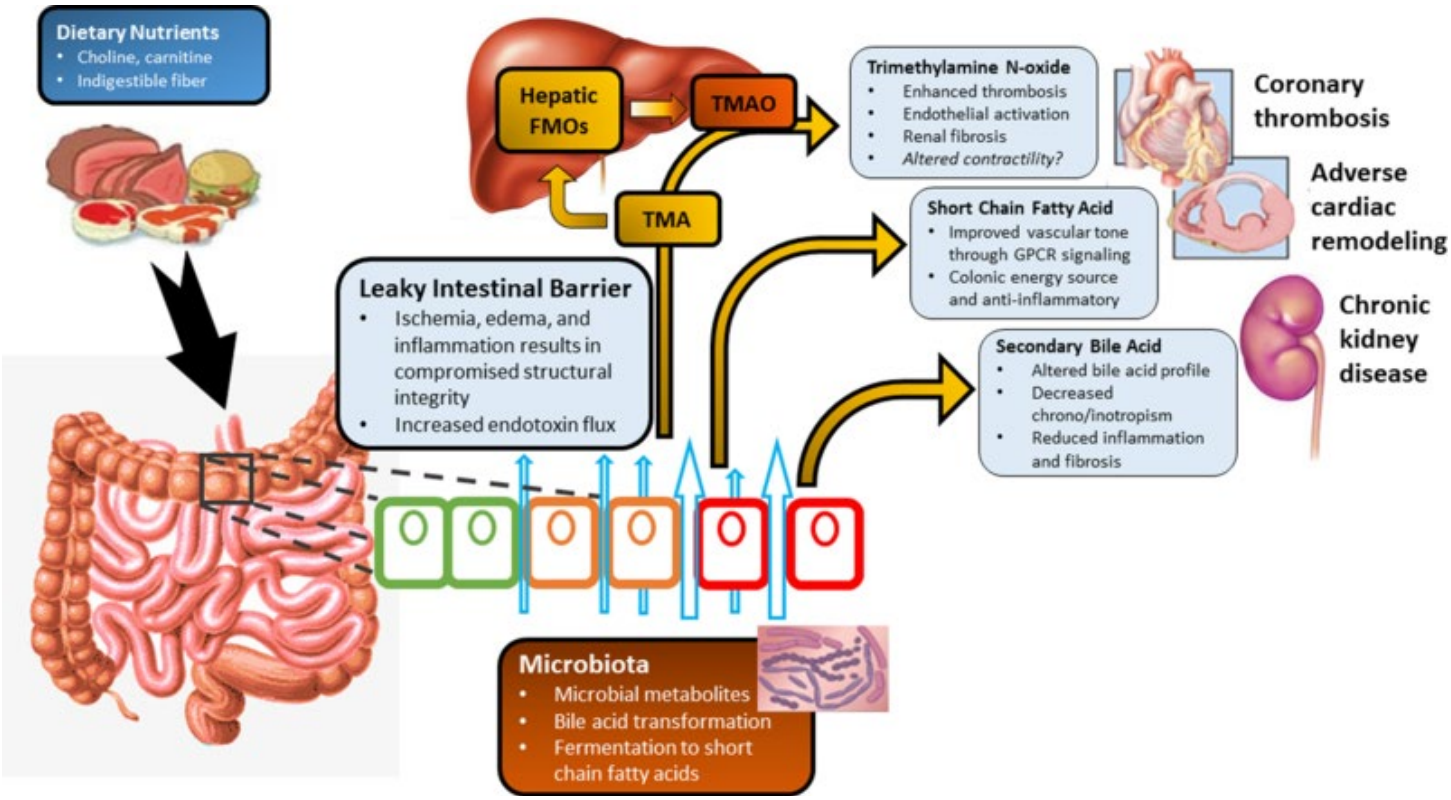
The diagram below, as well as the information contained in the scientific study from where the diagram is found, offers to us the following:

"Abstract: Advances in our understanding of how the gut microbiota contributes to human health and diseases have expanded our insight into how microbial composition and function affect the human host. Heart failure is associated with splanchnic circulation congestion, leading to bowel wall oedema and impaired intestinal barrier function. This situation is thought to heighten the overall inflammatory state via increased bacterial translocation and the presence of bacterial products in the systemic blood circulation. Several metabolites produced by gut microorganisms from dietary metabolism have been linked to pathologies such as atherosclerosis, hypertension, heart failure, chronic kidney disease, obesity, and type 2 diabetes mellitus. These findings suggest that the gut microbiome functions like an endocrine organ by generating bioactive metabolites that can

directly or indirectly affect host physiology. In this Review, we discuss several newly discovered gut microbial metabolic pathways, including the production of trimethylamine and trimethylamine N-oxide, short-chain fatty acids, and secondary bile acids, that seem to participate in the development and progression of cardiovascular diseases, including heart failure. We also discuss the gut microbiome as a novel therapeutic target for the treatment of cardiovascular disease, and potential strategies for targeting intestinal microbial processes."

Source: Tang, W.H.W., Li, D.Y. & Hazen, S.L. Dietary metabolism, the gut microbiome, and heart failure. Nat Rev Cardiol 16, 137–154 (2019). https://doi.org/10.1038/s41569-018-0108-7

Figure 1. Microbial-host meta-organismal pathway linking dietary metabolism, gut microbiota, and cardio-renal disease progression.




"Legend: Poor cardiac output in heart failure results in intestinal ischemia, edema, and inflammation which leads to a "leaky" intestinal barrier. This allows for increased passage of inflammatory bacterial products to enter the bloodstream causing chronic low-grade

inflammation. Furthermore, this alters the intestinal environment and impacts both the normal microbial community which resides in the gut and subsequently, the metabolic products from these bacteria. The metabolic pathways include fermentation of indigestible fiber to short chain fatty acids which have protective properties reducing inflammation and improving vascular tone. Dietary sources including choline, phosphatidylcholine, l-carnitine and other methylamine-containing nutrients provide substrates for microbiota mediated production of trimethylamine (TMA). TMA then enters the portal circulation and is converted by the hepatic host flavin-containing monooxygenase (FMO) family of enzymes to trimethylamine n-oxide (TMAO). TMAO can promote the development of atherogenesis, thrombosis, kidney disease, and heart failure. Additionally, the bacterial transformation of bile acids can result in altered bile acid profiles which then can affect systemic inflammatory and fibrotic processes. Collectively, these processes can influence the individual susceptibility, severity of heart failure."

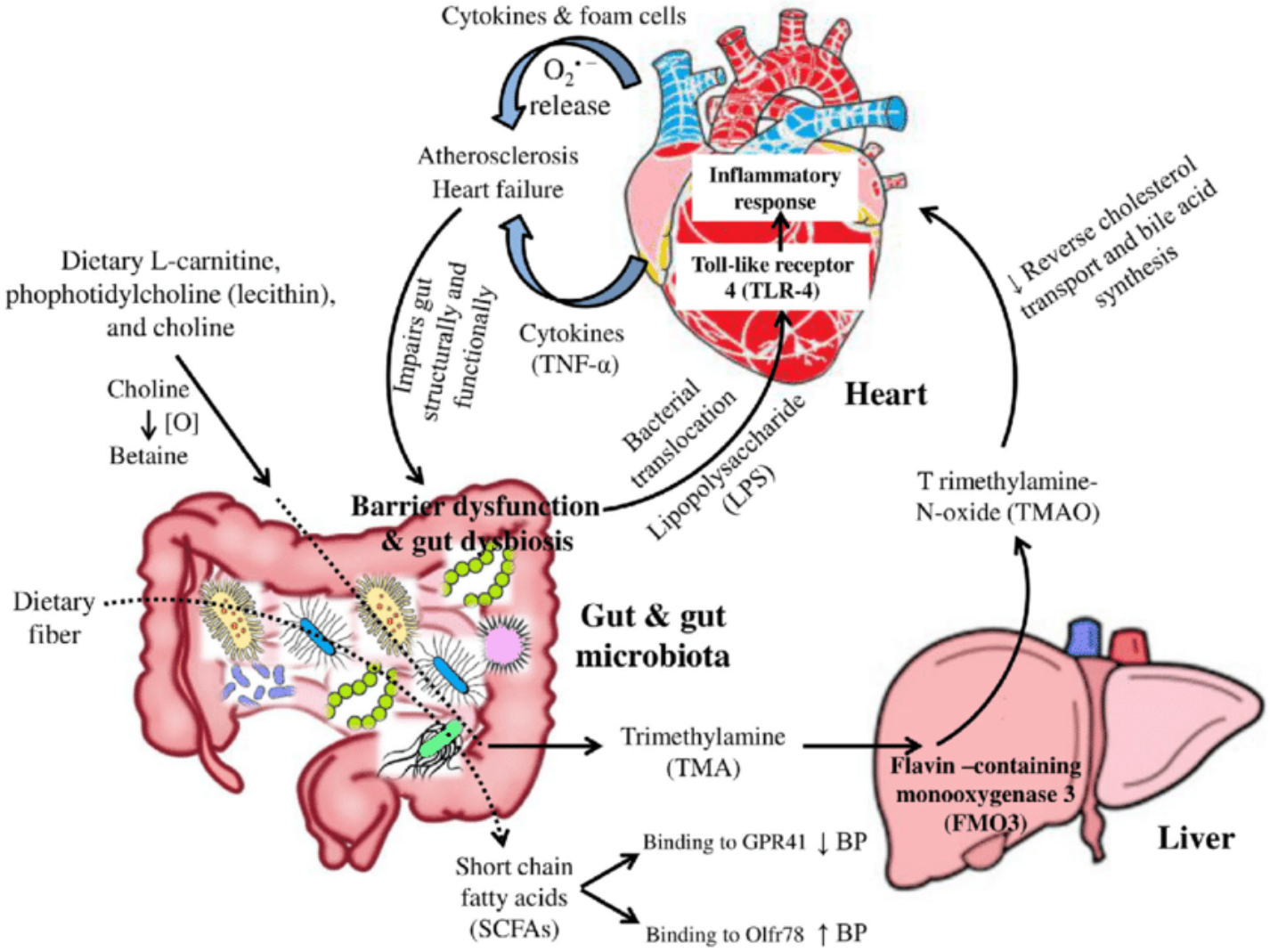
IFUS Point 1d:

We find in another schematic diagram supported by content from a study that links the following to human dis-ease:

- Cytokines and foam Cells
- Cytokines (specifically TNF-alpha)
- Toll-like receptor 4 (TLR-4)
- Tri-methylamine-N-oxide (TMAO)

Later in this White Paper, we will explore each of these as each relates to Chios Mastic Gum and ionic minerals. But, for now, let us once more establish a baseline of understanding as to what the science is suggesting to us in regard to the "Gut–organ axis: a microbial outreach and networking."

From this study, we are offered insight into a "Schematic representation of 'gut-heart' axis." As illustrated below we find connection to Cytokines, TNF-alpha, TLR-4, and TMAO:




Source: Shruti Ahlawat, et.al; "Gut–organ axis: A microbial outreach and networking," May 2020 Letters in Applied Microbiology 72(6), DOI:10.1111/lam.13333

The aforementioned study offers additional insight into the effect of microbial actions, interactions, and relationships within various Gut-organ axes (for purposes of this White Paper a focus on the Gut-Heart Axis). Screenshots focused specifically on GM alterations affecting the Gut-Heart Axis are provided below:

"Table 1: List of studies on disease-associated gut microbiota (GM) alterations for

establishing a link between gut and other organs via ‘gut–organ’ axis."

| | | | | | | |
|---|---|---|---|---|---|---|
| Gut–heart axis | | | | | | |
| 49. | Heart failure (HF) | 12 HF patients/ 12 healthy controls (HC); 12 HF patients (younger than 60 years)/ 10 HF patients (60 years or older) | ↓ *Dorea longicatena* and *Eubacterium rectale* in HF patients than in HC<br>↓ Bacteroidetes and *Faecalibacterium*<br>↑ Proteobacteria and *Lactobacillus* in older HF patients | 16S ribosomal RNA gene sequencing of faecal samples | HF patients have significantly altered GM, which varies further according to age | Kamo *et al*. (2017b) |
| 50. | Chronic heart failure (HF) | 30 mild CHF patients/30 moderate to severe CHF | ↑*Candida* species, *Campylobacter*, *Shigella*, *Salmonella* and *Yersinia* | – | Pathogenic bacteria and *Candida* were abundant in entire the CHF population | Pasini *et al*. (2016) |

(continued)



**Table 1** (continued)

| S. no. | Diseased state | No. of diseased *vs* healthy | Shift in flora | Method used | Outcome | Reference |
|---|---|---|---|---|---|---|
| | | patients/20 healthy controls (HC) | *enterocolitica* in entire CHF population | | | |
| 51. | Atherosclerotic cardiovascular disease (ACVD) | 218 ACVD patients/187 healthy controls (HC) | ↑ *Streptococcus* sp., *Lactobacillus salivarius*, *Solobacterium moorei*, *Atopobium parvulum*, *Ruminococcus gnavus* and *Eggerthella lenta*<br>↑ *Enterobacteriaceae* including *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella* sp.<br>↓ *Prevotella copri*, *Bacteroides* sp., *Alistipes shahii*, *Roseburia intestinalis* and *Faecalibacterium* cf. *prausnitzii* | Metagenomic shotgun sequencing | Profound imbalances in the composition and interspecies relationship in the GM of ACVD patients as compared to HC | Jie *et al*. (2017) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 52. | Symptomatic atherosclerosis | 12 Symptomatic atherosclerosis patients/13 healthy controls (HC) | ↑ *Collinsella* in symptomatic atherosclerosis patients; ↑ *Eubacterium* and *Roseburia* in HC | Metagenomic shotgun sequencing of faeces | Gut metagenomes were enriched in genes encoding peptidoglycan synthesis with characteristic changes in symptomatic atherosclerosis patients | Karlsson *et al*. (2012) |
| 53. | Hypertension | 60 Primary hypertension patients/60 healthy controls (HC) | ↑ *Parabacteroides merdae*, *Klebsiella* sp. and *Streptococcus* sp. in primary hypertension patients<br>↑ SCFAs-producing *Faecalibacterium prausnitzii* and *Roseburia* sp. in HC | Whole-metagenome shotgun sequencing | Hypertensive gut microbiome have higher membrane transport, LPS biosynthesis and steroid degradation | Yan *et al*. (2017) |
| 54. | Hypertension | 56 Prehypertension (pHTN)/99 Primary hypertension (HTN) patients/41 healthy controls (HC) | ↓ Microbial richness and diversity; ↑ *Klebsiella* and *Prevotella*; ↓ *Bifidobacterium*, *Butyrivibrio*, *Coprococcus* and *Oscillibacter* in both pHTN and HTN groups | Metagenomic sequencing on Illumina platform | The microbiome characteristic in pHTN group was quite similar to that in HTN | Li *et al*. (2017) |
| 55. | Myocardial infarction (MI) | 99 MI patients/ 103 patients at high CVD risk but free of coronary disease | ↓ Cholesterol-degrading bacteria (*Aerococcaceae*, *Norcardiaceae*, *Chryseobacterium*, *Gordonia*, *Propionibacterium* and *Rhodococcus*) in MI patients;<br>↑ Blood bacterial 16S rDNA concentration in patients with LDL cholesterol ≥1 g $l^{-1}$ as compared with controls | 16S qPCR- and 16S-targeted metagenomic sequencing of blood | This could represent potential predictive blood microbiome-based biomarkers of the MI | Amar *et al*. (2019) |



Shruti Ahlawat, et.al. go on to share with us: "Gut–heart axis: a bidirectional communication network:

Cardiovascular diseases are caused by various risk factors categorized as modifiable (diet

and lifestyle) and nonmodifiable (age and genetics). Several studies have shown that GM maintains a complex relationship with host physiological processes, thereby suggesting it as an extragenomic contributor to the CVDs risk (Tuohy et al. 2014). Most CVD risk factors induce dysbiosis that is associated with intestinal inflammation and reduced gut barrier integrity, which elevates the levels of gut bacteria–derived structural components and microbial metabolites in the circulation. This accelerates the development of CVDs (Battson et al. 2018). Several disorders that affect the heart such as metabolic syndrome or obesity have been linked to disturbed or inadequate postnatal microbiome acquisition or early exposure to environmental microorganisms during childhood with the presence of specific bacteria or bacterial families in their intestinal microbiome. Furthermore, the patients with intestinal diseases such as IBD have an increased risk for coronary heart disease despite lacking the classical risk factors. This indicates the additional connections that exist between the gut and the heart (Rogler and Rosano 2014). Above evidences together suggest the existence of a bi-directional communication network between the gut and the heart, that is, the 'gut–heart axis'.

Accumulating data suggest that the gut plays a crucial role in pathophysiology of heart failure (HF). The patients with HF develop disturbances such as peripheral vasoconstriction, reduced cardiac output and increased tissue congestion that structurally and functionally impairs gut with diminished intestinal blood flow, thickened bowel wall (of colon and terminal ileum), increased collagen accumulation (in small intestine) and hemodynamic alterations. These microcirculatory disturbances functionally damage IECs due to hypoxia-induced intestinal ischemia that impairs nutrient absorption and cause cachexia and malnutrition. Furthermore, barrier dysfunction leads to translocation of gut bacteria along with their products into the circulation (Kamo et al. 2017a). Upon entering the circulatory system, the gut bacteria-derived endotoxins such as LPS binds its receptor, that is, Toll-like receptor 4 (TLR-4) on cardiomyocytes. This binding is linked to induction of an inflammatory response with increased circulating cytokines (TNF-α), structural tissue damage, decreased contractility and impaired cardiac function. LPS also triggers the release of catecholamine by phagocytes and granulocytes that exert additional unfavourable effects on the gut perfusion (Rogler and Rosano 2014).

Furthermore, HF is also associated with microbial dysbiosis and aberrant production of GM-derived metabolites. Recently, GM profiling from HF patients suggested significantly altered GM with less abundant Dorea longicatena and Eu. rectale. The flora varies further according to age such that the older HF patients (60 years or above) had fewer quantities of Bacteroidetes and Faecalibacterium and larger proportions of Proteobacteria and Lactobacillus (Kamo et al. 2017b). Another study on chronic heart failure (CHF) patients revealed increased bowel wall thickness, increased permeability for sucralose and

lactulose/mannitol (Rogler and Rosano 2014), and reduced d-xylose absorption along with the higher levels of adherent bacteria in sigmoid colon mucosal biofilm of the patients. These changes together contributed to bowel ischemia, chronic inflammation and malnutrition (Sandek et al. 2007). Furthermore, compared to healthy controls, CHF patients were reported to had intestinal overgrowth of Candida species and pathogenic bacteria like Salmonella, Campylobacter, Shigella and Yersinia enterocolitica (Pasini et al. 2016) (Table 1). In addition, GM-derived metabolites were also shown to contribute to disease processes. Uremic toxins like TMAO, p-cresyl sulphate and indoxyl sulphate emerge from the microbial fermentation of dietary intake. Indoxyl sulphate has prohypertrophic and profibrotic effects on the heart while TMAO is a promising biomarker for predicting the CVD risk. A large cohort study suggested that the elevated plasma levels of TMAO were associated with an increased risk of myocardial infarction, stroke and death in patients undergoing elective coronary angiography. In addition, the HF patients were also shown to have significantly higher TMAO plasma levels than healthy controls (Kamo et al. 2017a).

The pathophysiology of another CVD, that is, atherosclerosis involves metabolic and inflammatory components that are influenced by alterations to GM (Battson et al. 2018). Emerging reports suggest a novel pathway that connects dietary lipid intake, GM and atherosclerosis. The production of betaine, choline and TMAO metabolites from the dietary phosphatidylcholine (lecithin) depends on the metabolism by GM, and TMAO is shown to have a strongest positive correlation with the CVDs risk (Tang and Hazen 2014). According to a study, TMAO reduces bile acid synthesis and also inhibits reverse cholesterol transport, which is associated with increased atherosclerosis. However, the exact mechanisms remain elusive (Bäckhed 2013). Moreover, it is believed that a chronic infection with Chlamydia pneumonia and Helicobacter pylori and subsequent immune responses are essential for the development of atherosclerosis. Furthermore, various studies support the association between increased serum LPS (endotoxin) levels and atherosclerosis. IBD or liver cirrhosis patients have impaired intestinal barrier function that results in higher serum LPS levels with an increased incidence of atherosclerosis. LPS influences lipoprotein metabolism by interacting with low-density lipoprotein (LDL), induces endothelial cell damage and stimulates the release of superoxide anions and the oxidation of LDL. Oxidized LDL favours the release of cytokines (IL-1 and TNF-α) from the macrophages that stimulates their transformation into foam cells. These features together contribute to the atherosclerosis development and progression (Rogler and Rosano 2014).

The presence of bacterial DNA of various species in the atherosclerotic plaques and the gut of same individuals suggests GM as a potential source of atherosclerotic bacteria. Therefore, GM is likely to participate in the pathogenesis and progression of coronary

artery disease. In an earlier work, Jie et al. identified higher proportions of Streptococcus sp. and Enterobacteriaceae in atherosclerotic CVD patients than in the healthy controls (Jie et al. 2017). Another study on symptomatic atherosclerotic patients revealed increased population of Collinsella and reduced proportions of Eubacterium and Roseburia in symptomatic atherosclerotic group when compared to healthy controls (Karlsson et al. 2012) (Table 1). Furthermore, as told earlier, GM is a risk factor for various diseases such as metabolic syndrome, obesity, diabetes and atherosclerosis that are proven to be connected with hypertension. The mechanism of hypertension is complex and multifactorial (Jin et al. 2019). Short-chain fatty acids (SCFAs) produced in gut by bacteria-induced anaerobic fermentation of dietary fibre has physiological functions in the regulation of blood pressure (BP) (Forkosh and Ilan 2019). The hyper- and hypotensive effects of SCFAs are mediated by binding to their receptors Olfr78 and GPR41 respectively (Fig. 3). A recent study on 56 pre-hypertension (pHTN), 99 primary hypertension (HTN) patients and 41 healthy controls (HC) demonstrated decreased SCFAs producing F. prausnitzii and Roseburia sp. in both pHTN and HTN groups (Li et al. 2017). Another report suggested the role of opportunistic pathogenic taxa (Parabacteroides merdae, Klebsiella sp. and Streptococcus sp.) in the pathogenesis of hypertension (Yan et al. 2017) (Table 1). Conclusively, the BP is closely associated with the diversity, richness and evenness of the GM and is affected by the Firmicutes/Bacteroidetes ratio (Jin et al. 2019)."

From this information we can plausibly suggest an over-simplification of sorts, that being:

- When properly nourished and balanced, GOOD Gut microbiota (GM) are linked to human health and the sequestration of BAD GM.

- When GOOD GM are improperly nourished and imbalanced, then BAD GM can thrive and are linked to human dis-ease.

- (i) What we eat, (ii) When we eat it, and (iii) How we eat it, when combined with proper supplementation can lead to a healthier GM…and subsequently a healthier human in mind and body. And, as stated above by W.H. Tang, et.al, (Dietary metabolism, the gut microbiome, and heart failure. Nat Rev Cardiol 16, 137–154 (2019)): "We also discuss the gut microbiome as a novel therapeutic target for the treatment of cardiovascular disease, and potential strategies for targeting intestinal microbial processes."

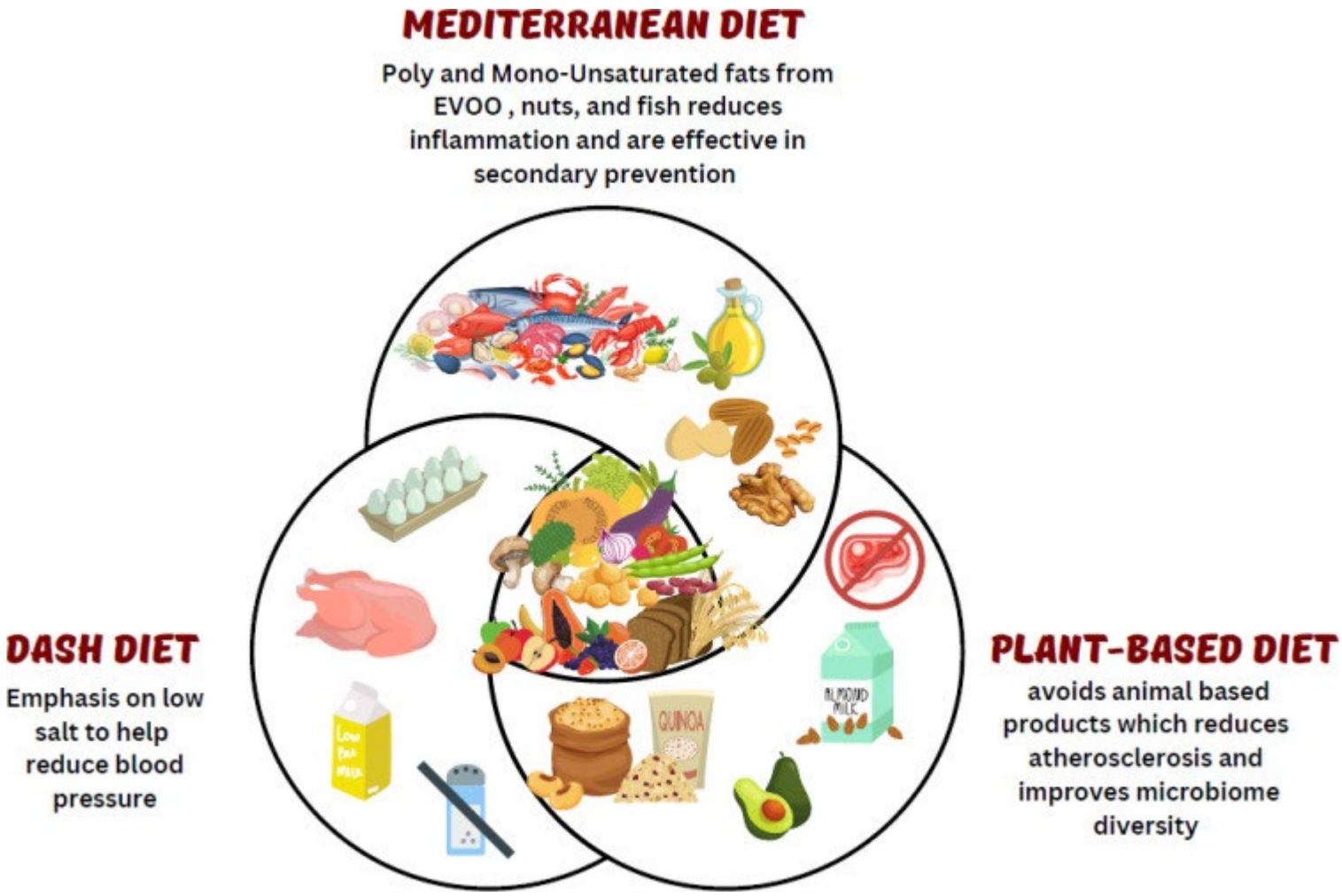
Later in this White Paper we will explore the anti-bacterial and prebiotic efficacy of Chios Mastic Gum and ionic minerals that could well serve as a "novel therapeutic" supplement (as Chios mastic gum is approved by the EU Health. The European Medicines Agency

(EMA) has approved it for traditional medicinal use, specifically for mild dyspeptic disorders and minor skin inflammations. This approval is based on scientific evidence supporting its therapeutic properties. Assessment report on Pistacia lentiscus L., resina (mastic) https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-pistacia-lentiscus-l-resin-mastic_en.pdf

For now, we will pivot a tad as we explore further support for the notion of what we eat, how we eat it, and when we eat it (as well as the role of effective supplementation).

IFUS Point 1e: Nutrition filled with proper mineralized hydration and active beneficial phytochemicals.

**Figure 1.**



Source: "The Heart Healthy Dietary Patterns: Mediterranean Diet, DASH Diet, and Healthy Plant-Based Diet." Vasc Health Risk Manag. 2023 Apr 21;19:237–253. doi: 10.2147/VHRM.S379874, states:

"The Mediterranean, DASH, and plant-based diets remain the leading heart healthy diets that match cardiology professional society recommendations. Emerging diets like the ketogenic diet and intermittent fasting are effective weight loss diets with unknown heart protective benefits, and require further studies on whether the benefits outweigh the risks. Adopting a heart-healthy diet is a foundational component for cardiovascular disease prevention, but barriers and limitations to adopting heart healthy diets exist and need to be assessed as part of cardiovascular risk assessment."

Interestingly enough, Chios Mastic Gum is produced specifically on the island of Chios, Greece, which is located in the Mediterranean Region (specifically, the Aegean Sea). Later in this White Paper, we will offer history and broader information of the impact of Chios Mastic Gum on human health in both mind and body.

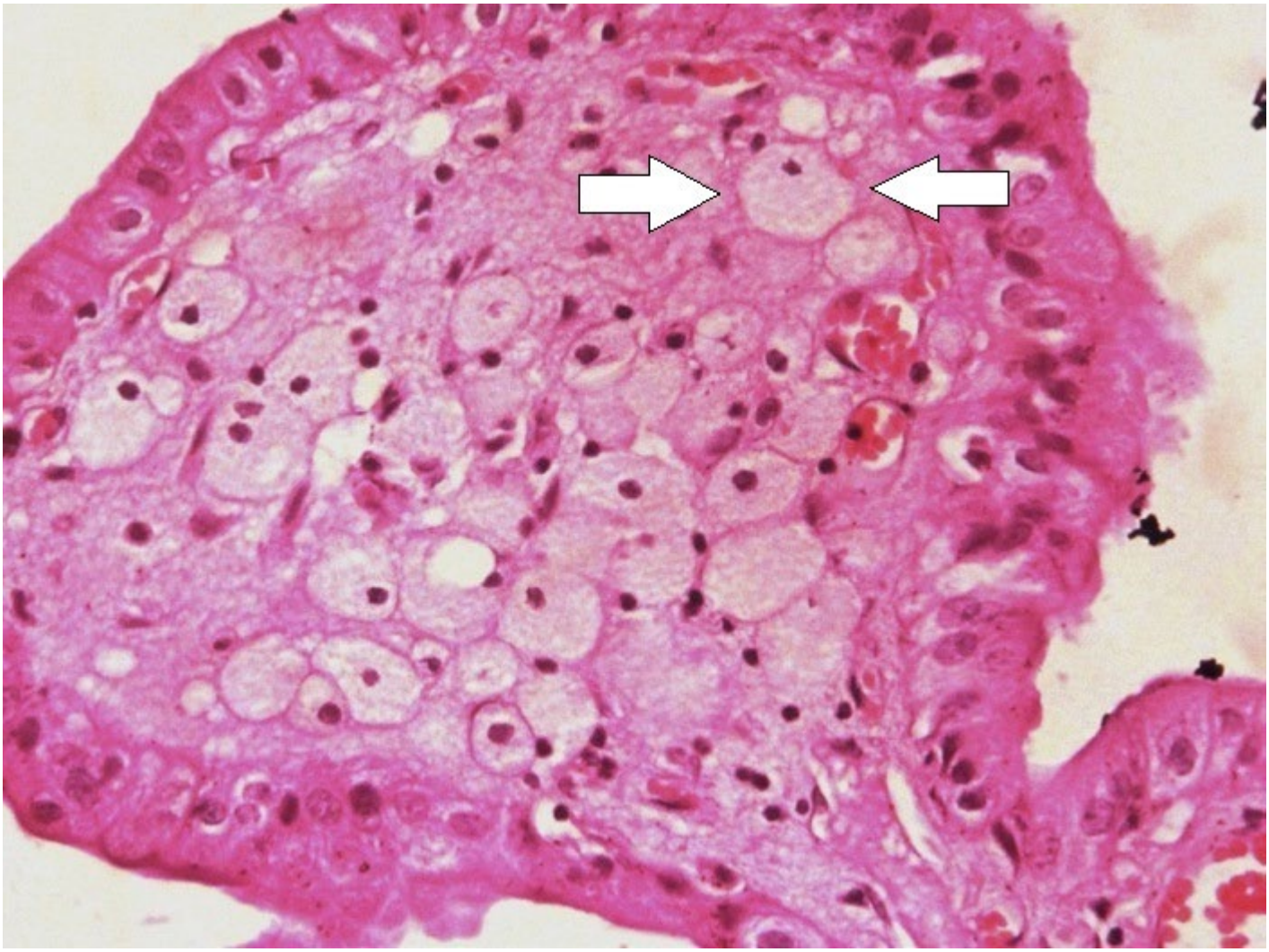
We will first shift into a deeper exploration of the “gut-heart' axis, specifically the connection to:

- Cytokines and foam Cells
- Cytokines (specifically TNF-alpha)
- Toll-like receptor 4 (TLR-4)
- Tri-methylamine-N-oxide (TMAO)

**(The Information Which Follows is Under Development and Should be Treated as such.**

IFUS Pont 1f: Brief Exploration into Cytokines and foam Cells

What are foam cells:



Histopathology of cholesterolosis, with annotated foam cell.jpg
https://commons.wikimedia.org/wiki/File:Histopathology_of_cholesterolosis,_with_annotated_foam_cell.jpg

“Foam cells, also called lipid-laden macrophages, are a type of cell that contain cholesterol. These can form a plaque that can lead to atherosclerosis and trigger myocardial infarction and stroke.[1][2][3]”

Ref. 1:

Ref 2:

Ref. 3

Foam cells are fat-laden cells with an M2 macrophage-like phenotype. They contain low density lipoproteins (LDL) and can be rapidly detected by examining a fatty plaque under a microscope after it is removed from the body.[4] They are named because the lipoproteins give the cell a foamy appearance.[5]

Ref. 5

Despite the connection with cardiovascular diseases they might not be inherently dangerous.[6]

Ref. 6

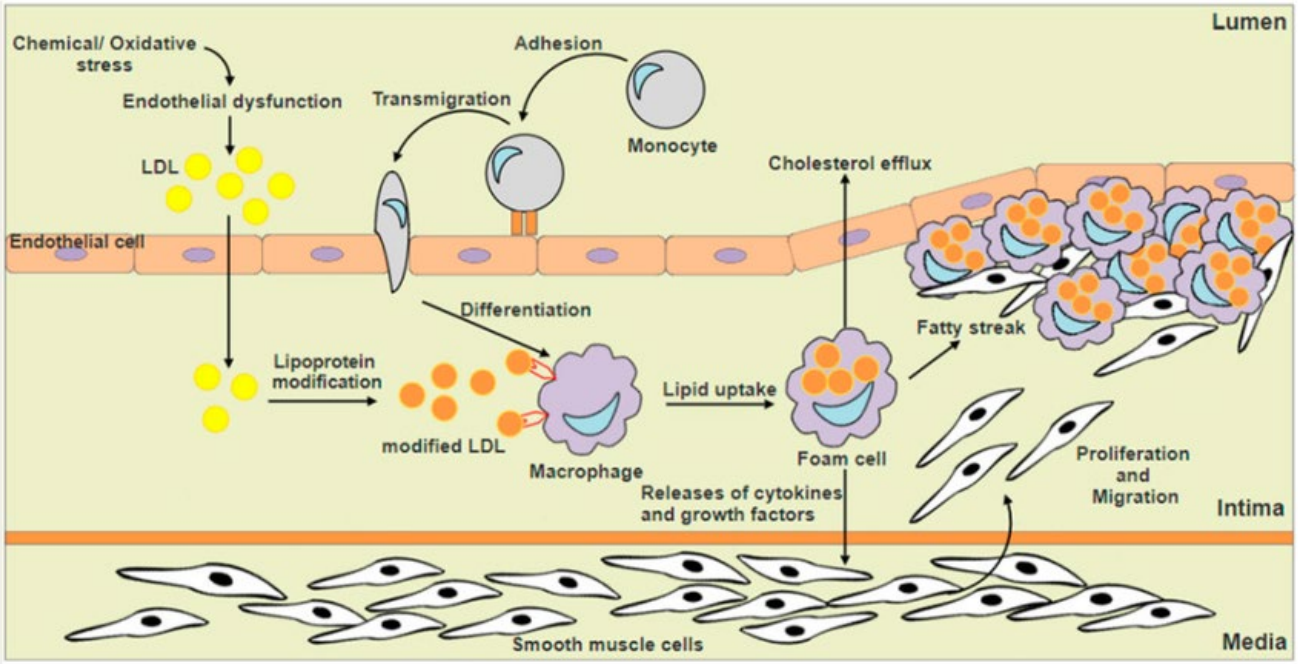
Some foam cells are derived from smooth muscle cells and present a limited macrophage-like phenotype.[7][8][9]

Ref 7

Ref 8

Ref 9

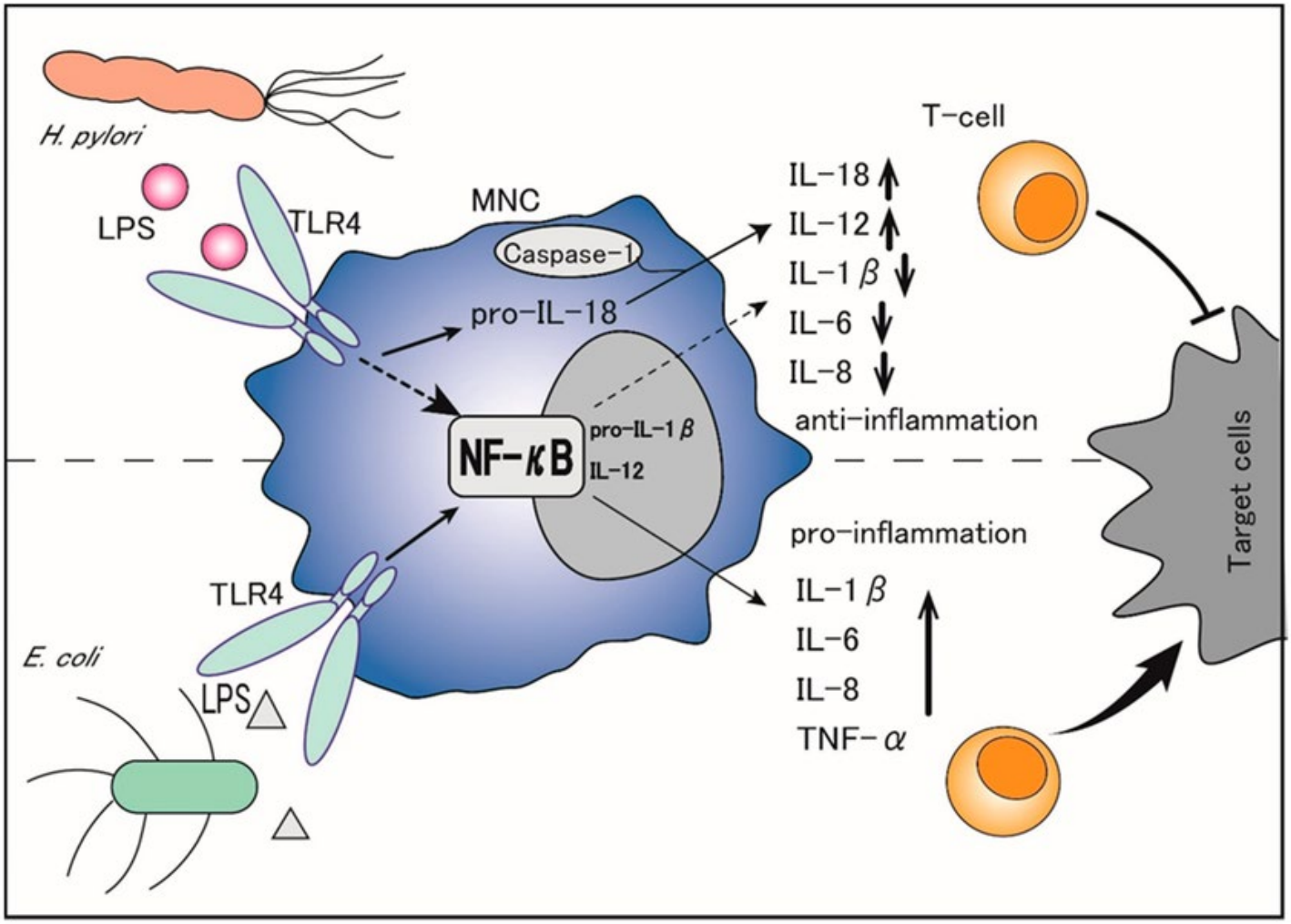
Macrophage-foam-cells-formation-and-fatty-streak-development-Increased-reactive-oxygen.tif (850×439)



https://www.researchgate.net/publication/320886082/figure/fig1/AS:11431281386851906@1745085435575/Macrophage-foam-cells-formation-and-fatty-streak-development-Increased-reactive-oxygen.tif

Relationship between foam cells and Cytokines:

IFUS Point 1g: Brief Exploration into Cytokines (specifically TNF-alpha)




Difference in inflammatory reactions between H. pylori lipopolysaccharide (LPS) and E. coli LPS. E. coli LPS stimulates mononuclear cell (MNC) to upregulate the production of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), whereas H. pylori LPS has an anti-inflammatory effect that selectively upregulates IL-18 and IL-12 but

inhibits other cytokines, leading to the suppression of T cell surveillance, which promotes gastric cancer initiation and progression.

> Source: Nozomi Ito, et.al., Helicobacter pylori-Mediated Immunity and Signaling Transduction in Gastric Can, Journal of Clinical Medicine (JCM), November 20209(11), DOI:10.3390/jcm9113699

IFUS Point 1g-1: *H. pylori* relationship to Cardio-Vascular Disease

Fang Y, Fan C, Xie H. Effect of Helicobacter pylori infection on the risk of acute coronary syndrome: A systematic review and meta-analysis. Medicine (Baltimore). 2019 Dec;98(50):e18348. doi: 10.1097/MD.0000000000018348. PMID: 31852134; PMCID: PMC6922357.Abstract

Background: Numerous studies have illustrated the association between Helicobacter pylori (H pylori) infection and acute coronary syndrome (ACS). However, the results are contradictory. Therefore, we conducted the meta-analysis to identify the association between H pylori and ACS.

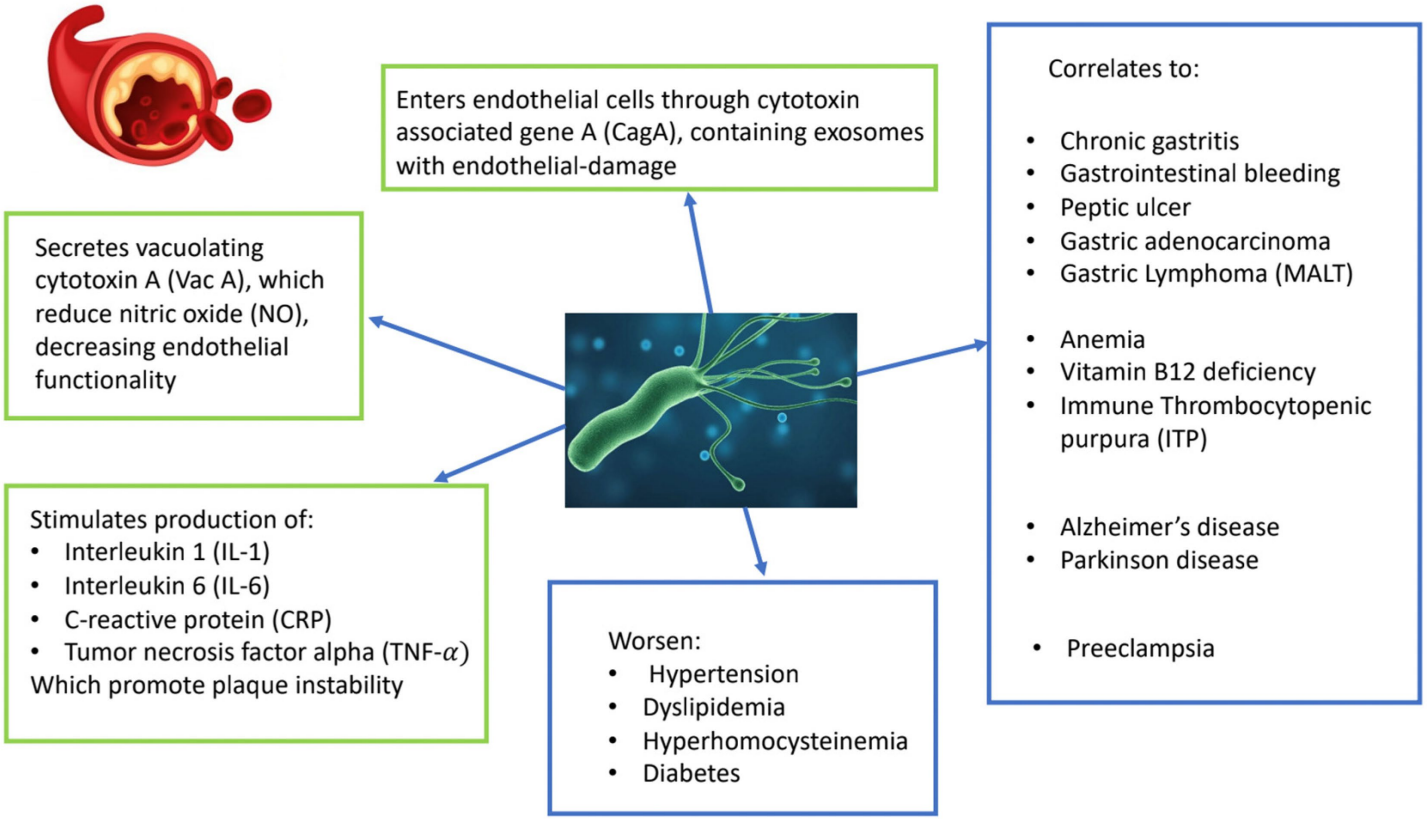
Methods: We performed a systematic search through electronic databases (Excerpta Medica Database, PubMed, Cochrane Library, and Web of Science). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with a random effect model. We also carried out the sensitivity analysis and publication bias.

Results: Forty-four eligible studies involving 7522 cases and 8311 controls were included. The pooled result showed that H pylori infection was associated with an increase risk of ACS (OR = 2.03, 95% CI 1.66–2.47). In addition, similar results were obtained in subgroups of study quality, area, human development index, and H pylori detection method. The OR for developing countries was significantly higher than developed countries (OR=2.58 vs OR=1.69). Moreover, H pylori with cytotoxin-associated antigen A was also significantly associated with an increase risk of ACS (OR=2.39, 95% CI 1.21–4.74).

Conclusion: The meta-analysis suggested that H pylori infection was associated with an increased risk of ACS, especially in developing countries. H pylori is easily screened and can be treated with a wide range of drugs. Thus, more high-quality and well-designed studies are needed to confirm whether the treatment of H pylori is an effective way to

reduce ACS risk.

IFUS Point 1g-2:




Source: Saviano, A.; Morabito Loprete, M.R.; Pignataro, G.; Piccioni, A.; Gasbarrini, A.; Franceschi, F.; Candelli, M. Helicobacter pylori, Atherosclerosis, and Coronary Artery Disease: A Narrative Review. Medicina 2025, 61, 346. https://doi.org/10.3390/medicina61020346

"Abstract: Coronary artery disease (CAD) is one of the leading causes of death worldwide, significantly contributing to mortality in both developed and developing nations. CAD arises from a combination of risk factors, including atherosclerosis, dyslipidemia, hypertension, diabetes, and smoking. In recent years, growing evidence has suggested a potential link between infectious agents and cardiovascular diseases. Among these, Helicobacter pylori (H. pylori) infection has been hypothesized for over a decade to play a role in the pathogenesis of CAD. This hypothesis is based on the bacterium's ability to

trigger host inflammatory or autoimmune responses, potentially contributing to the progression of atherosclerotic plaques and coronary events. The association between H. pylori infection and CAD is of considerable interest as it opens new avenues for prevention and management strategies in cardiovascular health. Understanding this relationship could lead to innovative approaches to reducing the burden of CAD, particularly in populations with a high prevalence of H. pylori. In this review, we aim to provide a comprehensive overview of the most recent evidence on the involvement of H. pylori in the development and prognosis of CAD. By analyzing and synthesizing current findings, we seek to shed light on unresolved questions and clarify the ambiguous aspects of this potential connection. Our goal is to contribute to a deeper understanding of how H. pylori, may influence cardiovascular disease and to inspire further research in this critical area."

"7. Conclusion: Despite the numerous pathophysiological mechanisms underlying the association between Helicobacter pylori (H. pylori) infection and coronary artery disease (CAD) that have been studied, clinical evidence demonstrating that eradication of the infection provides a tangible clinical benefit is still lacking. Research in this area should be encouraged through prospective studies, as identifying a risk factor as easily addressable as a bacterial infection could represent a crucial step in the prevention of the world's leading cause of mortality."

IFUS Point 1g-3:

Prasad G. Jamkhande, Surendra G. Gattani, Shaikh Ayesha Farhat, Helicobacter pylori and cardiovascular complications: a mechanism based review on role of Helicobacter pylori in cardiovascular diseases, Integrative Medicine Research, Volume 5, Issue 4, 2016, Pages 244-249, ISSN 2213-4220, https://doi.org/10.1016/j.imr.2016.05.005. (https://www.sciencedirect.com/science/article/pii/S221342201630035X)

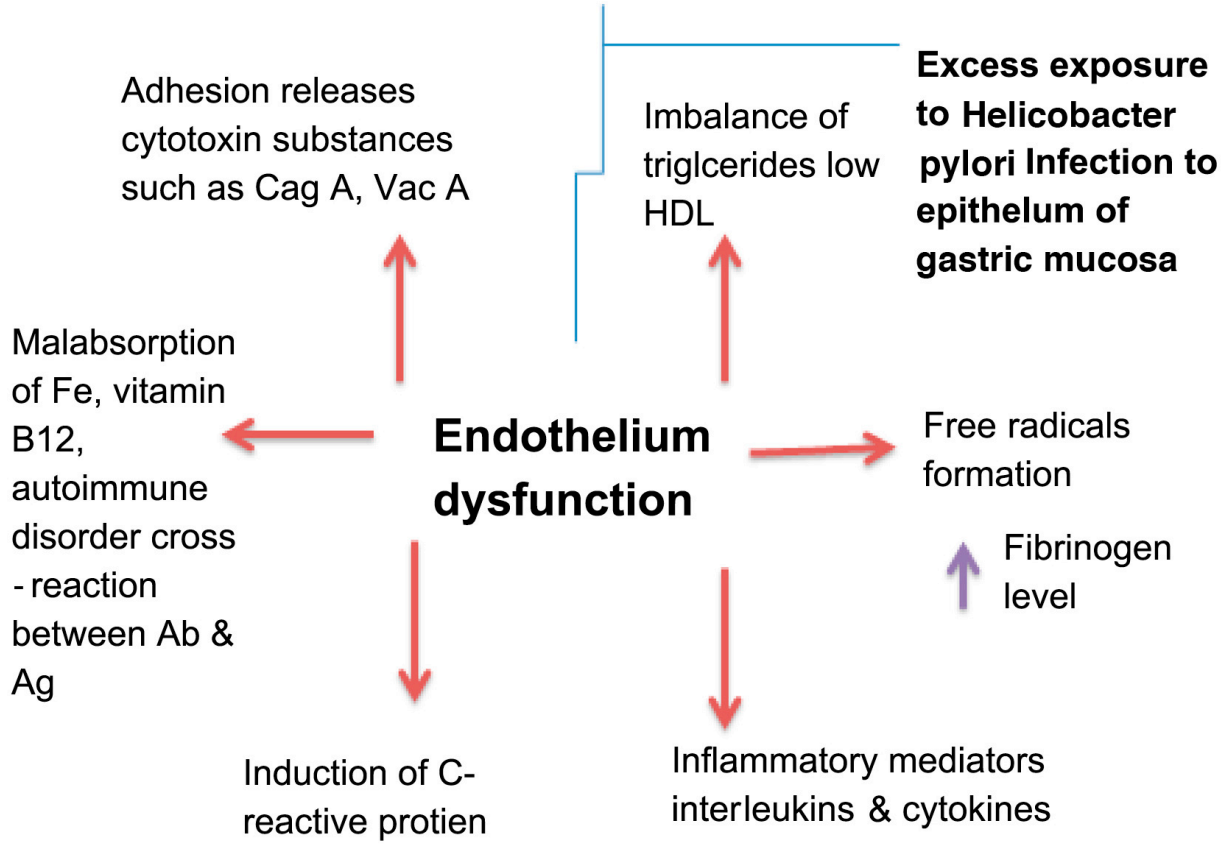
Abstract: Heart disease comprises a wide class of cardiovascular abnormalities, including ischemic heart disease, myocardial infarction, atherosclerosis, and coronary artery disease. It is the leading cause of death all over the world. Several traditional and novel risk factors, such as infectious and noninfectious agents, have been associated with heart disease. Out of these, Helicobacter pylori has been recently introduced as an important etiological factor for heart disease. Numerous seroepidemiological findings observed H. pylori antibodies in the blood of a patient with cardiovascular complications. The bacteria survive in the epithelial cells of gastric organs and cause digestive complications. Excess inflammatory pathogenesis and prognosis stimulate an immune response that further causes significant

disturbances in various factors like cytokines, fibrinogen, triglycerides, high density lipoprotein, C-reactive protein, heat shock protein, and white blood cell count, and provoke a number of problems such as atherosclerosis and prothrombic state, and cross-reactivity which eventually leads to heart diseases. H. pylori releases toxigenic nutrients, chiefly vacuolating cytotoxin gen A (Vac A) and cytotoxin associated gene A (Cag A), of which Cag A is more virulent and involved in the formation of cholesterol patches in arteries, induction of autoimmune disorder, and release of immune mediated response. Although numerous mechanisms have been correlated with H. pylori and heart disease, the exact role of bacteria is still ambiguous.

## 2. Pathological mechanism

No single factor could account for infection related heart diseases, as the infection is a multifactorial process. Several potential mechanisms and pathways allied with H. pylori contribute in infection-induced cardiovascular complications. Fig. 1 briefly focuses on different mechanisms by which H. pylori contributes to heart diseases.

Fig. 1. Helicobacter pylori infection induced immune response, HDL, high density lipoprotein.




## 3. Conclusion

Infection of H. pylori bacteria infection is directly or indirectly involved in the development of cardiovascular diseases. Several serological based findings have revealed the active role of H. pylori in heart diseases. Activation of inflammatory mediators, proinflammatory factors, release of toxins, abnormal lipid metabolism, altered iron metabolism, and autoimmune reaction are the leading mechanisms of H. pylori, which contributes in cardiovascular anomalies. The chief leading role in the cause of heart disease is the improper functioning of the immune system, both at the cellular and systemic level. Most of these findings are based on serological findings. Further studies are needed to recognize the exact involvement of H. pylori in these diseases.

## IFUS Point 1g-4:

In yet another study, we find more evidence of the effect of *H. pylori* on Cardio-Vascular Disease (CVD). The study to follow offers compelling evidence of the invasive and

destructive nature of *H. pylori*:

Yaslianifard, S., Sameni, F., Kazemi, K. et al. Beyond the gut: a comprehensive meta-analysis on Helicobacter pylori infection and cardiovascular complications. Ann Clin Microbiol Antimicrob 24, 18 (2025). https://doi.org/10.1186/s12941-025-00788-6

Abstract / Background: Helicobacter pylori (H. pylori) is known to induce chronic inflammatory conditions, and interactions between the host immune system and pathogen have diverted attention toward investigating its correlation with extra-gastrointestinal disorders.

Objective: The present study aimed to assess the rate of H. pylori infection in cardiovascular disease (CVD) through a systematic review and meta-analysis.

Methods: We conducted a large-scale meta-analysis to determine the prevalence rates of H. pylori infection in vascular diseases. Articles from PubMed/Medline, Web of Science, and Embase databases published between 2000 and 2023 were included for analysis. We used multiple independent observers to extract data, calculated the pooled frequency of H. pylori in vascular diseases using a random effect model, and reported the results as a weighted average based on the study population. The main outcome measures were presented with 95% confidence intervals (CI).

Results: In 87 included studies, the prevalence of H. pylori infection in vascular diseases was 56.7% worldwide. 14.25% of H. pylori isolates harbored the cagA gene. The predominant vascular complication was coronary artery disease (CAD) (31.07%), primarily documented in Europe. This meta-analysis revealed a declining emphasis on studying the association of H. pylori infection with vascular disease in recent times.

Conclusion: According to this meta-analysis, H. pylori infection has a high frequency in CVD and may increase the risk of vascular diseases. However, further research is required, particularly in nations with limited data." (See Table 4 below:)

Table 4 Type of vascular diseases in *H. pylori* positive patients (From: Beyond the gut: a comprehensive meta-analysis on *Helicobacter pylori* infection and cardiovascular complications)

| Variables (No. of studies) | No. of patients in continents | No. of patients /total (103) | No. of patients in countries |
|---|---|---|---|
| Atherosclerotic stroke (15) | Europe (1157) | 1521/6775 (22.45) | Germany (410)<br>England (414)<br>Italy (276)<br>Greece (57) |
| | Asia (364) | | Iran (59)<br>Iraq (56)<br>Japan (48)<br>China (101)<br>Korea (100) |
| Cerebral infarction (1) | Asia (33) | 33/6775 (0.48) | Japan (33) |
| Cerebral microbleeds (1) | Asia (31) | 31/6775 (0.45) | China (31) |
| CHD (8) | Asia (218) | 734/6775 (10.83) | Iran (81)<br>Japan (137) |
| | Europe (516) | | Italy (28)<br>Norway (86)<br>Germany (216)<br>Netherlands (186) |
| Aortic aneurysm (1) | Europe (67) | 67/6775 (0.98) | Turkey (67) |
| CAD (29) | Asia (800) | 2105/6775 (31.07) | Iran (324)<br>India (38) |

| Variables (No. of studies) | No. of patients in continents | No. of patients /total (103) | No. of patients in countries |
|---|---|---|---|
| | 38.00 | | Korea (242)<br>Israel (110)<br>Taiwan (86) |
| | Africa (113) | | Egypt (113) |
| | Europe (921) | | Italy (90)<br>Germany (270)<br>France (33)<br>Sweden (39)<br>Turkey (102)<br>Croatia (158)<br>Norway (51)<br>Spain (32)<br>Greece (146) |
| | America (271) | | USA (271) |
| Acute coronary syndrome (6) | Asia (112) | 608/6775 (8.97) | Japan (112) |
| | Europe (496) | | Ireland (139)<br>England (157)<br>Italy (108)<br>Spain (92) |
| Myocardial Infarction (15) | Asia (257) | 1115/6775 (16.45) | Taiwan (28)<br>Iran (78) |

| Variables (No. of studies) | No. of patients in continents | No. of patients /total (103) | No. of patients in countries |
|---|---|---|---|
| | | | Pakistan (82)<br>India (69) |
| | Europe (702) | | Sweden (163)<br>Italy (150)<br>England (389) |
| | America (84) | | USA (84) |
| | Africa (72) | | Egypt (72) |
| Cardiac syndrome X (2) | Asia (15) | 43/6775 (0.63) | Iran (15) |
| | Europe (28) | | Italy (28) |
| Ischemic Heart Disease (3) | Europe (103) | 103/6775 (1.52) | Italy (84)<br>England (19) |
| Stent implantation in a native coronary artery (1) | Europe (61) | 61/6775 (0.90) | France (61) |
| Peripheral arterial disease (2) | Asia (55) | 146/6775 (2.15) | Japan (55) |
| | Europe (91) | | Netherlands (91) |
| Idiopathic Dysrhythmias (1) | Europe (23) | 23/6775 (0.33) | Italy (23) |
| Atherosclerosis | Europe (51) | 185/6775 | Turkey (28) |

| Variables (No. of studies) | No. of patients in continents | No. of patients /total (103) | No. of patients in countries |
|---|---|---|---|
| (6) | | (2.73) | Italy (23) |
| | Asia (54) | | China (54) |
| | America (20) | | Argentina (20) |
| | Africa (60) | | Egypt (60) |

1. CHD: Coronary heart disease, CAD: Coronary Artery Disease

We find in the aforementioned data compelling data linking a part of the gut microbial biome of CVD.

IFUS Point 1g-5: References???

Another study offers another validating perspective of the effect of *H. pylori* infections linked to coronary heart disease. Once more we find a link to illustrating the impact of such microbial entities on the Gut-Heart Axis. Please note that these are statistical analyses, using proven methods to gather, interpret, and create plausible conclusions:

Li, B., Zhang, Y., Zheng, Y. et al. The causal effect of Helicobacter pylori infection on coronary heart disease is mediated by the body mass index: a Mendelian randomization study. Sci Rep 14, 1688 (2024). https://doi.org/10.1038/s41598-024-51701-8

Abstract: The association between Helicobacter pylori (H. pylori) infection and coronary heart disease (CHD) remains controversial, with an unclear causal link. This study employed bidirectional Mendelian randomization (MR) method, using H. pylori infection as the exposure, to investigate its causal relationship with CHD diagnosis, prognosis, and potential pathogenesis. H. pylori infection exhibited a causal association with body mass index (BMI) (β = 0.022; 95% CI 0.008–0.036; p = 0.001). Conversely, there was no discernible connection between H. pylori infection and the diagnosis of CHD (OR = 0.991; 95% CI 0.904–1.078; p = 0.842; IEU database; OR = 1.049; 95% CI 0.980–1.118; p = 0.178; FinnGen database) or CHD prognosis (OR = 0.999; 95% CI 0.997–1.001;

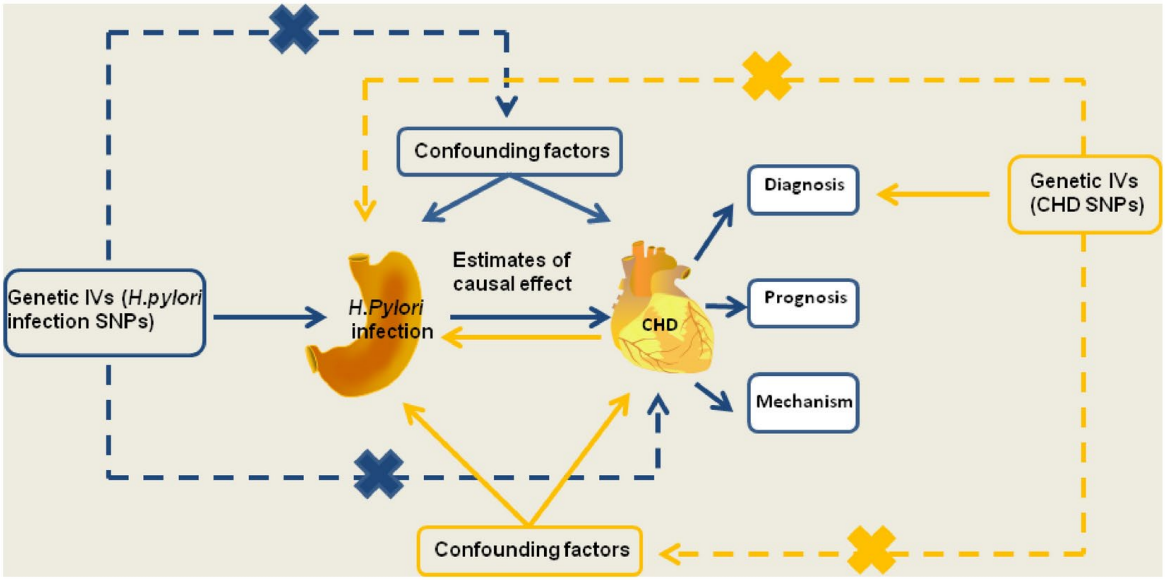
p = 0.391; IEU database; OR = 1.022; 95% CI 0.922–1.123; p = 0.663; FinnGen database). Reverse MR analysis showed no causal effect of CHD on H. pylori infection. Our findings further support that H. pylori infection exerts a causal effect on CHD incidence, mediated by BMI. Consequently, eradicating or preventing H. pylori infection may provide an indirect clinical benefit for patients with CHD.

Introduction: Coronary heart disease (CHD) is caused by atherosclerosis, which includes angina pectoris and myocardial infarction (MI) and is the leading cause of mortality in many countries1. The etiology, pathogenesis and prognosis of CHD are complex and have not been fully understood until recently. Helicobacter pylori (H. pylori) is a gram-negative bacterium that primarily inhabits the stomach and duodenum2. More than half of the world's population has been infected with H. pylori3. In addition to causing gastrointestinal diseases4, H. pylori can also induce systemic reactions, including abnormal glucose5 and lipid metabolism6, heightened blood hypercoagulability7,8, and chronic inflammatory reactions9,10,11, and is accompanied by vitamin (including vitamin B12, vitamin C, and vitamin D) deficiency12. While these reactions represent risk factors for CHD, it remains uncertain whether H. pylori influences the occurrence of CHD through these reactions.

However, the relationship between H. pylori infection and CHD is still controversial. Several studies have shown that H. pylori infection is not significantly related to the occurrence or severity of CHD13,14; however, some studies have shown that H. pylori infection is one of the main causes of CHD15,16. Studies have reported that eradication therapy for H. pylori can reduce the levels of peripheral blood inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) in patients. These inflammatory cytokines are implicated in the development of atherosclerosis and CHD, and their elevation increases the incidence of restenosis in patients after percutaneous transluminal coronary angioplasty (PTCA)17,18,19. The probability of MI in H. pylori-infected patients is twice that in uninfected individuals20. Another study used infrared radiation spectroscopy to measure the levels of triglycerides, C-reactive protein, homocysteine, low-density lipoprotein (LDL), and TNF-α in peripheral blood. The results showed that, compared with healthy individuals, CHD patients with H. pylori infection had elevated triglyceride levels and inflammation21. An Asian study also confirmed that H. pylori infection can increase the risk of CHD in the next 10 years22. At present, the evidence for a link between H. pylori infection and CHD is based on observational studies, and there may be some unknown confounding factors that affect judgment of the results. To address this controversial clinical issue, a study that removes confounding factors to accurately determine the causal relationship between H. pylori infection and CHD is urgently needed. In addition, although the infection rate of H. pylori is relatively high, H. pylori infection is not routinely screened, and many infected

individuals are unaware of having this infection. Exploring the causal relationship between the two will help determine whether routine screening and treatment of H. pylori is one of the prevention and treatment strategies for CHD.

**Figure 1:** From: The causal effect of *Helicobacter pylori* infection on coronary heart disease is mediated by the body mass index: a Mendelian randomization study




Schematic representation of the MR study on the causal relationship between *H. pylori* infection and CHD incidence. CHD, coronary heart disease; IVs, instrumental variables; *H. pylori*, *Helicobacter pylori*; SNP, single-nucleotide polymorphism.

IFUS Point 1g-6:

Fang Y, Fan C, Xie H. Effect of Helicobacter pylori infection on the risk of acute coronary syndrome: A systematic review and meta-analysis. Medicine (Baltimore). 2019 Dec;98(50):e18348. doi: 10.1097/MD.0000000000018348. PMID: 31852134; PMCID: PMC6922357.

"Results: Forty-four eligible studies involving 7522 cases and 8311 controls were included. The pooled result showed that H pylori infection was associated with an increase risk of ACS (OR = 2.03, 95% CI 1.66–2.47). In addition, similar results were obtained in subgroups of study quality, area, human development index, and H pylori detection method. The OR for developing countries was significantly higher than developed

countries (OR=2.58 vs OR=1.69). Moreover, H pylori with cytotoxin-associated antigen A was also significantly associated with an increase risk of ACS (OR=2.39, 95% CI 1.21–4.74).

Conclusion: The meta-analysis suggested that H pylori infection was associated with an increased risk of ACS, especially in developing countries. H pylori is easily screened and can be treated with a wide range of drugs. Thus, more high-quality and well-designed studies are needed to confirm whether the treatment of H pylori is an effective way to reduce ACS risk."

We find compelling, statistically viable data of the link between the Gut-Heart Axis from the information above, with a specific focus on just one of many parts of a complex microbial biome in the gut and the body at large…many of which are beneficial…and some of which are most harmful.

Understanding this aspect of human health is of paramount importance in providing guidance in both nutrition and supplementation. The aim should be creating a healthy outcome in mind and body for every human on the plant. Furthermore, much of this information can be leveraged through interpolation and extrapolation to animals…and even plants and the soil.

(ADD STUDY: Chios Mastic Gum linked to H Pylori Reduction/Elimination)

IFUS Point 1h: Brief Exploration into Toll-like receptor 4 (TLR-4)

As a basis of continued exploration we dive into yet another biomarker.

IFUS Point 1i: Brief Exploration into Tri-methylamine-N-oxide (TMAO)

IFUS Point 1j: Link Between the NLRP3 Inflammasome and Cardiovascular Disease

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**IFUS Point 2:**

**Reformat and remove what is not needed in this particular white paper**

IFUS Point 1: What do we think we know about Chios Mastic Gum and its Active Ingredients?

"Chios mastic gum is approved by the EU Health. The European Medicines Agency (EMA) has approved it for traditional medicinal use, specifically for mild dyspeptic disorders and minor skin inflammations. This approval is based on scientific evidence supporting its therapeutic properties. (ema.europa.eu: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-pistacia-lentiscus-l-resin-mastic_en.pdf) (Please ADD LINK)

2 February 2016
EMA/HMPC/46756/2015
Committee on Herbal Medicinal Products (HMPC)

# Assessment report on *Pistacia lentiscus* L., resina (mastic)

Final

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC (traditional use)

| | |
|---|---|
| Herbal substance(s) (binomial scientific name of the plant, including plant part) | *Pistacia lentiscus* L., resina (mastic) |
| Herbal preparation(s) | Powdered herbal substance |
| Pharmaceutical form(s) | Powdered herbal substance in solid dosage form for oral use<br>Powdered herbal substance in semi-solid dosage form for cutaneous use |
| Rapporteur(s) | I Chinou |
| Peer-reviewer | M Delbò |

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IFUS Point 1A: Since the dawn of recorded history, “mastic” has been cited for its nutritive and healing value. “Plants belonging to P. lentiscus are so far the most commonly used. Resin (mastic) has been used for gastrointestinal diseases in the Mediterranean and Middle East countries for the last 3000 years and continues to have alimentary applications until now.” (Bozorgi M., Memariani Z., Mobli M., Salehi S., Mohammad H., Shams-Ardekani M.R., Rahimi R. Five Pistacia species (P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus): A review of their traditional uses, phytochemistry, and pharmacology. Sci. World J. 2013;2013:219815. doi: 10.1155/2013/219815.)

IFUS Point 1B: “Mastic (Greek: Μαστίχα) is a resin obtained from the mastic tree (Pistacia lentiscus). In pharmacies and nature shops, it is called "arabic gum" (not to be confused with gum arabic) and "Yemen gum".”

IFUS Point 1C: “In Greece, it is known as the "tears of Chios," being traditionally produced on that Greek island, and, like other natural resins, is produced in "tears" or droplets.

> IFUS Point 1C-1: Originally liquid, it is sun dried into drops of hard brittle translucent resin.
>
> IFUS Point 1C-2: When chewed, the resin softens and becomes a bright white and opaque gum.
>
> IFUS Point 1C-3: The flavor is bitter at first, but after chewing, it releases a refreshing, slightly piney or cedar flavor.”

IFUS Point 1D: “Tschirch and Reutter (A. Pharm., 1904, 104) examined mastic carefully:

> IFUS Point 1D-1: They found an ethereal oil, possessing a pale-yellow color and a somewhat camphoraceous odor.
>
> IFUS Point 1D-2: There is also a bitter principle, which could not be isolated in pure form.”
>
> IFUS Point 1D-3: It is worthy to note that a “bitter principle” in qualitative chemical analysis is typically noted as being a “base” versus an “acid” as bases are inherently bitter.

IFUS Point 1E: Mastic has been used as a medicine since antiquity and is still used in traditional folk medicine of the Middle East.

IFUS Point 1E-1: In ancient Greece, it was given as a remedy for snakebite, and, in India and Persia, it was used to fill dental cavities. The first-century Greek physician Pedanius Dioscorides mentions the healing properties of mastic in his book *De Materia Medica*.

IFUS Point 1E-2: Hippocrates wrote that the mastic is good for prevention of digestive problems and colds.

IFUS 1E-3: Galenus suggested that mastic was useful for bronchitis and for improving the condition of the blood.

IFUS 1E-4: In medieval times, mastic was highly valued by sultans' harems as a breath freshener and a tooth whitener.

IFUS Point 1F: Historical and Present-Day Applications of Chios Mastic Gum with demonstrated efficacy include: (A) Anti-Inflammatory, (B) Anti-Microbial/Anti-fungal (C) Gastric/Digestive Improvement, (D)Anti-Oxidative, (E) Cardiovascular Improvements, (F) Liver Function Improvements and (G) Immunoregulatory and Cancer Treatment. "Chios Mastic Gum (CMG) has been found to have favorable effects on lipid and glucose metabolism, cardiovascular and hepatic health, inflammation, oxidative stress, body composition, and microbiota. It is attributed to the anti-inflammatory and anti-oxidative properties of its components. Clinical trials have shown its efficacy in treating inflammatory bowel disease (IBD). Additionally, CMG has demonstrated therapeutic effects on gastrointestinal disorders and cardiometabolic disease." (1,2,3)

IFUS Point 1F-1: Ref.(1) Soulaidopoulos S, Tsiogka A, Chrysohoou C, Lazarou E, Aznaouridis K, Doundoulakis I, Tyrovola D, Tousoulis D, Tsioufis K, Vlachopoulos C, Lazaros G. Overview of Chios Mastic Gum (Pistacia lentiscus) Effects on Human Health. Nutrients. 2022 Jan 28;14(3):590. doi: 10.3390/nu14030590. PMID: 35276949; PMCID: PMC8838553.

IFUS Point 1F-2: Ref.(2) Beneficial Clinical Effects of Chios Mastic Gum: A Review, Im JJ, et.al., Austin Biol. 2017; 2(1): 1022.

IFUS Point 1F-3: Ref.(3) Overview of Chios mastic gum (Pistacia lentiscus) effects on human health, https://aor.us/research-library/overview-of-chios-mastic-gum-pistacia-lentiscus-effects-on-human-health/

IFUS Point 1G: Anti-inflammatory efficacy of Chios Mastic Gum. There are more detailed studies in this paper However, this is but a sampling of studies to establish the point:

IFUS Point 1G-1: In a study published in "Nutr J. 2011 Jun 6;10:64. (doi: 10.1186/1475-2891-10-64.) and titled, "Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress," (Triantafyllou A, Bikineyeva A, Dikalova A, Nazarewicz R, Lerakis S, Dikalov S., Medical School of Athens, Athens, Greece.), the "Scavenging of superoxide radical was investigated by electron spin resonance and spin trapping technique using EMPO spin trap in xanthine oxidase system" was investigated

IFUS Point 1G-1.a: The study found that, "Superoxide production in endothelial and smooth muscle cells stimulated with TNF-α or angiotensin II and treated with vehicle (DMSO) or mastic gum (0.1-10 μg/ml) was measured by DHE and HPLC."

IFUS Point 1G-1.b: Furthermore, "Cellular H2O2 was measured by Amplex Red. Inhibition of protein kinase C (PKC) with mastic gum was determined by the decrease of purified PKC activity, by inhibition of PKC activity in cellular homogenate and by attenuation of superoxide production in cells treated with PKC activator phorbol 12-myristate 13-acetate (PMA)."

IFUS Point 1G-1.c: "Spin trapping study did not show significant scavenging of superoxide by mastic gum itself. However, mastic gum inhibited cellular production of superoxide and H2O2 in dose dependent manner in TNF-α treated rat aortic smooth muscle cells but did not affect unstimulated cells. TNF-α significantly increased the cellular superoxide production by NADPH oxidase, while mastic gum completely abolished this stimulation."

IFUS Point 1G-1.d: "Mastic gum inhibited the activity of purified PKC, decreased PKC activity in cell homogenate, and attenuated superoxide production in cells stimulated with PKC activator PMA and PKC-dependent angiotensin II in endothelial cells."

IFUS Point 1G-1.e: "Conclusion: We suggest that mastic gum inhibits PKC which attenuates production of superoxide and H2O2 by NADPH oxidases. This antioxidant property may have direct implication to the anti-inflammatory activity of the Chios mastic gum."

IFUS Point 1H: Antibacterial and antifungal efficacy of Chios Mastic Gum. There are more detailed studies in this paper. However, this is but a sampling of studies to establish the point:

IFUS Point 1H-1: Mastic contains antioxidants and also has antibacterial and antifungal properties. (Koutsoudaki C, Krsek M, Rodger A (October 2005). "Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia". *Journal of Agricultural and Food Chemistry* **53** (20): 7681–5. doi:10.1021/jf050639s. PMID 16190616.)

IFUS Point 1H-2: A Nottingham University study published in the New England Journal of Medicine claims that mastic can cure peptic ulcers by killing Helicobacter pylori bacteria. (Huwez FU, Thirlwell D, Cockayne A, Ala'Aldeen DA (December 1998). "Mastic gum kills Helicobacter pylori". *The New England Journal of Medicine* **339** (26): 1946. doi:10.1056/NEJM199812243392618. PMID 9874617.)

IFUS Point 1H-3: Other studies have indicated that mastic has only a modest ability to eliminate *H. pylori* but have also suggested that refining mastic by removing the polymer poly-β-myrcene may make the active components, particularly isomasticadienolic acid, more available and effective (Paraschos S, Magiatis P, Mitakou S, *et al.* (February 2007). "In vitro and in vivo activities of Chios mastic gum extracts and constituents against Helicobacter pylori". *Antimicrobial Agents and Chemotherapy* **51** (2): 551–9. doi:10.1128/AAC.00642-06. PMC 1797732. PMID 17116667.)

IFUS Point 1I: Chios Mastic Gum shown to have efficacy preventing tooth decay and gingivitis as chewing mastic reduces oral bacteria (1,2). Additionally, Mastic Gum is shown to have efficacy in oral cancer treatment and prevention (3).

IFUS Point 1I-1: Ref(1) Aksoy A, Duran N, Koksal F (June 2006). "In vitro and in vivo antimicrobial effects of mastic chewing gum against Streptococcus mutans and mutans streptococci". *Archives of Oral Biology* **51** (6): 476–81. doi:10.1016/j.archoralbio.2005.11.003. PMID 16343417.

IFUS Point 1I-2: Ref.(2) Takahashi K, Fukazawa M, Motohira H, Ochiai K, Nishikawa H, Miyata T (April 2003). "A pilot study on antiplaque effects of mastic chewing gum in the oral cavity". *Journal of Periodontology* **74** (4): 501–5. doi:10.1902/jop.2003.74.4.501. PMID 12747455.

IFUS Point 1I-3: Ref.(3) “Mastic gum displayed antibacterial and antimicrobial properties and inhibited plaque accumulation, constituting a beneficial adjuvant in caries prevention. In the treatment and prevention of periodontal diseases, Pistacia lentiscus essential oil provided effective antibacterial activity against a variety of periodontal bacteria as well as anti-inflammatory properties. For oral cancer, several clinical trials

revealed interesting results against cell proliferation, induction of apoptosis, and regulation of intracellular signaling pathways. This indicates the potential of Mastic gum to serve as a preventive and therapeutic agent for oral mucosa inflammation and oral cancer. No notable toxic or side effects were reported in the clinical trials reviewed." (Alwadi, M., Sidhu, A., Khaled, M.B. et al. Mastic (Pistacia lentiscus) gum and oral health: a state-of-the-art review of the literature. J Nat Med 77, 430–445 (2023). https://doi.org/10.1007/s11418-023-01704-y)

IFUS Point 1J: Efficacy of Chios Mastic Gum on Gastric/Digestive Disorders and Improvement. There are more detailed studies in this paper. However, this is but a sampling of studies to establish the point:

IFUS Point 1J-1: Mustafa S. Naouar, Lilia Zouiten Mekki, Lamia Charfi, Jalel Boubaker, Azza Filali, Preventive and curative effect of Pistacia lentiscus oil in experimental colitis, Biomedicine & Pharmacotherapy, Volume 83, 2016, Pages 577-583, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2016.07.021. (https://www.sciencedirect.com/science/article/pii/S0753332215304480)
"Abstract: To investigate the anti-inflammatory effect of the Pistacia lentiscus oil in experimental colitis model." (Note Lentisc Oil is also known as Mastic Oil)

"Materials and methods: Colitis was induced in male rats by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in all groups. The experimental groups consisted of: 5 rats received Lentisc oil 2months before colitis induction (preventive group), 5 rats received the oil on the day of colitis induction (curative group) and 5 control rats. Lentisc oil was extracted from the ripe fruit of the plant by the cold press method and was analyzed by spectro-chromatography. Lentisc oil has been inserted with a standard diet at the dose of 30mg oil/100g of food/rat."

"Results: The lentisc oil sample is composed mainly by Oleic acid (47.96%), Palmitic acid (27.94%) and Linoleic acid (20.22%). There was a significant difference between control rats and treated rats with lentisc oil concerned body mass ($p=0.009$), bleeding index ($p=0.005$ and $p=0.018$) and diarrhea ($p=0.012$). Histological examination revealed a clear difference between the control and preventive groups with disappearance of erosion, decreased of cryptitis, irregular crypts and crypt loss in the preventive group. Curative group showed a significant decrease of ulceration, hyperplasia, cryptitis, irregular crypts and crypt loss compared to the control group. There was an attenuation of inflammation in the preventive group compared to the curative group without statistically significant."

"Conclusion: Lentisc oil administration could provide a protective effect on intestinal inflammation in colitis rats induced by TNBS mainly when it is administered at a young age in preventive mode. This beneficial effect would involve a modification of arachidonic acid metabolism."

IFUS Point 1J-2: Chios mastic gum in inflammatory bowel disease treatment, https://peptiko.gr/en/chios-mastic-gum-in-inflammatory-bowel-disease-treatment/
"The potential of Pistacia lentiscus in IBD management: Recent explorations into complementary and traditional medicines have brought Pistacia lentiscus, particularly Chios mastic gum, into focus due to its unique effectiveness and moderate cost. Over 80% of the world's population relies on traditional medicine systems, where herbal products play a pivotal role. Chios mastic gum, derived from Pistacia lentiscus, stands out for its potential in treating various gastrointestinal disorders through:

- Maintaining intestinal epithelial barrier integrity
- Regulating macrophage activation
- Modulating immune responses
- Inhibiting TNF-alpha activity
- Scientific Evidence
- Animal Models"

"Scientific Evidence: Animal Models:
"Several studies utilizing animal models of IBD have demonstrated the anti-inflammatory effects of Pistacia lentiscus. These studies indicate that mastic gum can reduce the production of inflammatory cytokines such as TNF-α, IL-6, and IL-8, and promote histological improvement in colitis. Moreover, mastic gum's components, particularly terpenes and phenolic compounds, are believed to scavenge free radicals and regulate key inflammatory mediators of IBD."

"Scientific Evidence: Clinical Trials:
Clinical trials investigating the efficacy of Pistacia lentiscus, specifically mastic gum, in treating inflammatory bowel disease (IBD) provide critical insights into its potential therapeutic role. Although the body of evidence remains relatively small, these trials highlight mastic gum's promise in managing IBD symptoms, improving patients' quality of life, and possibly maintaining disease remission. Here's a closer look at the notable clinical trials conducted to date:"

1. "Efficacy of Chios Mastic Gum on Active Crohn's Disease: A pioneering study by Kaliora et al. in 2007 evaluated the effects of Chios mastic gum (CMG) on patients with active Crohn's Disease (CD). Ten patients with mild to moderately active CD received mastic gum capsules (2.2 g/day) for four weeks.

The trial observed significant reductions in the Crohn's Disease Activity Index (CDAI) and inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6), leading to remission in seven out of ten patients. This study underscored CMG's potential in regulating inflammation and oxidative stress in CD patients, suggesting its efficacy in inducing remission."

2. "Impact on Quality of Life and Inflammatory Markers: Building on preliminary findings, Papada et al. conducted a randomized controlled trial in 2019 to explore CMG's effects on IBD patients' quality of life and inflammatory markers. Sixty-eight patients with ulcerative colitis (UC) or Crohn's disease (CD) were randomized to receive either CMG (2.8 g/day) or a placebo for three months, alongside their stable medical treatment. The CMG group showed a significant decrease in fecal lysozyme, an indicator of lower disease activity, and improvements in the Inflammatory Bowel Disease Questionnaire (IBDQ) scores, reflecting enhanced quality of life. These outcomes suggest CMG's beneficial effects on disease activity and patients' well-being."
3. "Antioxidant Efficacy and Nutritional State Improvement: Another aspect of CMG's potential therapeutic effects was explored in a study focusing on its antioxidant efficacy. Patients treated with CMG showed significant reductions in oxidized low-density lipoprotein (oxLDL) and improvements in plasma amino acids profiles, indicative of CMG's antioxidant properties and its positive impact on the nutritional state of IBD patients."
4. Long-term Effects on Clinical Remission and Immunoregulatory Role: Further studies aimed to understand CMG's long-term effects on clinical remission and its potential immunoregulatory role. A study by Amerikanou et al. investigated CMG's regulatory effect on interleukin-17A (IL-17A) serum levels and alterations of the fecal metabolome in IBD patients. This trial found that CMG could modulate the gut microbiota composition, increase serum levels of beneficial amino acids, and potentially regulate Th17 cells' function and differentiation, suggesting a role in maintaining remission and regulating immune responses."

"Conclusions: The literature on Pistacia lentiscus, particularly mastic gum, showcases its potential to reduce pro-inflammatory cytokines and improve the clinical course of IBD. Despite the promising data, the evidence from randomized controlled studies remains limited, and larger, more definitive trials are needed to fully ascertain Chios mastic gum's therapeutic potential and efficacy in IBD treatment. The intriguing findings so far position mastic gum as a potential supplementary treatment to conventional IBD therapies, aiming to decrease disease activity, enhance nutritional status, and maintain clinical remission."

IFUS Point 1J-2a: An entire Chapter titled, "Chios mastic gum is a resin procured from the trunk as well as leaves of Pistacia lentiscus," Biomedicine & Pharmacotherapy, 2018, Chapter Vol. 2 (Subject Area: Medicine and Dentistry reveals:

"In a double-blind clinical trial carried out on 38 patients with symptomatic and endoscopically proved duodenal ulcer, patients were given either mastic gum (1 g daily) or a placebo for 2 weeks. 52 Symptomatic relief was obtained in 16 (80%) patients on mastic gum and 9 (50%) patients on placebo, whereas endoscopically proved healing occurred in 14 (70%) patients on mastic gum and 4 (22%) patients on placebo.

In another study, 52 patients with H. pylori infection were randomized to receive either 350 mg three times daily of pure mastic gum for 14 days (group A), 1.05 g three times daily of pure mastic gum for 14 days (group B), pantoprazole 20 mg twice daily plus pure mastic gum 350 mg three times daily for 14 days (group C), or pantoprazole 20 mg twice daily plus amoxicillin 1g twice daily plus clarithromycin 500 mg twice daily for 10 days (group D).53 Eradication of H. pylori was confirmed in 4 of 13 patients in group A and 5 of 13 in group B. No patient in group C achieved eradication, whereas 10 of 13 patients in group D did. These results confirm that mastic gum has some bactericidal activity on H. pylori in vivo but not enough to produce consistent clinical eradication."

IFUS Point 1J-2b: In a paper published by Malliga Raman Murali, Sangeetha Vasudevaraj Naveen, Chang Gue Son, Hanumantha Rao Balaji Raghavendran, "Current knowledge on alleviating Helicobacter pylori infections through the use of some commonly known natural products: bench to bedside, Integrative Medicine Research, Volume 3, Issue 3, 2014, Pages 111-118, ISSN 2213-4220, https://doi.org/10.1016/j.imr.2014.04.001. (https://www.sciencedirect.com/science/article/pii/S2213422014000249)
Mastic Gum: "Mastic gum is a natural resin that is excreted from the trunk and branches of the mastic bush (Pistacia lentiscus). Huwez et al (17) were the first to report on the bactericidal effect of mastic gum against H. pylori, and observed that crude mastic gum could kill H. pylori at a concentration of 0.06 mg/mL, regardless of whether the organism was sensitive or resistant to metronidazole. In addition, they also reported that the lowest concentration tested, 0.0075 mg/mL, could significantly inhibit the growth of H. pylori. This report was followed by a study by Marone et al, (18) who found that 50% and 90% of H. pylori strains were inhibited at a mastic gum concentration of 125 μg/mL and 500 μg/mL,

respectively. In addition, the morphological changes assessed through transmission electron microscopy were also reported, and it was noted that at a sub-MBC concentration, blebbing, morphological abnormalities, and cellular fragmentation were observed. These studies were further validated by Loughlin et al (19) through in vitro susceptibility testing and in vivo H. pylori eradication using specific-pathogen-free CD1 mice infected with H. pylori. In accordance with the previous reports, Loughlin et al (19) found that mastic gum exhibited good MIC and MBC against H. pylori SS1, with values of 7.80 mg/L and 31.25 mg/L, respectively. However, paradoxically, mastic gum failed to eradicate H. pylori infection in the infected mice model and did not produce any reduction in the bacterial load, although the mouse stomach was immediately examined after 7 days of treatment. The same year, Bebb et al (20) reported the role of mastic gum in H. pylori eradication in nine patients with H. pylori infection and proved the inefficiency of mastic gum in clearing H. pylori infections. In their study, mastic gum was administered at a high dose of 1 g four times a day for 14 days, and at the end of the treatment regime, all patients were still found to be H. pylori positive."

"Regardless of the contradictory reports on the activity of mastic gum, research on its anti-H. pylori activity has continued. For instance, Paraschos et al (21) performed both in vivo and in vitro assays of the anti-H. pylori activity of mastic gum. Their study differed from the previous studies in that the treatment regime used was longer (3 months, as opposed to 1 week in the study by Loughlin et al (19)) and the insoluble polymers were removed from mastic gum to ameliorate solubility. Administration of mastic gum at a concentration of 0.75 mg/day led to an approximately 30-fold reduction in H. pylori colonization in the infected mice; however, this eradication did not attenuate chronic inflammatory infiltration and chronic gastritis. Furthermore, the acidic and neutral fractions of mastic gum were tested for anti-H. pylori activity in vitro, and it was found that the acid fraction was the most active (MBC = 0.139 mg/mL) and that isomasticadienolic acid was the most active isolated compound (MBC = 0.202 mg/mL). These results show that administration of mastic gum may be effective in reducing H. pylori colonization, and that the major triterpenic acids in the acidic fraction may be responsible for such an activity. In another study, Dabos et al (22) administered two different doses of mastic gum (350 mg and 1.0 g) three times a day for 14 days, and noted that the H. pylori was eliminated at a rate of 30.8% and 38.5%, respectively. However, the authors had used only a small sample size (n = 13 participants) and did not include a detailed description of the difference between their study and the report by Bebb et al. (20)"

Ref.(17): FU Huwez, D Thirlwell, A Cockayne, DA Ala'Aldeen, Mastic gum kills Helicobacter pylori, N Engl J Med, 339 (26) (1998), p. 1946

Ref.(18): P Marone, L Bono, E Leone, S Bona, E Carretto, L Perversi, Bactericidal activity of Pistacia lentiscus mastic gum against Helicobacter pylori, J Chemother, 13 (6) (2001), pp. 611-614

Ref.(19): MF Loughlin, DA Ala'Aldeen, PJ Jenks, Monotherapy with mastic does not eradicate Helicobacter pylori infection from mice, J Antimicrob Chemother, 51 (2) (2003), pp. 367-371

Ref.(20): JR Bebb, N Bailey-Flitter, D Ala'Aldeen, JC Atherton, Mastic gum has no effect on Helicobacter pylori load in vivo, J Antimicrob Chemother., 5 (3) (2003), pp. 522-523

Ref.(21): S Paraschos, P Magiatis, S Mitakou, K Petraki, A Kalliaropoulos, P Maragkoudakis, et al., In vitro and in vivo activities of Chios mastic gum extracts and constituents against Helicobacter pylori, Antimicrob Agents Chemother, 51 (2) (2007), pp. 551-559

Ref.(22): KJ Dabos, E Sfika, LJ Vlatta, G Giannikopoulos, The effect of mastic gum on Helicobacter pylori: a randomized pilot study, Phytomedicine, 17 (3–4) (2010), pp. 296-299

IFUS Point 1J-2c:Yet, another study "The Role of Phytonutrients in Metabolic Disorders" by Shabana Bibi, Mohammad Mehedi Hasan, Partha Biswas, Anastasiia Shkodina, Muhammad Ajmal Shah, Ghulam Mujtaba Shah, Ajmal Khan, Ahmed Al-Harrasi, Chapter 7 - Phytonutrients in the management of lipids metabolism, Editor(s): Haroon Khan, Esra Küpeli Akkol, Maria Daglia, The Role of Phytonutrients in Metabolic Disorders, Academic Press, 2022, Pages 195-236, ISBN 9780128243565, https://doi.org/10.1016/B978-0-12-824356-5.00010-2.
(https://www.sciencedirect.com/science/article/pii/B9780128243565000102)

IFUS Point 1K: Anti-oxidative efficacy of Chios Mastic Gum.

"Various studies also show mastic is used in the manufacture of adhesive bandages, acts as an antioxidant and helps to protect human cells against oxidative damage, to show cytotoxicity

against cancer cells, without harming healthy cells, used as a remote astringent in cases of internal hemorrhage, used to treat albuminuria and diabetes, used to treat psoriasis and external hemorrhoids."

IFUS Point 1K-1: Ottria, R.; Xynomilakis, O.; Casati, S.; Abbiati, E.; Maconi, G.; Ciuffreda, P. Chios Mastic Gum: Chemical Profile and Pharmacological Properties in Inflammatory Bowel Disease: From the Past to the Future. Int. J. Mol. Sci. 2023, 24, 12038. https://doi.org/10.3390/ijms241512038

Abstract: Chios mastic gum, the product of the tree Pistacia lentiscus var. Chia, has been used for more than 2500 years in traditional Greek medicine for treating several diseases, thanks to the anti-inflammatory and antioxidant properties of its components. Despite the long-time use of mastic in gastroenterology and in particular in chronic-inflammation-associated diseases, to date, the literature lacks reviews regarding this topic. The aim of the present work is to summarize available data on the effects of P. lentiscus on inflammatory bowel disease. A comprehensive review of this topic could drive researchers to conduct future studies aimed at deeply investigating P. lentiscus effects and hypothesizing a mechanism of action. The present review, indeed, schematizes the possible bioactive components of mastic gum. Particular care is given to P. lentiscus var. Chia medica.

Conclusion: In conclusion, the data from the literature show that CMG reduces pro-inflammatory cytokines such as IL-6 [89] and TNF-α [90] and increases the levels of interleukin-a17A [98], which is considered a protective key factor in the development and relapse of IBD. These data have been corroborated by randomized controlled studies showing that P. lentiscus may also reduce free AA in plasma [94], a surrogate for inflammation and cell homeostasis [96], and may play a key role in pathways regulating intestinal health.

Ref.(89); Kaliora, A.C.; Stathopoulou, M.G.; Triantafillidis, J.K.; Dedoussis, G.V.Z.; Andrikopoulos, N.K. Chios mastic treatment of patients with active Crohn's disease. World J. Gastroenterol. 2007, 13, 748–753.

Ref.(90): Kaliora, A.C.; Stathopoulou, M.G.; Triantafillidis, J.K.; Dedoussis, G.V.Z.; Andrikopoulos, N.K. Alterations in the function of circulating mononuclear cells derived from patients with Crohn's disease treated with mastic. World J. Gastroenterol. 2007, 13, 6031–6036.

Ref.(98): Amerikanou, C.; Dimitropoulou, E.; Gioxari, A.; Papada, E.; Tanaini, A.; Fotakis, C.; Zoumpoulakis, P.; Kaliora, A.C. Linking the IL-17A immune response with NMR-based faecal metabolic profile in IBD patients treated with Mastiha. Biomed. Pharmacother. 2021, 138, 111535.

Ref.(94): Papada, E.; Forbes, A.; Amerikanou, C.; Torović, L.; Kalogeropoulos, N.; Tzavara, C.; Triantafillidis, J.K.; Kaliora, A.C. Antioxidative Efficacy of a Pistacia lentiscus Supplement and Its Effect on the Plasma Amino Acid Profile in Inflammatory Bowel Disease: A Randomised, Double-Blind, Placebo-Controlled Trial. Nutrients 2018, 10, 1779.

Ref.(96): Nakaya, M.; Xiao, Y.; Zhou, X.; Chang, J.-H.; Chang, M.; Cheng, X.; Blonska, M.; Lin, X.; Sun, S.-C. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity 2014, 40, 692–705.

On account of these data, it has been argued that CMG may be used as a supplement to decrease disease activity, improve nutritional status, and maintain clinical remission in IBD patients.

Unfortunately, despite the large amount of preliminary data on the effect of P. lentiscus on biochemical markers of inflammation and homeostasis, the scientific evidence of its clinical effectiveness in IBD is still scanty and mainly based on a few randomized controlled studies. These studies showed that P. lentiscus may improve IBD quality of life, although to the same extent as placebo, and its effects on IBD activity, assessed by scores tools, although with some benefits, still remain uncertain [91]. However, it should be acknowledged that the sample sizes of these trials are small and that the true extent of P. lentiscus's potential benefit is difficult to assess because it has been associated with different drugs, as usually happens for most supplementary treatments. Therefore, large prospective trials are still needed."

Ref.(91): Papada, E.; Gioxari, A.; Amerikanou, C.; Forbes, A.; Tzavara, C.; Smyrnioudis, I.; Kaliora, A.C. (Regulation of faecal biomarkers in inflammatory bowel disease patients treated with oral mastiha (Pistacia lentiscus) supplement: A double-blind and placebo-controlled randomised trial. Phytother. Res. 2019, 33, 360–369.

IFUS Point 1K-2: "There is now substantial evidence to suggest that mastiha demonstrates a plethora of favorable effects, mainly attributed to the anti-inflammatory

and anti-oxidative properties of its components." Soulaidopoulos S, Tsiogka A, Chrysohoou C, Lazarou E, Aznaouridis K, Doundoulakis I, Tyrovola D, Tousoulis D, Tsioufis K, Vlachopoulos C, Lazaros G. Overview of Chios Mastic Gum (Pistacia lentiscus) Effects on Human Health. Nutrients. 2022 Jan 28;14(3):590. doi: 10.3390/nu14030590. PMID: 35276949; PMCID: PMC8838553. (Note: "Mastika or mastiha is a liqueur seasoned with mastic, a resin with a slightly pine or cedar-like flavor gathered from the mastic tree, a small evergreen tree native to the Mediterranean region.")

IFUS Point 1K-3: Triantafyllou A, Bikineyeva A, Dikalova A, Nazarewicz R, Lerakis S, Dikalov S. Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress. Nutr J. 2011 Jun 6;10:64. doi: 10.1186/1475-2891-10-64. PMID: 21645369; PMCID: PMC3127998.

"Conclusion: We suggest that mastic gum inhibits PKC which attenuates production of superoxide and H2O2 by NADPH oxidases. This antioxidant property may have direct implication to the anti-inflammatory activity of the Chios mastic gum."

IFUS Point 1K-4: Abstract: "In vitro antioxidant and antimutagenic activities of two polyphenols isolated from the fruits of Pistacia lentiscus was assessed. Antioxidant activity was determined by the ability of each compound to scavenge the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPHradical dot), to inhibit xanthine oxidase and to inhibit the lipid peroxidation induced by H2O2 in K562 cell line. Antimutagenic activity was assayed with SOS chromotest using Escherichia coli PQ37 as tester strain and Comet assay using K562 cell line. 1,2,3,4,6-Pentagalloylglucose was found to be more effective to scavenge DPPHradical dot radical and protect against lipid peroxidation. Moreover, these two compounds induced an inhibitory activity against nifuroxazide and aflatoxin B1 mutagenicity. The protective effect exhibited by these molecules was also determined by analysis of gene expression as response to an oxidative stress. For this purpose, we used a cDNA-microarray containing 82 genes related to cell defense, essentially represented by antioxidant and DNA repair proteins. We found that 1,2,3,4,6-pentagalloylglucose induced a decrease in the expression of 11 transcripts related to antioxidant enzymes family (GPX1, TXN, AOE372, SHC1 and SEPW1) and DNA repair (POLD1, APEX, POLD2, MPG, PARP and XRCC5). The use of Gallic acid, induced expression of TXN, TXNRD1, AOE372, GSS (antioxidant enzymes) and LIG4, POLD2, MPG, GADD45A, PCNA, RPA2, DDIT3, HMOX2, XPA, TDG, ERCC1 and GTF2H1 (DNA repair) as well as the repression of GPX1, SEPW1, POLD1 and SHC1 gene expression." (Afef Abdelwahed, Ines Bouhlel, Ines Skandrani, Kita Valenti, Malika Kadri, Pascal Guiraud, Régine Steiman, Anne-Marie Mariotte, Kamel Ghedira, François

Laporte, Marie-Geneviève Dijoux-Franca, Leila Chekir-Ghedira, Study of antimutagenic and antioxidant activities of Gallic acid and 1,2,3,4,6-pentagalloylglucose from Pistacia lentiscus: Confirmation by microarray expression profiling,

Chemico-Biological Interactions, Volume 165, Issue 1, 2007, Pages 1-13, ISSN 0009-2797, https://doi.org/10.1016/j.cbi.2006.10.003. (https://www.sciencedirect.com/science/article/pii/S0009279706002894)

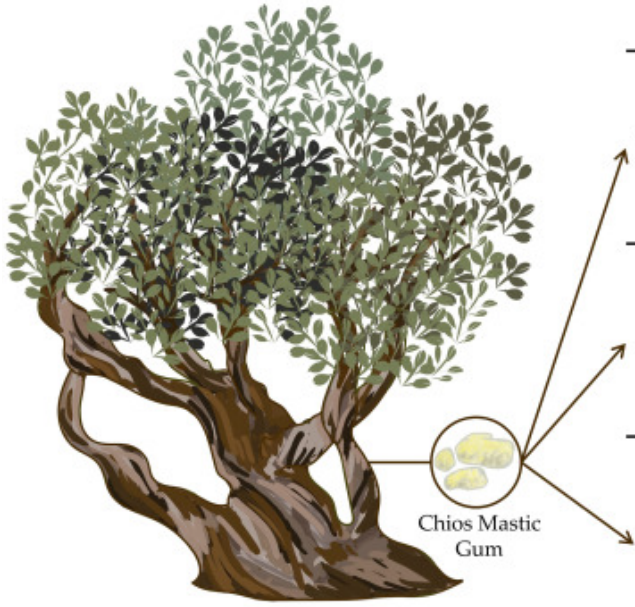
IFUS Point 1L: Efficacy of Chios Mastic Gum on Cardiovascular Health. There are more detailed studies in this paper However, this is but a sampling of studies to establish the point:

IFUS Point 1L-1: A study found that high consumption of Chios mastic powder results in decreased levels of total serum cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B, SGOT, SGPT and gamma-GT. (Triantafyllou, A.; Chaviaras, N.; Sergentanis, T. N.; Protopapa, E.; Tsaknis, J. (2007). "Chios mastic gum modulates serum biochemical parameters in a human population". *Journal of Ethnopharmacology* **111** (1): 43–49. doi:10.1016/j.jep.2006.10.031. PMID 17150319.)

IFUS Point 1L-2: Blomquist, S.A.; Fernandez, M.L. Chios Mastic Gum: A Promising Phytotherapeutic for Cardiometabolic Health. Nutrients 2024, 16, 2941. https://doi.org/10.3390/nu16172941

IFUS Point 1L-2a: Figure 1: Impacts of Chios mastic gum on disease mechanisms and cardiometabolic outcomes. Abbreviations used: * adjunct metabolic syndrome treatments only, ** CMG-gene interactions only, 11β-HSD1 = 11-beta-hydroxysteroid dehydrogenase, adipo = adiponectin, ALT = alanine aminotransferase, AMPKα = AMP-activated protein kinase alpha, Apo(B) = apolipoprotein B, AST = aspartate aminotransferase, BF = body fat, CD36 = cluster of differentiation 36, CRP = C-reactive protein, FG = fasting glucose, GGT = gamma-glutamyl transferase, Gpx = glutathione peroxidase, GR = glucocorticoid receptor, GSH = glutathione, HDL = high density lipoprotein, HOMA = HOMA-IR (homeostatic model assessment for insulin resistance), IL-10 = interleukin 10, IL-6 = interleukin 6, Ins = insulin, LDL = low density lipoprotein, LPC = lysophosphatidylcholine, LPE = lysophosphatidylethanolamine, Lp(a) = Llipoprotein(a), MAP = mean arterial pressure, Microbiota div. = microbiota diversity, NAFLD = non-alcoholic fatty liver disease, NF-κB = nuclear factor kappa B, NOX-2 = NADPH oxidase 2, NRF-2 = nuclear factor erythroid 2-related factor 2, oxLDL = oxidized low-density lipoprotein, p65 = p65 subunit of NF-κB, PEPCK = phosphoenolpyruvate carboxykinase, PPARα = peroxisome proliferator-activated receptor alpha, PPARγ = peroxisome proliferator-activated receptor gamma, pPP =

peripheral pulse pressure, SBP = systolic blood pressure, TAS = total antioxidant status, TC = total cholesterol, TG = triglycerides, TNF-α = tumor necrosis factor alpha, VF = visceral fat, Wt = weight.




| | Inflammation & immunity | Oxidative stress & antioxidants | Cardiovascular & hepatic | Metabolic & microbiota |
|---|---|---|---|---|
| **Humans** | ↓ IL-6 + gene expression**<br>↓ TNF-a gene expression**<br>↓ IL-10** | ↓ oxLDL<br>↓ NOX-2<br>↑ TAS**<br>↓ or ↑ Gpx** | ↓ SBP<br>↓ ALT*, AST*<br>↓ GGT*<br>↓ pPP | ↓ TG, LDL, TC<br>↓ BF*, VF*, Wt*<br>↓ Ins, FG, HOMA<br>↑ HDL, adipo*<br>↑ Microbiota div.<br>↓ Lp(a), Apo(B)<br>↓ LPC, LPE<br>↓ Cholic acid<br>↓ Hemoglobin** |
| **Animals** | ↓ CRP<br>↓ IL-6 | | ↓ Hepatic steatosis, NAFLD, fibrosis, ALT<br>↓ SBP, DBP, MAP<br>↓ Renin<br>↓ Infarct size<br>Improved cardiac indices | ↓ Glucose<br>↓ TG, LDL, TC<br>↓ Total lipids<br>↑ HDL<br>↑ Microbiota div. |
| ***In vitro, In silico*** | ↓ Monocyte attachment<br>↓ Adhesion molecules<br>↓ NF-κB, p65<br>↓ Cell migration | ↓ oxLDL<br>↓ NRF-2<br>↓ CD36<br>↑ GSH | | ↓ PEPCK<br>↓ GR<br>↓ PPARα<br>↓ AMPKα<br>11β-HSD1 inhibition<br>PPARγ agonist |

IFUS Point 1M: Chios Mastic Gum Shows Antibacterial Efficacy.

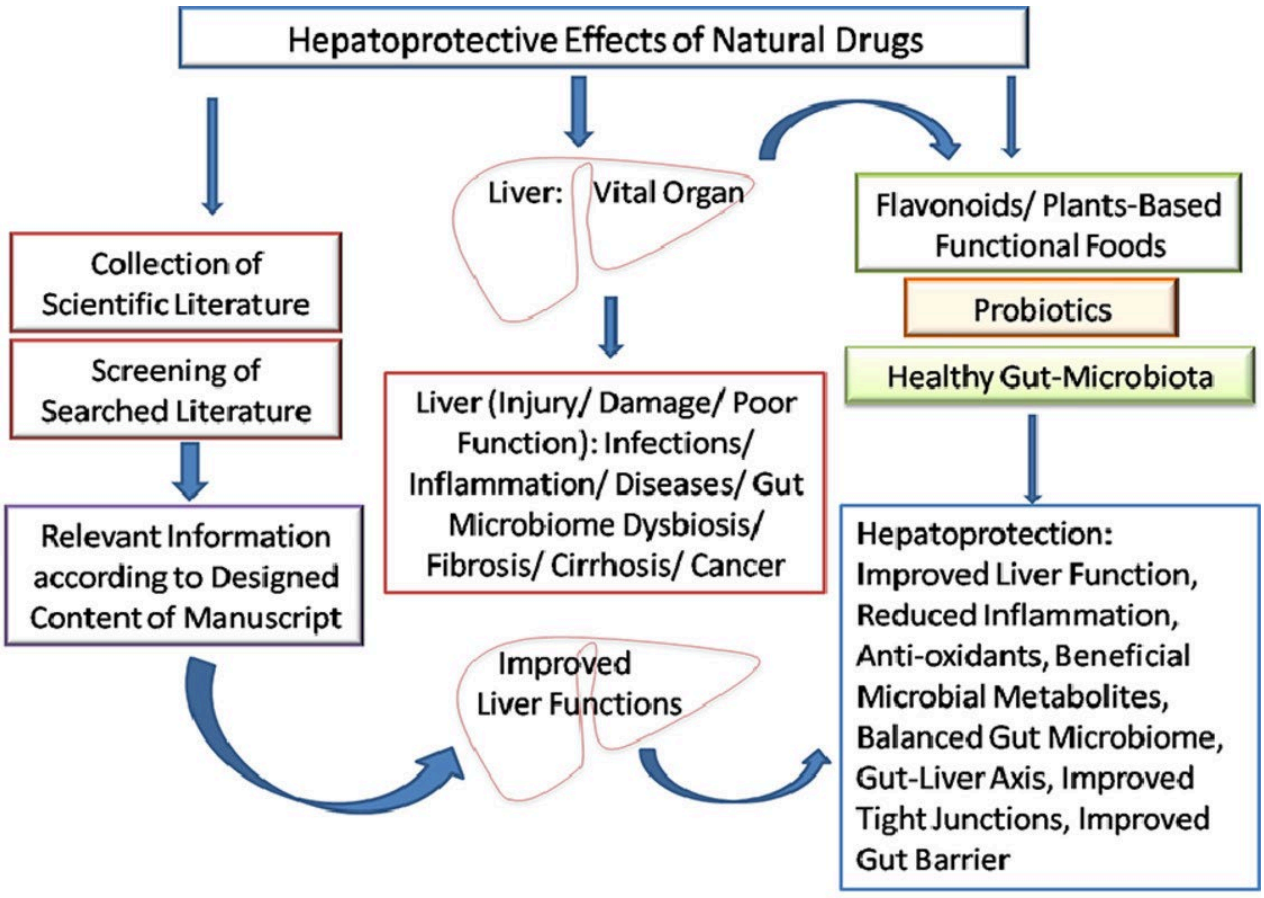
In summary: "Commonly held benefits of mastic include:
Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil have been identified: verbenone, alpha-terpineol, and linalool. The sensitivity to these compounds was different for different bacteria tested (Escherichia coli, Staphylococcus aureus, and Bacillus subtilis), which suggests that the antibacterial efficacy of mastic oil is due to a number of its components working synergistically. (J Agric Food Chem. 2005 Oct 5; 53(20):7681-5. Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia., Koutsoudaki C, Krsek M, Rodger A., Department of Chemistry and Department of Biological Sciences, University of Warwick, Warwick, Coventry CV4 7AL, United Kingdom.)

IFUS Point 1M-1: Gum of Chios mastic (Pistacia lentiscus var. chia) is a natural antimicrobial agent that has found extensive use in pharmaceutical products and as a nutritional supplement.

(Triantafyllou A, Bikineyeva A, Dikalova A, Nazarewicz R, Lerakis S, Dikalov S. Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress. Nutr J. 2011 Jun 6;10:64. doi: 10.1186/1475-2891-10-64. PMID: 21645369; PMCID: PMC3127998.)

IFUS Point 1N: Efficacy of Chios Mastic Gum on Liver Function. There are more detailed studies in this paper However, this is but a sampling of studies to establish the point:

IFUS Point 1N-1: "Hepatoprotective effects of natural drugs: Current trends, scope, relevance and future perspectives," Sonal Datta, et.al., Phytomedicine, Volume 121, December 2023, 155100




**General presentation showing major reasons for liver damage and role of flavonoids and probiotics as hepatoprotective agents.**

IFUS Point 1N-2: The hepatoprotective effect of the boiled and non-boiled aqueous extracts of Pistacia lentiscus, Phillyrea latifolia, and Nicotiana glauca, that are alleged to be effective in the

treatment of jaundice in Jordanian folk medicine, was evaluated in vivo using carbon tetrachloride (CCl4) intoxicated rats as an experimental model. Plant extracts were administrated orally at a dose of 4 ml/kg body weight, containing various amounts of solid matter. Only total serum bilirubin level was reduced by treatment with non-boiled aqueous extract of N. glauca leaves, while the boiled and non-boiled aqueous extracts of the N. glauca flowers were non effective. Bilirubin level and the activity of alkaline phosphatase (ALP) were both reduced upon treatment with boiled aqueous extract of P. latifolia without reducing the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Aqueous extract of P. lentiscus (both boiled and non-boiled) showed marked antihepatotoxic activity against CCl4 by reducing the activity of the three enzymes and the level of bilirubin. The effect of the non-boiled aqueous extract was more pronounced than that of the boiled extract. (Sana Janakat, Hela Al-Merie, Evaluation of hepatoprotective effect of Pistacia lentiscus, Phillyrea latifolia and Nicotiana glauca, Journal of Ethnopharmacology, Volume 83, Issues 1–2, 2002, Pages 135-138, ISSN 0378-8741, https://doi.org/10.1016/S0378-8741(02)00241-6. (https://www.sciencedirect.com/science/article/pii/S0378874102002416)

IFUS Point 1N-2.a: As a note, "Hepatoprotection is the ability to protect the liver by a chemical substance via restoring the function of catalase, glutathione peroxidase, and superoxide dismutase to normal levels [1,3,5].

Ref.(1): Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya-Martínez MT, Gutiérrez-Salinas J, Bautista M, Morales-González Á, García-Luna y González-Rubio M, Aguilar-Faisal JL, Morales-González JA. Review of natural products with hepatoprotective effects. World J Gastroenterol. 2014 Oct 28;20(40):14787-804. doi: 10.3748/wjg.v20.i40.14787. PMID: 25356040; PMCID: PMC4209543.

Ref.(3): Sonal Datta, Diwakar Aggarwal, Nirmala Sehrawat, Mukesh Yadav, Varruchi Sharma, Ajay Sharma, Abdulrazzaq N. Zghair, Kuldeep Dhama, Aanchal Sharma, Vikas Kumar, Anil K. Sharma, Hailian Wang, Hepatoprotective effects of natural drugs: Current trends, scope, relevance and future perspectives, Phytomedicine, Volume 121, 2023, 155100, ISSN 0944-7113, https://doi.org/10.1016/j.phymed.2023.155100. (https://www.sciencedirect.com/science/article/pii/S0944711323004609)
Highlights:

- The present review highlights the potential of natural products as hepatoprotective agents.

- Drugs-induced liver injury (DILI) has been discussed, and the role of phytochemicals in ameliorating liver injury has been summarised.

- The review covers the role of plant secondary metabolites, probiotics, prebiotics, postbiotics, and synbiotics in hepatoprotection.

- Mechanistic insights into flavonoid-mediated hepatoprotection are also discussed.

- This review aims to provide a systematically organized, state-of-the-art understanding of the role of phytochemicals in hepatoprotection.

Abstract: Background - The liver is a well-known player in the metabolism and removal of drugs. Drug metabolizing enzymes in the liver detoxify drugs and xenobiotics, ultimately leading to the acquisition of homeostasis. However, liver toxicity and cell damage are not only related to the nature and dosage of a particular drug but are also influenced by other factors such as aging, immune status, environmental contaminants, microbial metabolites, gender, obesity, and expression of individual genes Furthermore, factors such as drugs, alcohol, and environmental contaminants could induce oxidative stress, thereby impairing the regenerative potential of the liver and causing several diseases. Persons suffering from other ailments and those with comorbidities are found to be more prone to drug-induced toxicities. Moreover, drug composition and drug-drug interactions could further aggravate the risk of drug-induced hepatotoxicity. A plethora of mechanisms are responsible for initiating liver cell damage and further aggravating liver cell injury, followed by impairment of homeostasis, ultimately leading to the generation of reactive oxygen species, immune-suppression, and oxidative stress.

Objective: To summarize the potential of phytochemicals and natural bioactive compounds to treat hepatotoxicity and other liver diseases.

Study design: A deductive qualitative content analysis approach was employed to assess the overall outcomes of the research and review articles pertaining to hepatoprotection induced by natural drugs, along with analysis of the interventions.

Methods: An extensive literature search of bibliographic databases, including Web of Science, PUBMED, SCOPUS, GOOGLE SCHOLAR, etc., was carried out to understand the role of hepatoprotective effects of natural drugs.

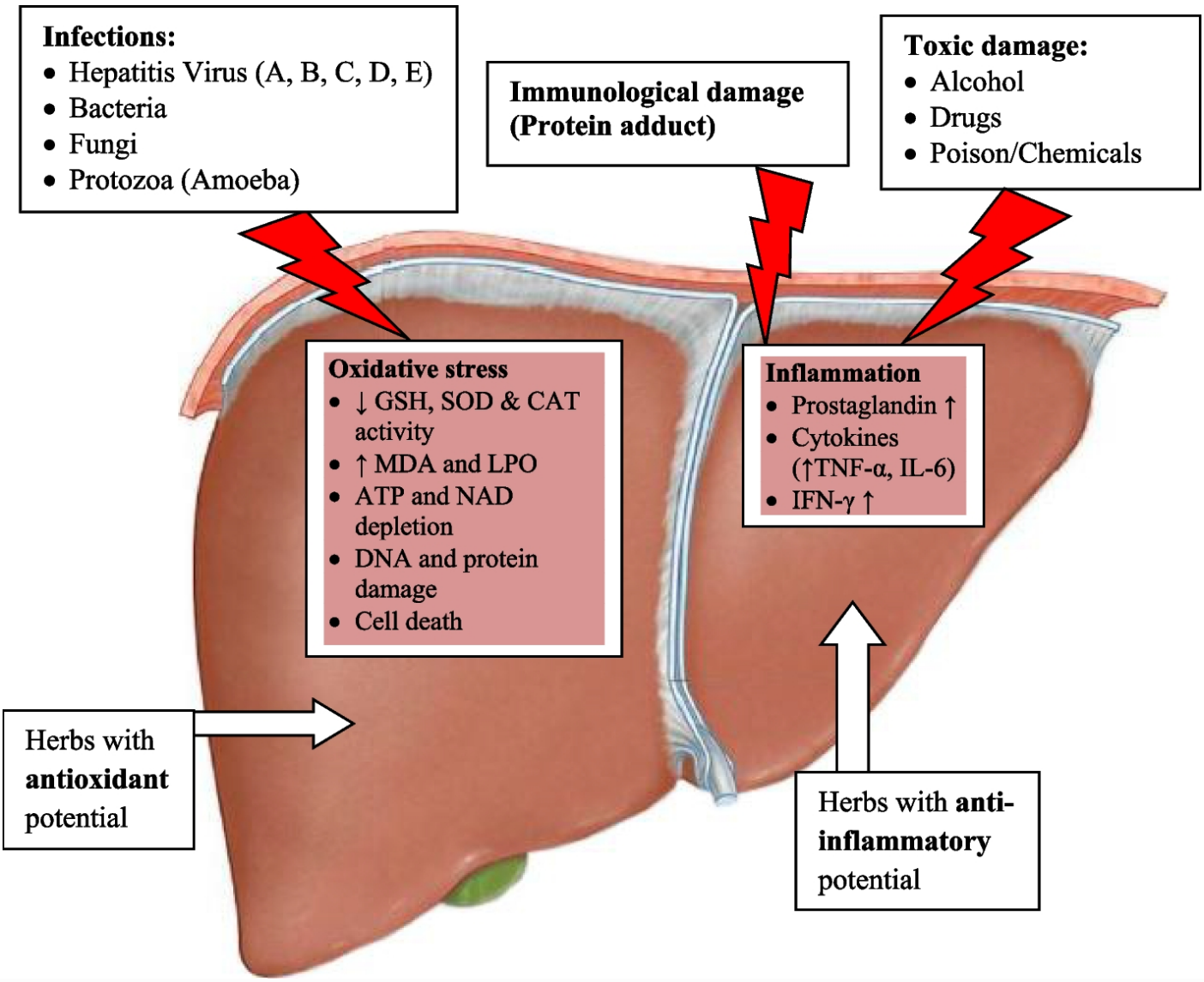
Results: Bioactive natural products, including curcumin, resveratrol, etc., have been seen as neutralizing agents against the side effects induced by the drugs. Moreover, these natural products are dietary and are readily available; thus, could be supplemented along with drugs to reduce toxicity to cells. Probiotics, prebiotics, and synbiotics have shown promise of improving overall liver functioning, and these should be evaluated more extensively for their hepatoprotective potential. Therefore, selecting an appropriate natural product or a bioactive compound that is free of toxicity and offers a reliable solution for drug-induced liver toxicity is quintessential.

Conclusions: The current review highlights the role of natural bioactive products in neutralizing drug-induced hepatotoxicity. Efforts have been made to delineate the possible underlying mechanism associated with the neutralization process.

IFUS Point 1N-3: Pandey, B., Baral, R., Kaundinnyayana, A. et al. Promising hepatoprotective agents from the natural sources: a study of scientific evidence. Egypt Liver Journal 13, 14 (2023). https://doi.org/10.1186/s43066-023-00248-w

IFUS Point 1N-3.a: Fig. 2: Promising hepatoprotective agents from the natural sources: a study of scientific evidence: The mechanism by which herbal remedies protect against liver injury from different toxins and injurious stimuli (Al-Asmari AK, Al-Elaiwi AM, Athar MT, Tariq M, Al Eid A, Al-Asmary SM (2014) A review of hepatoprotective plants used in Saudi traditional medicine. Ev-Based Complement Altern Med 2014:1-22)

IFUS Point 1N-3.a.1: Fig. 2 From: Promising hepatoprotective agents from the natural sources: a study of scientific evidence. The mechanism by which herbal remedies protect against liver injury from different toxins and injurious stimuli. )Al-Asmari AK, Al-Elaiwi AM, Athar MT, Tariq M, Al Eid A, Al-Asmary SM (2014) A review of hepatoprotective plants used in Saudi traditional medicine. Ev-Based Complement Altern Med 2014:1-22)


Infections:
• Hepatitis Virus (A, B, C, D, E)
• Bacteria
• Fungi
• Protozoa (Amoeba)
Immunological damage (Protein adduct)
Toxic damage:
• Alcohol
• Drugs
• Poison/Chemicals
Oxidative stress
• ↓ GSH, SOD & CAT activity
• ↑ MDA and LPO
• ATP and NAD depletion
• DNA and protein damage
• Cell death
Inflammation
• Prostaglandin ↑
• Cytokines (↑TNF-α, IL-6)
• IFN-γ ↑
Herbs with antioxidant potential
Herbs with anti-inflammatory potential

IFUS Point 1N-3.a.2: Table 3: Clinical trial with different promising hepatoprotective leads in patients with different liver diseases (Promising hepatoprotective agents from the natural sources: a study of scientific evidence)

| S. no | Name of hepatoprotective lead | Condition | Study design (*n*) | Treatment (*n*) | Duration | Outcome with hepatoprotective leads | References |
|---|---|---|---|---|---|---|---|
| 1 | Silymarin | Epileptic children having antiepileptic treatment and experienced drug-induced liver injury (DILI) | Randomized clinical trial (55) | Randomized children were administered either silymarin (5 mg/kg per day) or folic acid (1 mg per day) for 1 month | Three months | Folic acid group had significantly decreased ALT, AST, and GGT levels compared to the patients in the silymarin groups. Both treatments were safe and effective in the management of DILI, but folic acid seems to be superior | [110] |
| | | Patients with severe preeclampsia whose pregnancy was terminated | Randomized clinical trial (30) | Case group received 70 mg of silymarin, and control group received the placebo at 3 and 24 h after the termination of pregnancy | Three days | Hepatic enzymes ALT and AST level decreased significantly during 36 and 60 h after the termination of pregnancy in the study group compared to control group. This indicates that silymarin improves liver disorder in severe preeclampsia | [121] |
| | | Non-cirrhotic patients with non-alcoholic steatohepatitis (NASH) | Randomized double-blind placebo control phase II clinical trial (78) | Silymarin treatment group 400 mg (26 patients) and 700 mg (27 patients) and placebo group (25 patients) in dosing frequency thrice a day | 48 weeks | Significant variation was not observed in side effect among the treatment group and patient treated with silymarin in patients. Whether NASH is treated with silymarin is inconclusive due to the lack of substantial number of patients who meet the histological criteria and therefore required additional clinical trial | [115] |
| | | New cases of pulmonary tuberculosis patients | Randomized double-blind clinical trial (70) | Test group received silymarin 140 mg three times a day + standard antituberculosis treatment, and control group received standard antituberculosis treatment only | Two weeks | Silymarin was proved to be safe without adverse event, but measurable hepatoprotective effect was not observed among patients receiving tuberculosis treatment | [99] |
| | | Liver disease (78% with daily alcohol use) | Double-blind control study (97) | Test group (47) received silymarin 420 mg/day, and control group (50) received placebo | 4 weeks | Liver function parameters, liver histology, ALT, and AST were observed to be improved | [145] |
| | | Alcoholic liver disease (ALD) (50% with cirrhosis) | Double-blind comparative study (116) | Test group (57) received silymarin 420 mg/day, and control group (59) received placebo | 3 months | No significant effects | [166] |
| | | ALD or non-alcoholic fatty liver disease (NAFLD) (70% with cirrhosis) | Randomized control trial (170) | Test group (87) received silymarin 420 mg/day, and control group (59) received placebo | Median 41 months | Ameliorate the survival rate, and difference in survival was observed mostly in patients with ALD and liver cirrhosis and those with less serious ailments (Child class A) | [46] |
| | | ALD | 36 | Test group (17) received silymarin, and control group (19) received placebo | 6 months | Decrease in ALT, bilirubin and procollagen synthesis | [45] |
| | | ALD | Double-blind protocol NA (not available) | Test group received silymarin 420 mg/day, and control group received placebo | 6 months | Improvement in antioxidative system by decreasing MDA and increasing GSH in liver cell | [113] |
| | | ALD (72% with cirrhosis) | Double-blind randomized control trial (59) | Test group (25) received silymarin 280 mg/day, and control group (34) received placebo | 15 months | Blood glucose level was observed to get improved (including fasting); HbAIC and MDA daily dose of insulin administration get decreased in ALT and AST | [18] |
| | | Insulin-treated type 2 diabetic mellitus with alcoholic cirrhosis | Randomized clinical trial (60) | Test group (30) received silymarin 680 mg/day + standard treatment, and control group (30) received standard treatment only | 12 months | No measurable effect was observed on progression, and survival of cells involved in liver disease | [171] |
| | | ALD with cirrhosis | Double-blind randomized control multicenter trial (200) | Test group (103) received silymarin 450 mg/day, and control group (97) received placebo | 2 years | Decrease MDA and aminoterminal propeptide of procollagen type 3 | [127] |
| | | ALD with cirrhosis | Randomized double-blind placebo control clinical trial (49) | Test group (24) received silymarin 450 mg/day, and control group (25) received placebo | 6 months | Slight upsurge in the level of glutathione and down surge of lipid peroxidation were observed in the patient with ALD was silymarin administration | [88] |
| 2 | Picroside | Liver disease patients | Double-blinded placebo randomized clinical trial | Test group received the herbal formulation contains picroside, and control group received placebo | NA | Reduction in bilirubin value by 2.5 mg was achieved by 27.4 days for the picroside herbal formulation treatment group, whereas 75.9 days are required for the placebo group | [6] |
| | | Hypolipidemic patients with liver disease | Double-blind placebo study | Test group receive 2 gm kutaki formulation along with atorvastatin 20 mg two times a day, and control group received 500-mg starch powder with atorvastatin 20 mg in same duration | NA | Test group showed significant increase in the liver function enzyme parameters as compared to the placebo group | [105] |
| 3 | Phyllanthin | Oxidative stress, liver damage, and patients with hangover symptoms | Randomized placebo-controlled trial | Test group received 750 mg/day; *Phyllanthus amarus* ethanol extract and control group received placebo (15) | Ten days | *Phyllanthus amarus* treatment group showed significant control over hangover, inflammation, and liver function following intoxication by reducing blood alcohol and upregulating cytokine IL-8 and IL-10 as compared to control group | [49] |
| | | Patients suffering from liver disease | Clinical study (107) | Test group received 3 g of *Phyllanthus amarus* powder for three times for a day orally with water (98) | 45 days | Significant decrease in SGPT and bilirubin and increase in hemoglobin with patient treated with test drug | [128] |
| 4 | Glycyrrhizin | A healthy individual consume alcohol (vodka) nightly for 12 days | Randomized, double-blind, placebo, cross over study (12; six males and six females) | Test group received the glycyrrhizin product with daily alcohol, and control group received alcohol alone daily | 12 days | Plasma glutathione level and ALP significantly decreased in alcohol control group suggested that consumption of glycyrrhizin product during the alcohol consumption may improve the liver health compared with the consumption of alcohol alone | [29] |
| | | Digestive tract cancer patients | Clinical trial (84) | Test group treated with the saponin (160 mg i.v once a day) with standard cancer chemotherapy, and control group received only standard cancer chemotherapy alone | NA | Test group showed significantly lower liver transaminase level and higher level of neutrophile, granulocyte, and white blood cells when compared with the standard chemotherapy control group | [55] |
| | | Patients with hepatitis E and severe jaundice | Clinical trial (78) | Test group received magnesium isoglycyrrhizinate, and control group received an intravenous injection of 150 mg of magnesium once a day | 6 weeks | Use of magnesium isoglycyrrhizinate in test group showed improved effective against the hepatitis E virus infection with severe jaundice as compared to the control group | [182] |
| | | Patients with chronic hepatitis B | Clinical trial (64) | Test group received magnesium isoglycyrrhizinate, and control group received an intravenous injection of saponin once a day | 4 weeks | Liver function was improved in both patients group, but no statistically significant difference observed in test group and control group | [19] |
| 5 | Curcumin | Patients with liver cirrhosis | Randomized double-blind placebo control trial (70) | Test group (35) received 1000 mg/day curcumin, and control group received the placebo | 3 months | Curcumin supplement showed beneficial effect in decreasing disease activity score and severity of cirrhosis in patients with liver cirrhosis as compared to placebo group | [120] |
| | | Chronic alcoholic patients | Randomized double-blind placebo control trial (48) | One group received curcumin-galactomannoside complex 500 mg/day, and another group received placebo drug per day | 8 weeks | Curcumin-galactomannoside complex group showed significant decrease in liver function markers such as transaminase and GGT Increase in endogenous antioxidant (GSH, SOD, GPx) and decrease in inflammatory markers (IL-6 and CRP) level as compared to placebo group | [159] |
| | | Type 2 diabetic mellitus (T2DM) patients and measure glycemic, hepatic, and inflammatory biomarker measure | Randomized double-blind placebo control trial (100) | Test group received standard treatment, dietary advice plus curcuminoids 500 mg/day coadministered with piperine 5 mg/day, and control group received the standard treatment, dietary advice plus placebo drug | 3 months | Intervention group showed significant reduction in serum level of glucose, HbA1c, and low serum level of ALT and AST as compared to the placebo group. The study concluded that curcuminoid mixed with piperine when coadministered with diabetic medicine shows improved hepatoprotective and glycemic control in T.DM patients | [124] |
| | | Patients with non-alcoholic fatty liver disease (NAFLD) | Double-blind randomized clinical trial (46; 21 males and 25 females) | Intervention group received six turmeric capsules containing 500 mg in each, and control group received placebo drug daily | 12 weeks | Turmeric consumption decreased the serum level of glucose, insulin, HOMA-IR, and leptin as compared to the placebo group. This may be useful in control of NAFLD complications | [116] |
| | | Patients diagnosed with non-alcoholic fatty liver disease (NAFLD) | Randomized double-blind placebo control trial (102) | Intervention group received 1000 mg/day curcumin in 2 divided dose ($n$ = 50), and control group received the placebo drug ($n$ = 52). Both groups received the dietary and lifestyle advice before the start of clinical trial | 8 weeks | Curcumin supplement group showed reduction of body mass Index, waist circumference, decrease level of AST and ALT as compared to the placebo drug treatment group. This indicated that intervention improve the liver fat and normalize liver biomarker level in patients with NAFLD | [125] |
| | | Non-alcoholic fatty liver disease (NAFLD) | Randomized double-blind placebo control trial (80) | Intervention group received amorphous curcumin powder 500 mg/day (equivalent to 70 mg/day curcumin), and control group received the placebo drug | 8 weeks | Curcumin-treated group showed reduction of liver fat, body mass index, total cholesterol, low-density lipoprotein, triglyceride, AST, ALT, glucose, glycated hemoglobin as compared to the placebo drug-treated group. This indicated that curcumin amorphous powder improves liver of patients with NAFLD | [137] |
| 6 | Berberine | Non-alcoholic fatty liver disease (NAFLD) | Randomized parallel controlled open-label clinical trial (184) | Lifestyle intervention group or placebo group ($n$ = 62) (60) is lifestyle intervention plus pioglitazone 15 mg qd ($n$ = 60), lifestyle intervention plus 0.5 g berberine t.i.d. ($n$ = 62) | 16 weeks | As compared to the placebo and pioglitazone treatment group, intervention group showed significant reduction in serum lipid profile, body weight, HOMA-IR which help to ameliorate NAFLD and related metabolic disorder by directly regulating the hepatic lipid metabolism | [185] |

IFUS Point 1N-3.a.3: Table 2 Some major phytoconstituents with hepatoprotective activity From: Promising hepatoprotective agents from the natural sources: a study of scientific evidence

| S. no | Name of bioactive phytoconstituent | Plant name | Parts used | Extract used | Dose used | Hepatotoxicity-inducing agents | Finding of study | Mechanism of hepatoprotective activity | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Phenylethanoid glycoside: acteoside | *Plantago major* L | Aerial parts | Aqueous methanolic | 500 mg/kg orally | CCl4 | Inhibited the serum elevation of ALT, AST, ALP, and GGT enzymes as well as total and direct bilirubin. Decrease LPO and increased GSH in the liver | Blocks the P50-mediated CCL4 bioactivation and exhibits superoxide-free radical scavenging effects | [40, 192] |
| 2 | Pentacyclic triterpene: alpha-amyrin | *Alstonia scholaris* Linn | Stem bark | Ethanol extract | 20 mg/kg/day orally | CCl4 | Decreased serum liver markers like GGT, AST, ALT, LDH, ALP, acid phosphatase (ACP), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GDH), and total bilirubin, total protein<br>Increased glutathione, ceruloplasmin, β-carotene, vitamin C, and vitamin E<br>Increased hepatic antioxidants like SOD, CAT, GPx, GR, GST, LPO, 5′-ribonucleotidase, acid ribonuclease, succinic dehydrogenase | Exhibits antifibrotic, anti-inflammatory, antiapoptotic, and free radical scavenging effects | [149, 162] |
| 3 | Triterpenoid: asiatic acid | *Potentilla chinensis* | | | 4 or 8 mg/kg/day orally | Lipopolysaccharide/D-galactosamine | Decreased the serum ALT and AST and showed improvement of liver pathology | Inhibits MAPK and NF-κB via the partial induction of PDCD4 and upregulation of Nrf2 in an AMPK/GSK3β pathway activation-dependent manner resulting in the inhibition of oxidative stress and inflammation | [91, 126] |
| 4 | Pentacyclic triterpenoid saponin: asiaticoside | *Centella asiatica* | | | 5, 10, and 20 mg/kg/day orally | Lipopolysaccharide/D-galactosamine | Decreased the elevated serum level of ALT, hepatocytes apoptosis, caspase-3, improvement of liver pathological injury in dose-dependent manner. Also reduced the elevation of phospho-p38 MAPK, phospho-JNK, phospho-ERK protein, and TNF-alpha mRNA expression in liver tissue | Inhibits TNF alpha and MAPKs | [13, 191] |
| 5 | Saponin: cristatain | *Celosia cristata* L | Seeds | 50% ethane | 1, 2, and 4 mg/kg/day orally | CCl4 and N,N-dimethyl formamide | Significantly reduced in the values of AST, ALT and ALP of serum and histopathological examinations compared to controls | Downregulates caspase-3 and caspase-8 activities and prevents hepatic cell apoptosis. Exhibits antioxidant activities through scavenging hydroxyl and DPPH-free radicals | [4, 177] |
| 6 | Oleanolic acid saponins: celosin A and celosin B | *Semen celosiae* | Seeds | Ethanolic extract | 1, 2, and 4 mg/kg orally | CCl4 | Inhibited the serum elevation of AST, ALT, and ALP while improve the serum level of GSH_PX, MDA, CAT, and SOD | Both have significant hepatoprotective effects due to the antioxidant property by decreasing the serum liver biochemical markers and liver antioxidant enzymes | [183, 184] |
| 7 | Sesquiterpene glycoside: cichotyboside | *Cichorium intybus* | Seeds | - | | CCl4 | Exhibited a significant anti-hepatotoxic activity by reducing the elevated levels of liver enzymes such as AST and ALT | Reduces liver weight and liver protein; inhibits oxidative stress by increasing reduced glutathione content and decreasing lipid peroxidation | [64, 94] |
| 8 | Flavonol glycoside: visconoside C | *Cleome viscosa* L | Leaves | Methanolic extract | 100 µM | CCl4-induced hepatotoxicity on HepG2 cells | Exhibited a significant hepatoprotective activity by antioxidant mechanism and quercetin taken as standard control | Shows free radical scavenging activity and normalizes impaired membrane function activity | [118, 151] |
| 9 | Dehydrocavidine | *Corydalis saxicola* | | - | 1, 0.5 and 0.25 mg/kg/day intraperitoneally | CCl4-induced hepatic fibrosis in rats | Inhibited the serum level of ALT, AST, ALP, TB and increased the SOD, CAT, GPx | Alleviates liver damage by reducing the formation of fibrous septa, decreasing the MDA concentration, reducing oxidative stress, promoting collagenolysis, and regulating fibrosis-related genes | [175] |
| 10 | Isoflavones: puerarin | Kudzu roots/*Pueraria lobelia* | Roots | | 30, 60, and 120 mg/kg/day orally | Chronic alcohol-induced liver injury | Decreased the serum levels of ALT, AST, ALP, and intpatic contents of alcohol dehydrogenase (ADH); aldehyde dehydrogenase (ALDH) were elevated | Inhibits endogenous activities of CYP2E1, CYP1A2, and CYP3A which potentially sustain metabolic homeostasis | [28, 84] |
| 11 | Rubiadin | *Rubia cordifolia* Linn | | - | 50, 100, and 200 mg/kg orally | CCl4-induced hepatic damage in rats | Normalized serum level of ALT, AST, ALP, and GGT and decrease activities of glutathione S-transferase and glutathione reductase | Shows glutathione mediated detoxification as well as free radical scavenging effect | [103, 138] |
| 12 | Ursolic acid | *Hedyotis corymbosa* L | Whole plant | Ethane | 0.75 mg/ml/kg body weight orally | Paracetamol-induced liver injury | Decreased serum enzyme levels of ALT, AST, ALP, total bilirubin, and also normalized histological architecture of the liver compared to the paracetamol-treated group | Suppresses the nuclear factor-kappa beta (NF-κB) activation, inhibits Cytochrome P4502E1 expression, enhances hepatic-glutathione regeneration capacity, and upregulates metallothionein expression | [56, 157] |
| 13 | Coumarin: wedelolactone | *Eclipta prostrata* L | NA | NA | 50 and 100 mg/kg intraperitoneally | Concanavalin A-induced hepatitis in mice | Significantly reduced leukocyte infiltration and T-cell activation in liver. Suppress the activity of nuclear factor-kappa B, tumor necrosis factor, interferon gamma, and interleukin (IL)-6 | Inhibits the infiltration of leukocytes into the liver via suppression of NF-κB signaling pathway | [89] |
| 14 | Betulinic acid and ricinine | *Tetracarpidium conophorum* | Seeds | Hexane fraction | 100 mg/kg betulinic acid and 50 mg/kg ricinine orally | CCl4-induced hepatotoxicity | Both betulinic acid and ricinine exhibited hepatoprotective potential in CCl4 rat model in vivo. Both possess strong affinity and better interaction in the active sites of hepatitis B virus DNA polymerase | Improves tissue redox system, decrease lipid peroxidation, and maintain antioxidant system | [122, 189] |
| 15 | Coumarin analogues: meranzin hydrate I | *Citrus grandis* | Pericarp | 70% ethanol and 30% water | 20-µM concentration | D-Galactosamine-induced cell survival inhibition in LO2 cells by MTT assay | Increased superoxide dismutase and glutathione peroxidase and decreased the level of malondialdehyde in liver toxic model | Modulates cellular antioxidant pathway and improves the free radical scavenging property | [164] |

IFUS Point 1N-3.a.4: Does Pistacia lentiscus var. chia contain Phyllanthin? “The resin contains Phyllanthin: Pistacia lentiscus var. chia, also known as Chios Mastic

Gum (CMG), is an evergreen shrub that produces aromatic resin. The resin contains Phyllanthin, which has been recognized for its therapeutic indications in mild dyspeptic disorders and skin inflammation/healing of minor wounds (1). Additionally, the oleoresin of Pistacia lentiscus var. Chia contains Oleanonic acid, which has PPARγ activation properties. (3)"

Ref.(1) Vasiliki K. Pachi, Eleni V. Mikropoulou, Petros Gkiouvetidis, Konstantinos Siafakas, Aikaterini Argyropoulou, Apostolis Angelis, Sofia Mitakou, Maria Halabalaki, Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review, Journal of Ethnopharmacology, Volume 254, 2020, 112485, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2019.112485. (https://www.sciencedirect.com/science/article/pii/S0378874119331174)

Ref.(3) Floris S, Di Petrillo A, Pintus F, Delogu GL. Pistacia lentiscus: Phytochemistry and Antidiabetic Properties. Nutrients. 2024 May 27;16(11):1638. doi: 10.3390/nu16111638. PMID: 38892571; PMCID: PMC11174566.

IFUS Point 1N-3.a.5: What effect does Phyllanthin on Liver Function?

IFUS Point 1N-3.a.5.1: Based on a study published by George A, Udani JK, Yusof A (2019) "Effects of Phyllanthus amarus PHYLLPROTM leaves on hangover symptoms: a randomized, double-blind, placebo-controlled crossover study," (Pharm Biol 57:145–153), "Oxidative stress, liver damage, and patients with hangover symptoms" in a "Randomized placebo-controlled trial" of a "Test group received 750 mg/day; Phyllanthus amarus ethanol extract and control group received placebo." (Benić MS, Nežić L, Vujić-Aleksić V, Mititelu-Tartau L (2022) Novel therapies for the treatment of drug-induced liver injury: a systematic review. Front Pharmacol 12:785790) for "Ten days".

Results: "Phyllanthus amarus treatment group showed significant control over hangover, inflammation, and liver function following intoxication by reducing blood alcohol and upregulating cytokine IL-8 and IL-10 as compared to control group"

IFUS Point 1N-3.a.5.2: In another study, "Patients suffering from liver disease" (Clinical study: Mohammed E, Peng Y, Wang Z, Qiang X, Zhao Q

(2022) Synthesis, antiviral, and antibacterial activity of the glycyrrhizic acid and glycyrrhetinic acid derivatives. Russ J Bioorg Chem 48:906–918). The "Test group received 3 g of Phyllanthus amarus powder for three times for a day orally with water" (Manns MP, Wedemeyer H, Singer A, Khomutjanskaja N, Dienes HP, Roskams T, Goldin R, Hehnke U, Inoue H, Group ESS (2012) Glycyrrhizin in patients who failed previous interferon alpha-based therapies: biochemical and histological effects after 52 weeks. J Viral Hepatitis. 19:537–546) for "45 days."

Results: "Significant decrease in SGPT and bilirubin and increase in hemoglobin with patient treated with test drug" Parés A, Planas R, Torres M, Caballería J, Viver JM, Acero D, Panés J, Rigau J, Santos J, Rodés J (1998) Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. J Hepatol 28:615–621

FIX THIS IFUS Point 1N-3.a.6: Pistacia lentiscus var. Chia contains natural pentacyclic triterpenoids, including Oleanolic acid, which has PPARγ activation properties (1,3).

Ref.(1): Abstract: Pistacia species contain oleoresins with bioactive triterpenes. In this study triterpenes, including minor components, were identified and quantified in both neutral and acidic fraction of Pistacia lentiscus var. Chia resin, grown exclusively in Chios island (Greece), collected traditionally, as well as by the use of stimulating agents (liquid collection). It was proved that these two resin samples were composed of several different minor triterpenes. In the traditional collection of the resin, 36 triterpenes were identified, 23 of which are new minor compounds (five in the acidic and eighteen in the neutral fraction). In the liquid collection resin eight compounds were identified in the acidic and 11 in the neutral fraction, while seven compounds were not contained in resin traditionally collected. The main triterpenes in both resin samples collected traditionally and by use of stimulating agents were in the following order: isomasticadienonic acid (24 and 22.5% w/w of triterpenic fraction respectively), masticadienonic acid (9.3 and 14.7% w/w of triterpenic fraction) and 28-norolean-17-en-3-one (19 and 36% w/w of triterpenic fraction respectively). The aim of this study was to compare the qualitative and quantitative composition of triterpenes in the resin samples collected using the traditional and new liquid techniques, and examine whether the

collection technique influences the contained triterpenes in P. lentiscus var. Chia resin samples. Finally, since there is confusion on interpreting mass spectra of triterpenes we present an analytical review on the base peaks, main fragments and fragmentation mechanism/pattern of several skeleton penta- and tetra- cyclic triterpenes reported in P. lentiscus resin. Also, a biosynthetic route for triterpene skeletons contained in P. lentiscus resin was approached. (Biomedical Chromatography, GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of Pistacia species. Part I. Pistacia lentiscus var. Chia, A. N. Assimopoulou, V. P. Papageorgiou, First published: 14 January 2005 https://doi.org/10.1002/bmc / Chapter 7 - Phytonutrients in the management of lipids metabolism)

Ref.(3) Floris S, Di Petrillo A, Pintus F, Delogu GL. Pistacia lentiscus: Phytochemistry and Antidiabetic Properties. Nutrients. 2024 May 27;16(11):1638. doi: 10.3390/nu16111638. PMID: 38892571; PMCID: PMC11174566.

IFUS Point 1N-3.a.6: What effect do triterpenes, including Oleanolic acid have on Liver Function?

“Oleanolic acid (OA), a natural pentacyclic triterpenoid found in edible and medicinal plants, has hepatoprotective, anti-inflammatory, and antioxidant activities (3,5). It reduces hyperglycemia and lipid accumulation by activating PPARγ (3,4). PPARγ is associated with adipocyte differentiation and modulates the transcription of relevant target genes (3).”

Ref.(3) Zhang G, Zhang H, Dong R, Zhao H, Li J, Yue W, Ma Z. Oleanolic acid attenuates obesity through modulating the lipid metabolism in high-fat diet-fed mice. Food Sci Nutr. 2024 Aug 29;12(10):8243-8254. doi: 10.1002/fsn3.4408. PMID: 39479652; PMCID: PMC11521747.

Ref.(5) Wang, Y., Liu, K. Therapeutic potential of oleanolic acid in liver diseases. Naunyn-Schmiedeberg's Arch Pharmacol 397, 4537–4554 (2024). https://doi.org/10.1007/s00210-024-02959-2

Ref.(4) Claro-Cala CM, Jiménez-Altayó F, Zagmutt S, Rodriguez-Rodriguez R. Molecular Mechanisms Underlying the Effects of Olive Oil Triterpenic Acids in Obesity and Related Diseases. Nutrients. 2022 Apr 12;14(8):1606. doi: 10.3390/nu14081606. PMID: 35458168; PMCID: PMC9024864.

IFUS Point 1N-3.a.7: Does Pistacia lentiscus var. chia contain cristatain? “Pistacia lentiscus var. chia contains cristatain (2). It is also known as Chios Mastic Gum (CMG) and is produced by the evergreen shrub Pistacia lentiscus var. Chia (3,4,).”

> Ref.(2) Chemical Profiling of Pistacia lentiscus var. Chia Resin and Essential Oil: Ageing Markers and Antimicrobial Activity, Vasiliki K. Pachi, et.al., Processes 2021, 9(3), 418; https://doi.org/10.3390/pr9030418, Submission received: 29 January 2021 / Revised: 18 February 2021 / Accepted: 21 February 2021 / Published: 25 February 2021, https://www.mdpi.com/2227-9717/9/3/418
>
> Ref.(3) Floris S, Di Petrillo A, Pintus F, Delogu GL. Pistacia lentiscus: Phytochemistry and Antidiabetic Properties. Nutrients. 2024 May 27;16(11):1638. doi: 10.3390/nu16111638. PMID: 38892571; PMCID: PMC11174566.
>
> Ref.(4) Vasiliki K. Pachi, Eleni V. Mikropoulou, Petros Gkiouvetidis, Konstantinos Siafakas, Aikaterini Argyropoulou, Apostolis Angelis, Sofia Mitakou, Maria Halabalaki, Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review, Journal of Ethnopharmacology, Volume 254, 2020, 112485, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2019.112485. (https://www.sciencedirect.com/science/article/pii/S0378874119331174)

IFUS Point 1N-3.a.8: What effect does cristatain demonstrate hepatoprotective activity? The “Saponin: cristatain” from “Celosia cristata L Seeds” extracted with “50% ethane” and dosed “1, 2, and 4 mg/kg/day orally” and added with the “Hepatotoxicity-inducing agents CCl4 and N,N-dimethyl formamide” is shown to “Significantly reduced in the values of AST, ALT and ALP of serum and histopathological examinations compared to controls” as it “Downregulates caspase-3 and caspase-8 activities and prevents hepatic cell apoptosis. Exhibits antioxidant activities through scavenging hydroxyl and DPPH-free radicals.” (4,177)

Ref. (4) Al-Snai A, Mousa H, Majid WJ (2019) Medicinal plants possessed hepatoprotective activity. IOSR J Pharmacy 9:26–56

Ref.(177) Wang Y, Jiang Y, Fan X, Tan H, Zeng H, Chen P, Huang M, Bi H (2015) Hepato-protective effect of resveratrol against acetaminophen-induced liver

injury is associated with inhibition of CYP-mediated bioactivation and regulation of SIRT1-p53 signaling pathways. Toxicol Lett 236:82–89

IFUS Point 1N-3.a.9: Does Pistacia lentiscus var. chia contain Oleanolic acid Saponins? “Pistacia lentiscus var. Chia contains Oleanolic acid saponins (1,2,3,4). These compounds have anti-inflammatory and antioxidant properties and act as modulators of peroxisome proliferator-activated receptors (PPARs)(3).”

Ref.(1) Floris S, Di Petrillo A, Pintus F, Delogu GL. Pistacia lentiscus: Phytochemistry and Antidiabetic Properties. Nutrients. 2024 May 27;16(11):1638. doi: 10.3390/nu16111638. PMID: 38892571; PMCID: PMC11174566.

Ref.(2) Ottria R, Xynomilakis O, Casati S, Abbiati E, Maconi G, Ciuffreda P. Chios Mastic Gum: Chemical Profile and Pharmacological Properties in Inflammatory Bowel Disease: From the Past to the Future. Int J Mol Sci. 2023 Jul 27;24(15):12038. doi: 10.3390/ijms241512038. PMID: 37569412; PMCID: PMC10419108.

Ref.(3) Soulaidopoulos, Stergios & Tsiogka, Aikaterini & Chrysohoou, Christina & Lazarou, Emilia & Aznaouridis, Konstantinos & Doundoulakis, Ioannis & Tyrovola, Dimitra & Tousoulis, Dimitris & Tsioufis, Konstantinos & Vlachopoulos, Charalambos & Lazaros, George. (2022). Overview of Chios Mastic Gum (Pistacia lentiscus) Effects on Human Health. Nutrients. 14. 590. 10.3390/nu14030590.

Ref.(4) Vasiliki K. Pachi, Eleni V. Mikropoulou, Petros Gkiouvetidis, Konstantinos Siafakas, Aikaterini Argyropoulou, Apostolis Angelis, Sofia Mitakou, Maria Halabalaki, Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review, Journal of Ethnopharmacology,Volume 254, 2020, 112485, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2019.112485. (https://www.sciencedirect.com/science/article/pii/S0378874119331174

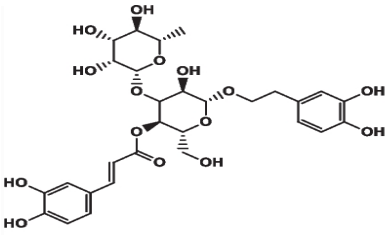
IFUS Point 1N-3.a.10: What effect do the Oleanolic acid saponins: celosin A and celosin B have on hepatoprotective effects? The “Oleanolic acid saponins: celosin A and celosin B” from “Semen celosiae Seeds” and derived be “Ethanolic extract”, when dosed “1, 2, and 4 mg/kg orally” with the “Hepatotoxicity-inducing agents CCl4” is shown to “Inhibited the serum elevation of AST, ALT, and ALP while

improve the serum level of GSH_PX, MDA, CAT, and SOD.””Both have significant hepatoprotective effects due to the antioxidant property by decreasing the serum liver biochemical markers and liver antioxidant enzymes.” (183,184)

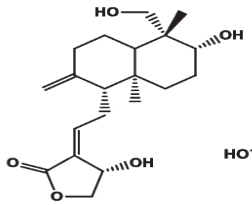
Ref.(183) Xiaoling L, Liming Y, Xiaohong W (2014) Efficacy of magnesium isoglycyrrhizinate in treatment of hepatitis E with severe jaundice. J 临床肝胆病杂志 30:537–539

Ref.(184)Xu G-B, Xiao Y-H, Zhang Q-Y, Zhou M, Liao S-G (2018) Hepatoprotective natural triterpenoids. Eur J Med Chem 145:691–716

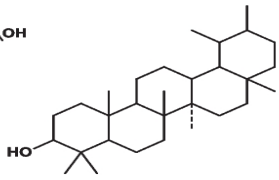
IFUS Point 1N-3.a.11: **Fig. 1** From: Promising hepatoprotective agents from the natural sources: a study of scientific evidence. Chemical structure of some potent bioactive phytochemical with hepatoprotective activity (https://eglj.springeropen.com/articles/10.1186/s43066-023-00248-w/figures/1)



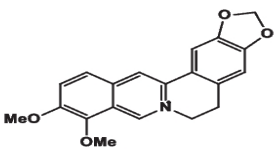
Acteoside



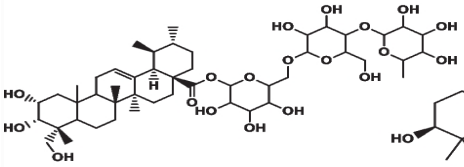
Andrographolide



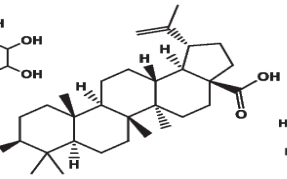
Alpha amyrin



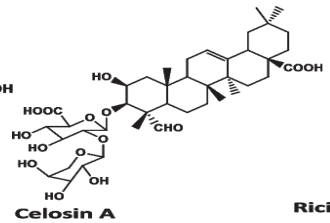
Berberine



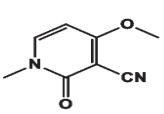
Asiaticoside



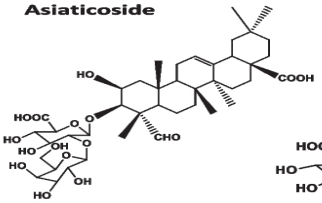
Betulinic acid



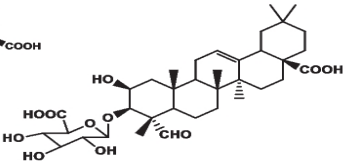
Celosin A



Ricinine



Celosin B



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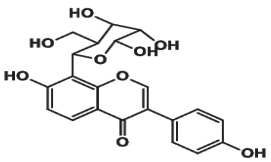

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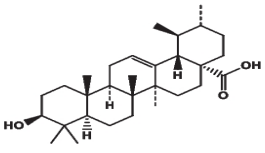
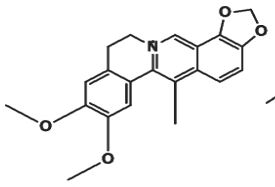
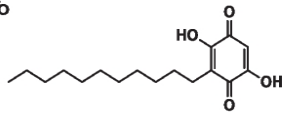


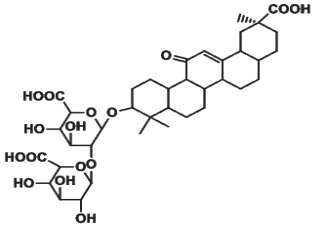
Ursolic acid



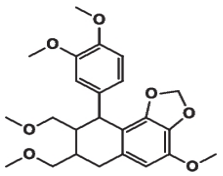
Dehydrocavadine



Embeline



Glycyrrhizin



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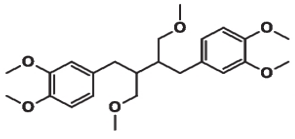
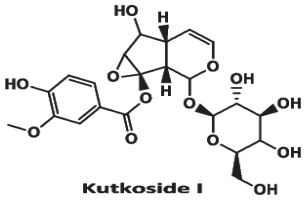
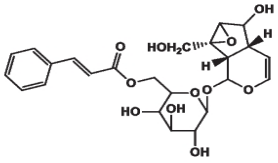


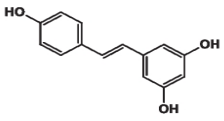
Phyllanthin



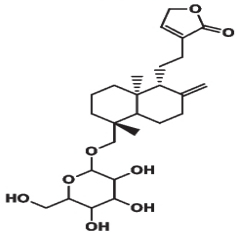
Kutkoside I



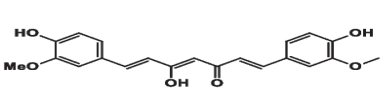
Picroside I



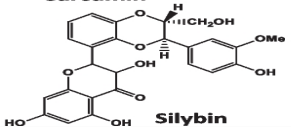
Resveratrol



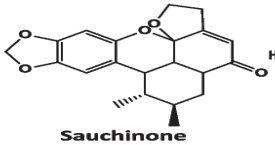
Neoandrographolide



Curcumin

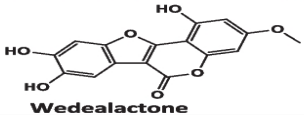


Silybin



Sauchinone

Silychristin



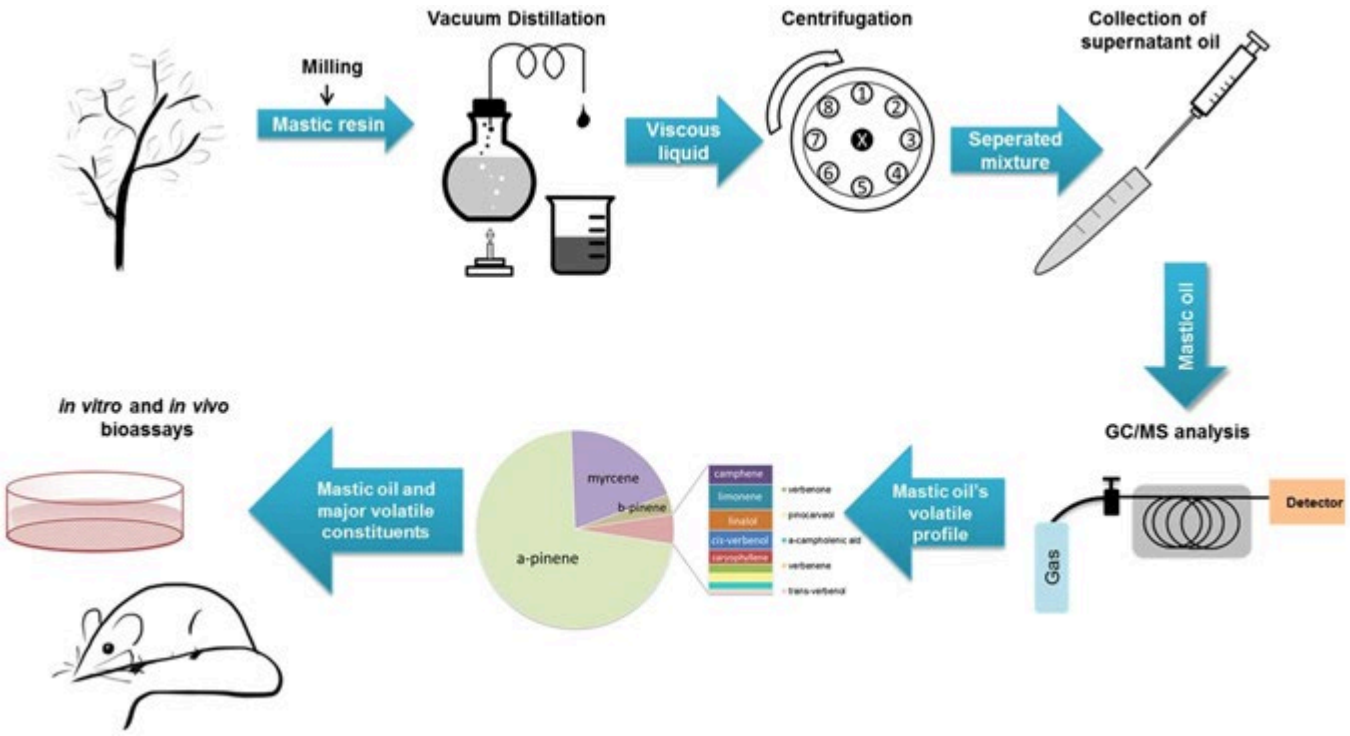
Wedealactone

IFUS Point 1O: Efficacy of Chios Mastic Gum on Immunulatory and Cancer Treatment.
"Pistacia lentiscus and its isolated phenolic compounds have anticancer activity against various cancer cells, including lung, cervical, prostate, gastric, colon, liver, renal, skin, and breast cancer cells." There are more detailed studies in this paper However, this is but a sampling of studies to establish the point.

IFUS Point 1O-1: Ref(1): Spyridopoulou K, Tiptiri-Kourpeti A, Lampri E, Fitsiou E, Vasileiadis S, Vamvakias M, Bardouki H, Goussia A, Malamou-Mitsi V, Panayiotidis MI, Galanis A, Pappa A, Chlichlia K. Dietary mastic oil extracted from Pistacia lentiscus var. chia suppresses tumor growth in experimental colon cancer models. Sci Rep. 2017 Jun 19;7(1):3782. doi: 10.1038/s41598-017-03971-8. PMID: 28630399; PMCID: PMC5476564.

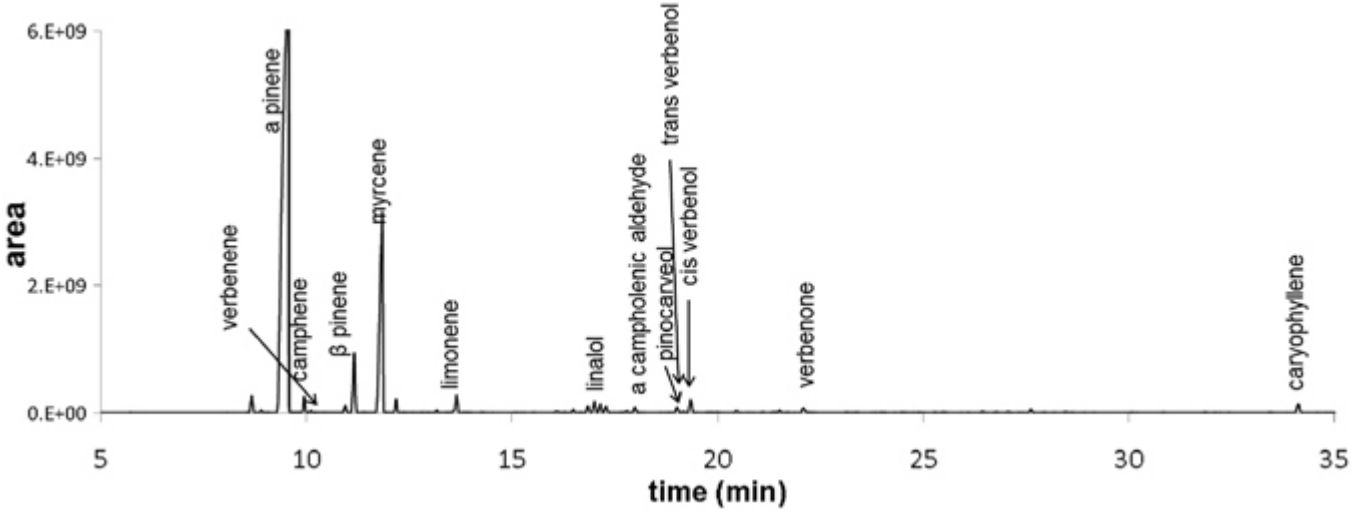
IFUS Point 1O-1a: Abstract: Plant-derived bioactive compounds attract considerable interest as potential chemopreventive anticancer agents. We analyzed the volatile dietary phytochemicals (terpenes) present in mastic oil extracted from the resin of Pistacia lentiscus var. chia and comparatively investigated their effects on colon carcinoma proliferation, a) in vitro against colon cancer cell lines and b) in vivo on tumor growth in mice following oral administration. Mastic oil inhibited - more effectively than its major constituents- proliferation of colon cancer cells in vitro, attenuated migration and downregulated transcriptional expression of survivin (BIRC5a). When administered orally, mastic oil inhibited the growth of colon carcinoma tumors in mice. A reduced expression of Ki-67 and survivin in tumor tissues accompanied the observed effects. Notably, only mastic oil -which is comprised of 67.7% α-pinene and 18.8% myrcene- induced a statistically significant anti-tumor effect in mice but not α-pinene, myrcene or a combination thereof. Thus, mastic oil, as a combination of terpenes, exerts growth inhibitory effects against colon carcinoma, suggesting a nutraceutical potential in the fight against colon cancer. To our knowledge, this is the first report showing that orally administered mastic oil induces tumor-suppressing effects against experimental colon cancer. (See Figures 1 & 2 plus Table 1)

## Figure 1.



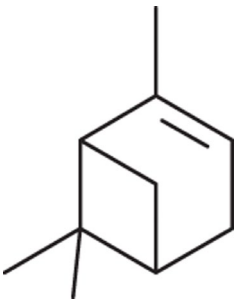
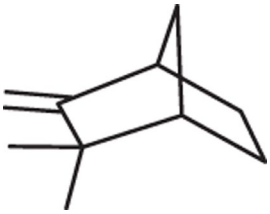
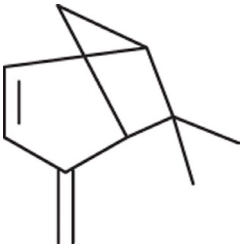
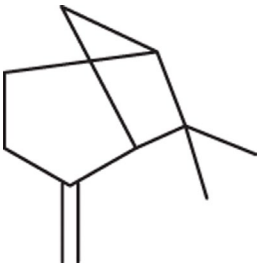
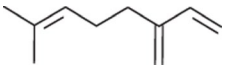

Schematic representation of the MO extraction procedure and analysis of its constituents. MO was extracted from the resin of the plant *Pistacia lentiscus* var. *chia* through vacuum distillation and its volatile profile was analyzed by GC/MS. MO and its identified major constituents were comparatively tested for their potential anticancer properties *in vitro* and *in vivo*.

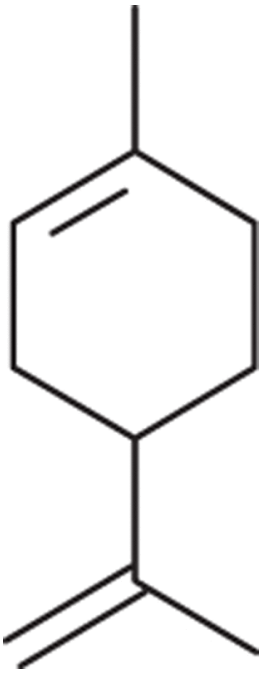
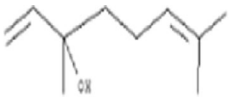
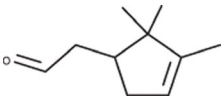
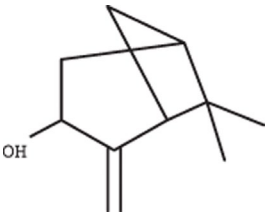
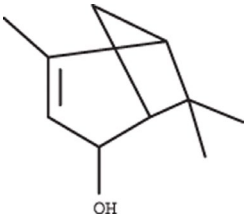
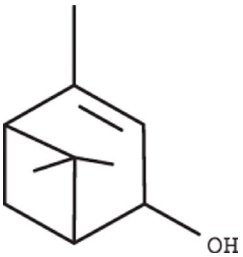
## Figure 2.

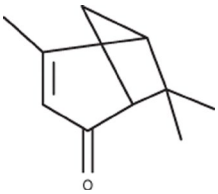
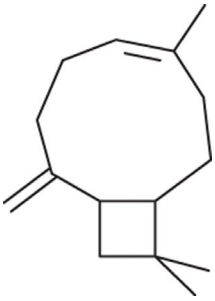


Gas chromatogram of extracted MO. Analysis of volatile compounds in mastic oil was performed by the capillary GC-MS on an Agilent mass selective detector system. Compound identification (labeled signals) was based on a comparison of the retention indices and mass spectra with those of authentic samples.

Table 1.
Volatile compounds present in MO documented by GC-MS analysis.

| **KRI*** | **compounds** | **relative (%) area** | **structure** | **formula** | **MW**** |
|---|---|---|---|---|---|
| 920 | ***α* -pinene** | 67.71 |  | $C_{10}H_{16}$ | 136.24 |
| 934 | camphene | 0.70 |  | $C_{10}H_{16}$ | 136.24 |
| 937 | verbenene | 0.07 |  | $C_{10}H_{14}$ | 134.22 |
| 958 | ***β* -pinene** | 3.05 |  | $C_{10}H_{16}$ | 136.24 |
| 976 | **myrcene** | 18.81 |  | $C_{10}H_{16}$ | 136.24 |

| KRI* | compounds | relative (%) area | structure | formula | MW** |
|---|---|---|---|---|---|
| 1010 | **limonene** | 0.89 |  | $C_{10}H_{16}$ | 136.24 |
| 1086 | **linalol** | 0.73 |  | $C_{10}H_{18}O$ | 154.25 |
| 1094 | *α*-campholenic ald | 0.26 |  | $C_{10}H_{16}O$ | 152.23 |
| 1113 | pinocarveol | 0.32 |  | $C_{10}H_{16}O$ | 152.23 |
| 1117 | *trans*-verbenol | 0.07 |  | $C_{10}H_{16}O$ | 152.23 |
| 1120 | *cis*-verbenol | 0.69 |  | $C_{10}H_{16}O$ | 152.23 |

| KRI* | compounds | relative (%) area | structure | formula | MW** |
|---|---|---|---|---|---|
| 1168 | verbenone | 0.32 |  | $C_{10}H_{14}O$ | 150.22 |
| 1405 | caryophyllene | 0.50 |  | $C_{15}H_{24}$ | 204.36 |

*KRI: Kovats Retention Indices; **MW: molecular weight.
Mastic oil inhibits colon cancer cell proliferation *in vitro* more effectively than its major

IFUS Point 1O-1b: Mastic oil inhibits colon cancer cell proliferation in vitro more effectively than its major constituents: MO and the monoterpenes α-pinene, β-pinene, myrcene, limonene and linalol were examined for their antiproliferative activity against human and murine colon cancer cell lines. MO inhibited growth of human and murine cells in vitro, in a concentration and time-dependent manner (Table 2, Fig. 3a,b). In addition, cytotoxic activity of MO and its monoterpenes was evidenced using the Trypan Blue exclusion test and by flow cytometry with propidium iodide (data not shown).

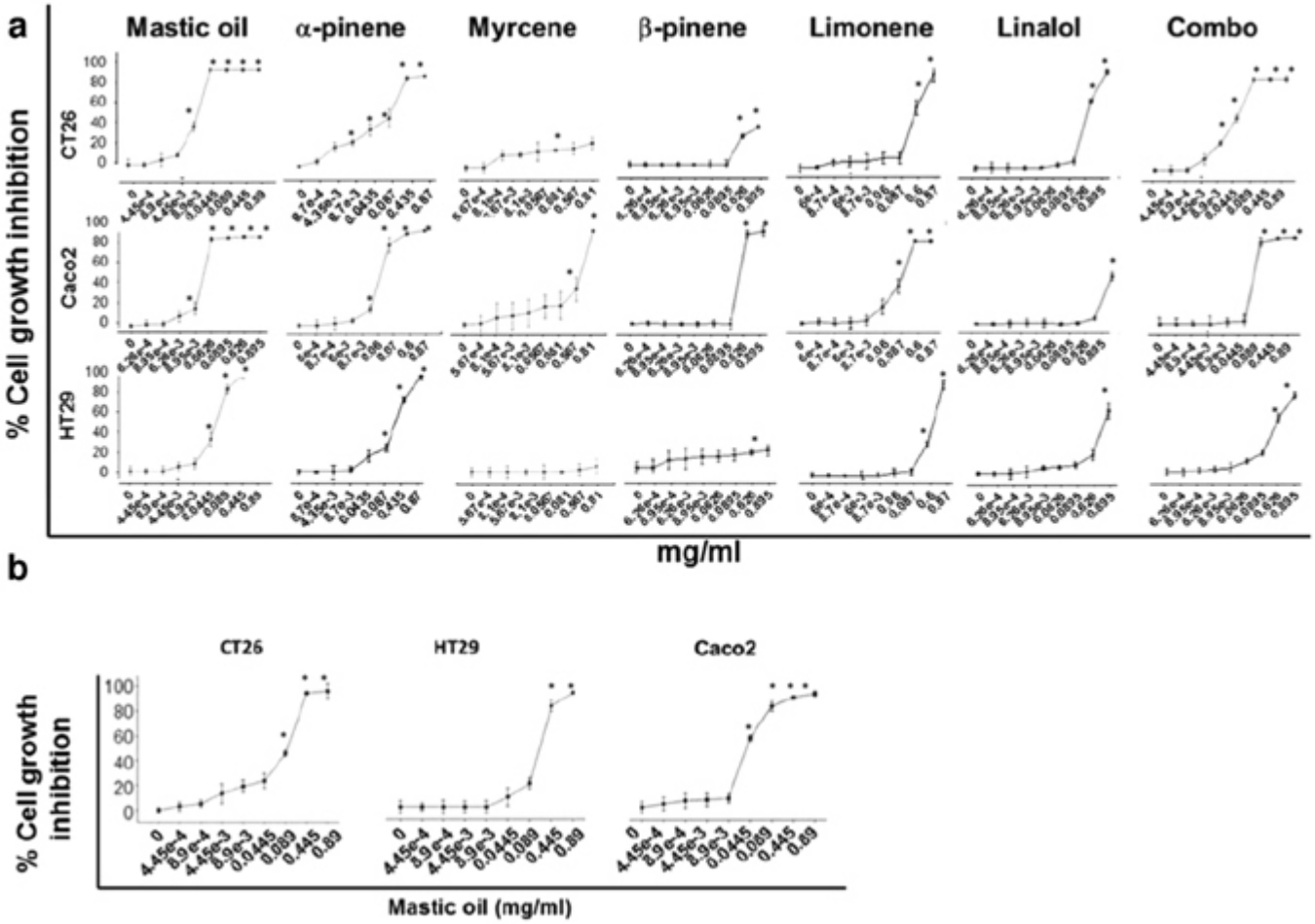
## Table 2.

$IC_{50}$ values (efficient concentration that causes a 50% decrease in cell viability) of mastic oil (48 h and 72 h) and its constituents (72 h) against colon cancer cell lines. Data are representative of at least three independent experiments and are presented as mean ± SD (n = 6).

| Cell line | Mastic oil $IC_{50}$, 48 h (mg/ml) | Mastic oil $IC_{50}$, 72 h (mg/ml) | α-pinene $IC_{50}$, 72 h (mg/ml) | Myrcene $IC_{50}$, 72 h (mg/ml) | β-pinene $IC_{50}$, 72 h (mg/ml) | Limonene $IC_{50}$, 72 h (mg/ml) | Linalol $IC_{50}$, 72 h (mg/ml) | Combo $IC_{50}$, 72 h (mg/ml) |
|---|---|---|---|---|---|---|---|---|
| CT26 | 0.1335 ± 0.0540 | 0.0104 ± 0.0004 | 0.2433 ± 0.0835 | *n.d.** | *n.d.* | 0.4915 ± 0.0425 | 0.1540 ± 0.0267 | 0.0251 ± 0.0077 |
| Caco-2 | 0.0368 ± 0.0225 | 0.0176 ± 0.0035 | 0.0720 ± 0.0012 | 0.6300 ± 0.0150 | 0.3700 ± 0.0701 | 0.0901 ± 0.0042 | *n.d.* | 0.0760 ± 0.0065 |
| HT29 | 0.1751 ± 0.0028 | 0.0762 ± 0.0057 | 0.4837 ± 0.1211 | *n.d.* | *n.d.* | 0.6966 ± 0.0122 | 0.8428 ± 0.0126 | 0.4600 ± 0.0335 |

* *n.d.*: not detected (not possible to determine efficient concentration that causes 50% decrease in cell viability).

Figure 3.

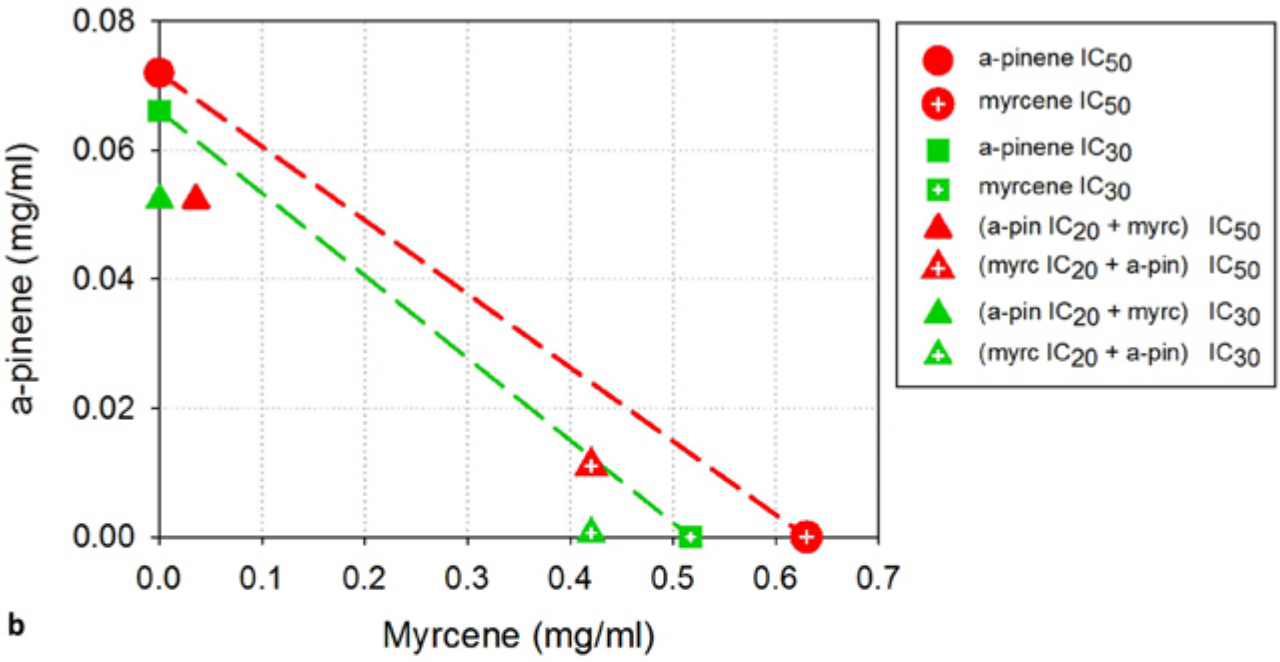



MO inhibits colon cancer cell proliferation *in vitro* more effectively than its major constituents. Antiproliferative effect of increasing doses of MO and its main constituents at (**a**) 72 h or (b) 48 h (only for MO) on murine CT26 and human HT29 and Caco-2 colon cancer cells, determined by the SRB assay. All data shown are representative of at least 3 independent experiments. Values represent mean (n = 6) ± SD.

IFUS Point 1O-1c: Combined cytotoxic effects of α-pinene and myrcene on Caco-2 cells (See Figure 4)

Figure 4.

a




b

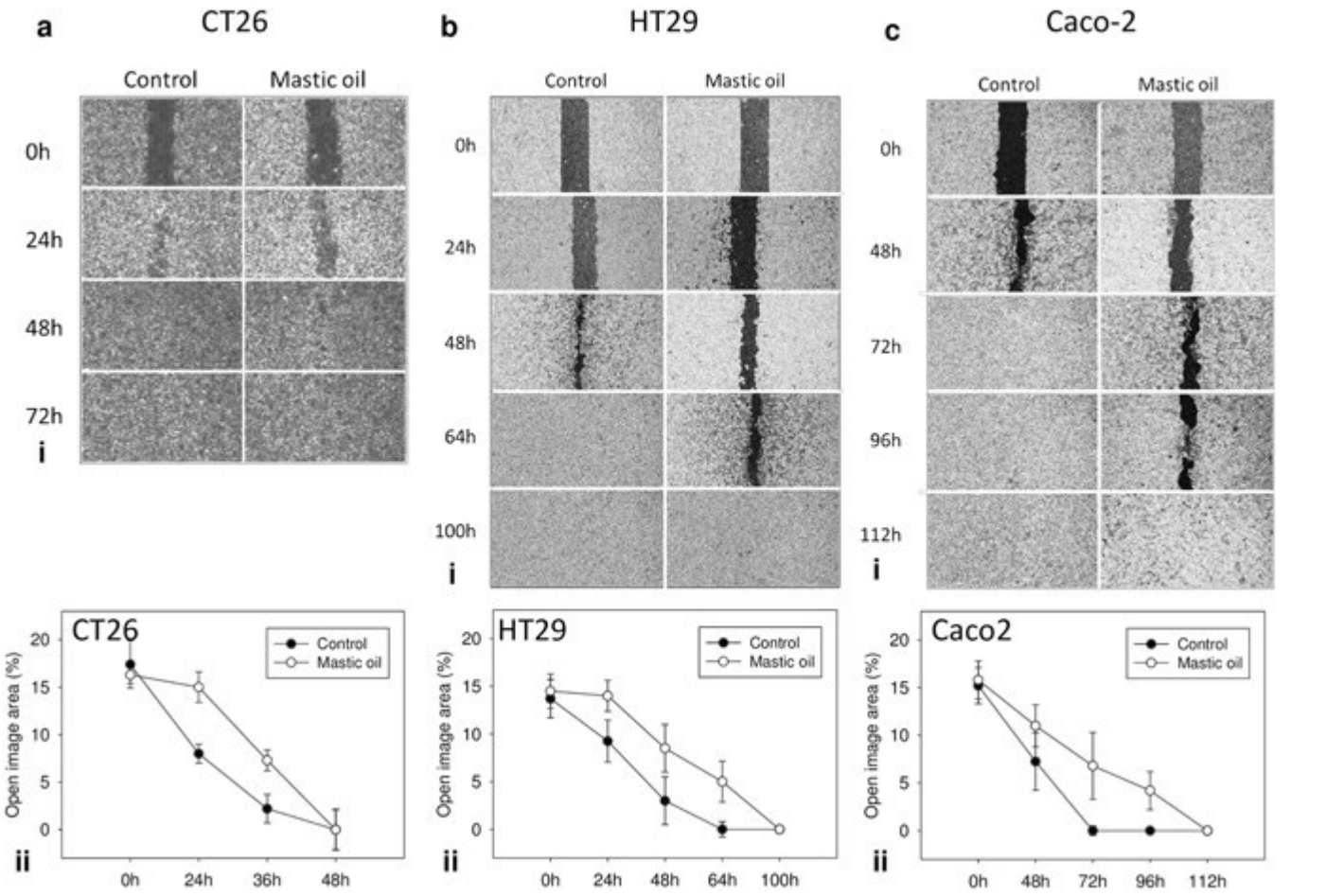
| a-pinene + myrcene 0.4204 mg/ml | | | | myrcene +a-pinene 0.0523 mg/ml | | | |
|---|---|---|---|---|---|---|---|
| **IC** | **myrc (mg/ml)** | **a-pin (mg/ml)** | **Combination index** | **IC** | **myrc (mg/ml)** | **a-pin (mg/ml)** | **Combination index** |
| **30** | 0.4204 | 6.09E-04 | **0.822** | **30** | 8.10E-04 | 0.0523 | **0.793** |
| **50** | 0.4204 | 0.011 | **0.820** | **50** | 0.0358 | 0.0523 | **0.783** |

Synergistic Caco-2 cell growth inhibition by the combination of α-pinene and myrcene. (**a**) Isobologram showing the interaction between α-pinene and myrcene in inhibiting cell growth of Caco-2 cells. Cells were treated for 72 h with α-pinene and/or myrcene and their viability was estimated with the SRB assay. Red symbols denote the $IC_{50}$ values of α-pinene (circle), myrcene (crossed circle), the combination of α-pinene's $IC_{20}$ and myrcene (triangle), and the combination of myrcene's $IC_{20}$ and α-pinene (crossed triangle). Green symbols denote the $IC_{30}$ values of α-pinene (square), myrcene (crossed square), the combination of α-pinene's $IC_{20}$ and myrcene (triangle), and the combination of myrcene's $IC_{20}$ and α-pinene (crossed triangle). Dashed lines indicate additive effects. Solid data points below the line of the same color, indicate a synergistic effect, whereas points above the line, indicate antagonism. (**b**)

$IC_{30}$ and $IC_{50}$ values of the different combinations of myrcene and α-pinene, and the estimated combination index values.

IFUS Point 1O-1d: Mastic oil attenuates migration of colon cancer in vitro (See Figure 5)

Figure 5




Open in a new tab

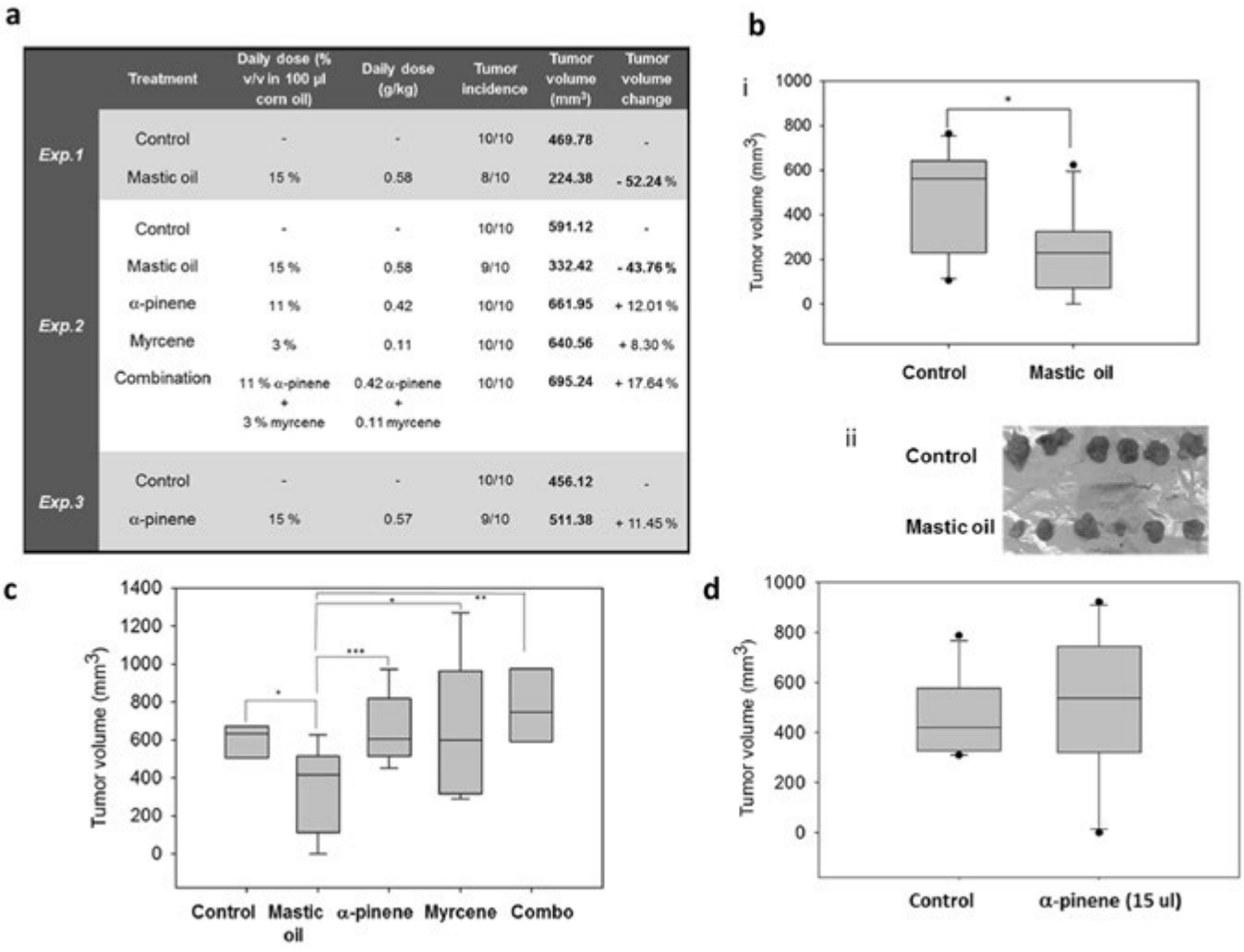
Effect of MO on migration of colon cancer cells. Wound-healing assay for (ai) CT26, (bi) HT29 and (ci) Caco-2 cells treated with MO (0.015 mg/ml for CT26, 0.02 mg/ml for HT29 and 0.004 mg/ml for Caco-2) or dimethylsulfoxide (DMSO) for control. Migration of cells was monitored with an optical microscope at the indicated time points. Quantification of the percentage of wound closure by ImageJ software analysis for (aii) CT26, (bii) HT29 and (cii) Caco-2 cells. Data are presented as the mean ± SD of three independent experiments.

IFUS Point 1O-1e: Oral administration of Mastic oil inhibits in vivo growth of colon carcinoma in mice (See Figure 6)

Figure 6.

a

| | Treatment | Daily dose (% v/v in 100 µl corn oil) | Daily dose (g/kg) | Tumor incidence | Tumor volume ($mm^3$) | Tumor volume change |
|---|---|---|---|---|---|---|
| *Exp.1* | Control | - | - | 10/10 | 469.78 | - |
| | Mastic oil | 15 % | 0.58 | 8/10 | 224.38 | - 52.24 % |
| *Exp.2* | Control | - | - | 10/10 | 591.12 | - |
| | Mastic oil | 15 % | 0.58 | 9/10 | 332.42 | - 43.76 % |
| | α-pinene | 11 % | 0.42 | 10/10 | 661.95 | + 12.01 % |
| | Myrcene | 3 % | 0.11 | 10/10 | 640.56 | + 8.30 % |
| | Combination | 11 % α-pinene + 3 % myrcene | 0.42 α-pinene + 0.11 myrcene | 10/10 | 695.24 | + 17.64 % |
| *Exp.3* | Control | - | - | 10/10 | 456.12 | - |
| | α-pinene | 15 % | 0.57 | 9/10 | 511.38 | + 11.45 % |

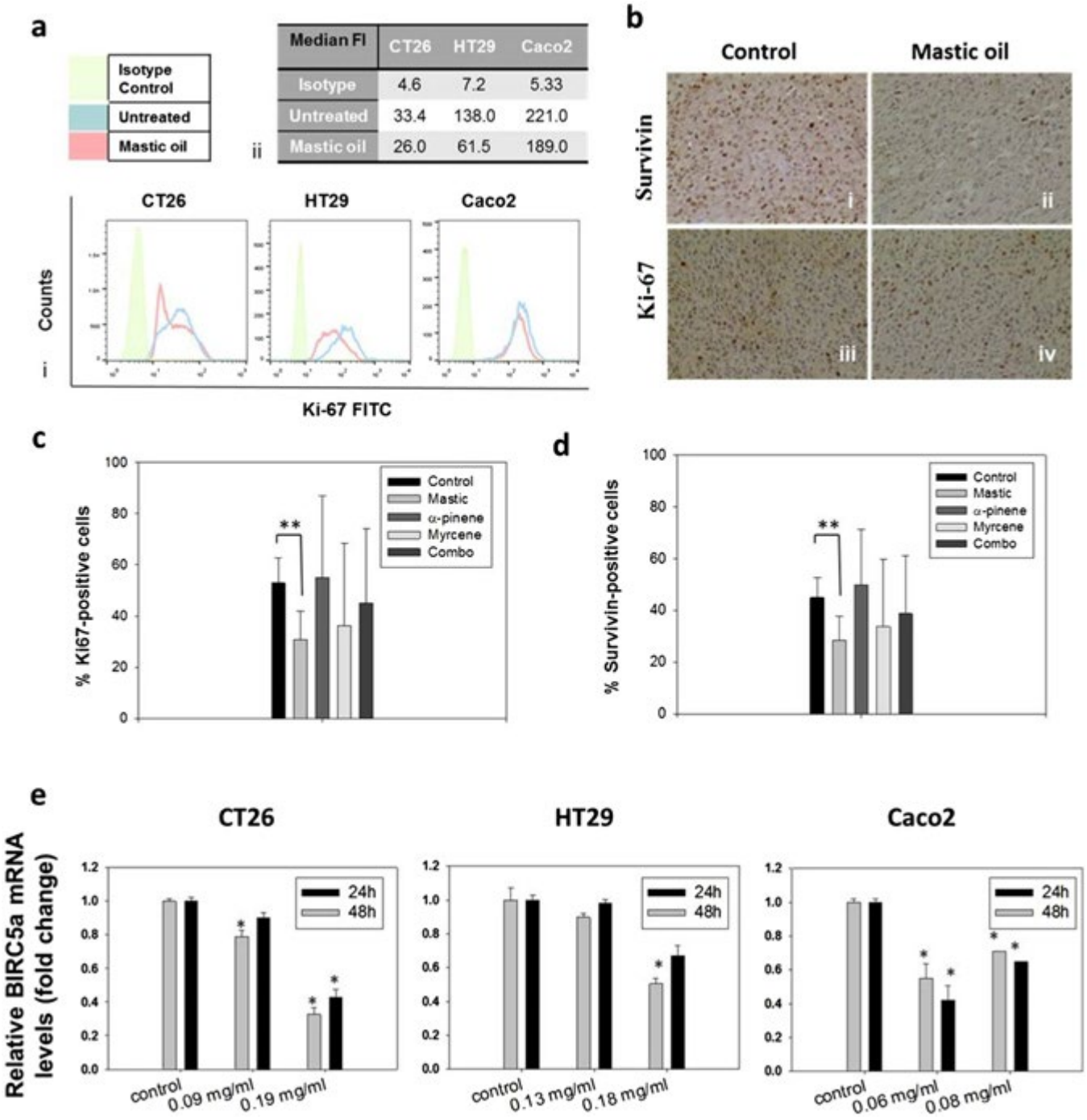



Oral administration of MO inhibits *in vivo* growth of colon carcinoma in mice. MO or α-pinene, myrcene or a combination of α-pinene and myrcene (combo) were administered *per os* daily to BALB/c mice for 13 days. On the tenth day mice were inoculated subcutaneously with CT26 cancer cells and 7 days later tumors were harvested from euthanized animals. (**a**) Presentation of results from three independent experiments (n = 10 per group) following oral administration of MO or its major monoterpenes. A statistically significant reduction of ≈43–52% in tumor volume (Exp.1: $p = 0.017$, Student's *t*-test, Exp.2: $p = 0.016$, (one-way ANOVA)) was observed

only in MO- treated mice as compared to control. (**b**) Mean tumor volume (bi) or photographic observation (bii) of tumors excised from mice that received MO or corn oil (control) (Exp.1). (**c**) Mean tumor volume of tumors excised from MO- or α-pinene (11% v/v)- or myrcene- or combo (a combination of α-pinene and myrcene)- treated mice (Exp.2) (**d**) Mean tumor volume from tumor bearing mice treated daily with a higher concentration (15% v/v) of α-pinene (Exp.3).

IFUS Point 1O-1f: Mastic oil reduces protein expression of Ki-67 and survivin (BIRC5a) in colon cancer cells (see Figure 7)

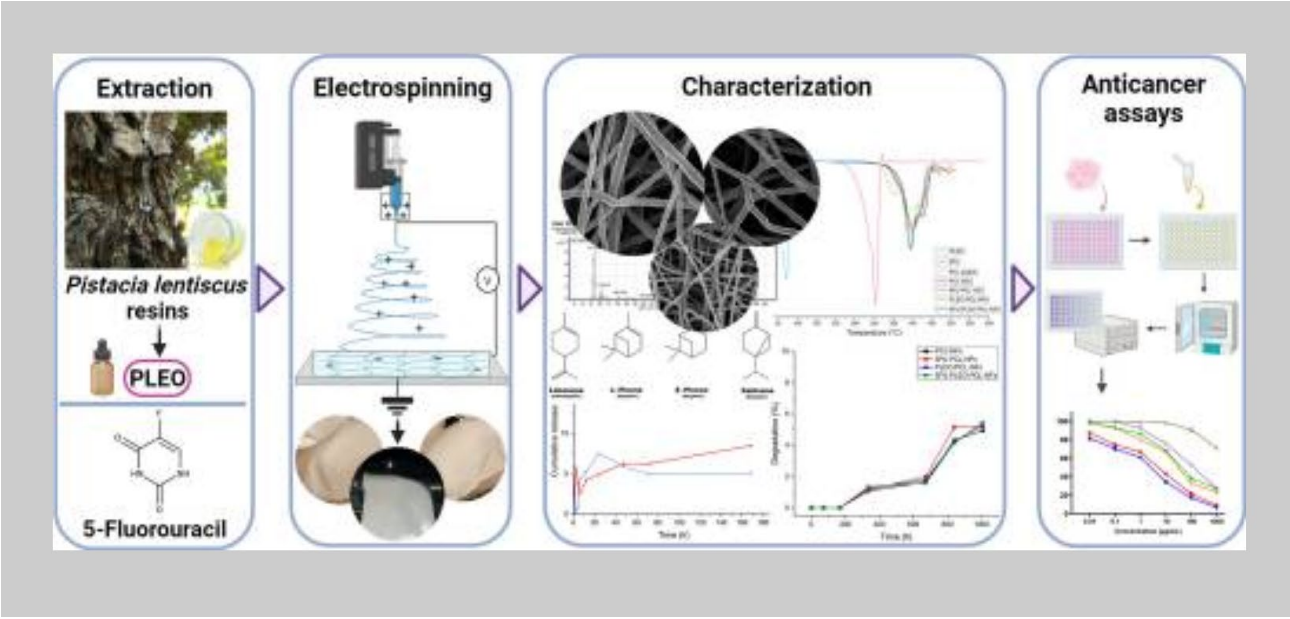
**Figure 7.**




MO inhibits protein expression of Ki-67 and protein and transcriptional expression of survivin (BIRC5a). (**a**) Flow cytometric analysis of Ki-67 protein expression in MO-treated CT26, HT29 or Caco-2 colon cancer cell lines compared to non-treated cells. Results are representative of three independent experiments. (**b**–**d**) Immunohistochemical analysis on tumors excised from mice treated *per os* with MO or its major constituents. Representative images (**b**) showing the effect of administration of MO on survivin (bi,bii) and Ki-67 (biii,biv) protein expression.

Results showing the percentage of Ki-67-positive (**c**) or survivin-positive (**d**) cells in CT26 tumors excised from BALB/c mice treated with MO or its constituents. Statistically significant differences were observed in (**c**) the number of Ki-67 positive ($p = 0.003$, Student's *t*-test) or (**d**) the number of survivin-positive ($p = 0.049$, Student's *t*-test) cells in tumor tissue from MO-treated mice as compared to control mice. Each bar represents the mean number of positive cells ± SD in tumor sections from at least three mice. (**e**) Relative gene expression (mean fold change) of *BIRC5α* in CT26, HT29 or Caco-2 cells treated with MO for 24 or 48 hours, as compared to non-treated cells. Mean fold change (±SD) is relative to control cells recovered before treatment. Endogenous expression of *ACTB* was used as internal reference. Results are representative of three independent experiments and are presented as mean values of triplicates ± SD. Asterisks indicate statistically significant differences ($p < 0.05$, Student's *t*-test).

IFUS Point 1O-2: Alabrahim OAA, Azzazy HME. Synergistic anticancer effect of Pistacia lentiscus essential oils and 5-Fluorouracil co-loaded onto biodegradable nanofibers against melanoma and breast cancer. Discov Nano. 2024 Feb 14;19(1):27. doi: 10.1186/s11671-024-03962-5. PMID: 38353827; PMCID: PMC10866856.




IFUS Point 1O-2a: "Conclusions and future perspectives: One of the most common cancer treatments is the administration of chemotherapeutics which cause severe and chronic toxicities and chemoresistance. Many natural compounds derived from various plant extracts have exerted potent anticancer activities. Particularly, EOs have been established with great potential for various preventive and therapeutic strategies amongst different tumors. Various tumorous tissues have shown great

recession after being targeted and treated with EOs of different plants. Several malignancies were targeted by EOs, such as leukemia, hepatoma, breast tumors, gastric malignancies, glioma, and pulmonary and colorectal cancers. EOs of P. lentiscus (PLEO) have demonstrated promising antimicrobial, antioxidant, anticancer, and anti-inflammatory properties. However, their poor solubility, bioavailability, and stability have limited their therapeutic beneficence."

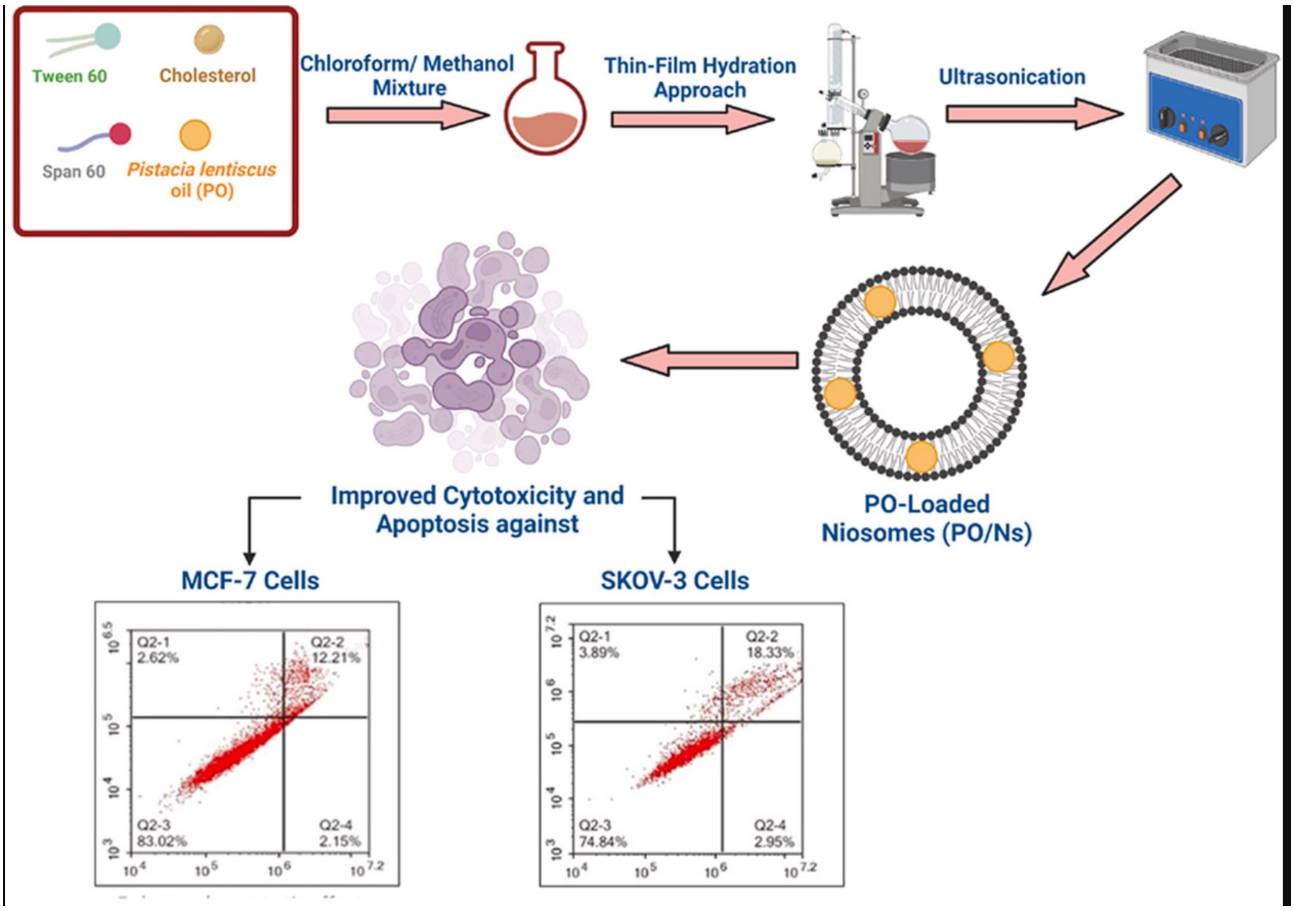
IFUS Point 1O-2a.1: Please note the last statement of the "Conclusions and future perspectives: . However, their poor solubility, bioavailability, and stability have limited their therapeutic beneficence." What if Nutri-Mastic™, which allows for mastic to become soluble and shows itself to be stable for years in IFUS' containers can provide the bioavailability lacking in this treatment option?

IFUS Point 1O-3: Ref(3): Sherif Ashraf Fahmy, Nada K. Sedky, Asmaa Ramzy, Manal M.M. Abdelhady, Obaydah Abd Alkader Alabrahim, Samir N. Shamma, Hassan Mohamed El-Said Azzazy, Green extraction of essential oils from Pistacia lentiscus resins: Encapsulation into Niosomes showed improved preferential cytotoxic and apoptotic effects against breast and ovarian cancer cells, Journal of Drug Delivery Science and Technology, Volume 87, 2023, 104820, ISSN 1773-2247, https://doi.org/10.1016/j.jddst.2023.104820. (https://www.sciencedirect.com/science/article/pii/S177322472300672X)

IFUS Point 1O-3a: "Abstract: The essential oils extracted from Pistacia lentiscus resins possess promising antiproliferative effects against ovarian and breast cancer cells because of their high content of bioactive compounds. However, Pistacia lentiscus essential oil (PO) has many drawbacks that hinder its application in cancer therapy, such as increased volatility, hydrophobicity, and limited bioavailability. In this study, niosomal formulation loaded with PO was prepared with improved stability and cytotoxicity against two cancer cell lines. In this regard, PO was extracted from the resins of Chios mastic utilizing a green approach, hydrodistillation. Its chemical composition was analyzed using gas-chromatography–mass spectrometry. PO loaded-niosomes (PO/Ns) were prepared using the thin-film hydration method. The prepared nanovesicles were spherical and had an average size of 112.38 ± 13.1 nm, poly- dispersity index of 0.13 ± 0.06, and ζ potential (ZP) of -13.09 ± 2.9 mV. PO/Ns had high entrapment efficiency (EE) of 93.4 ± 15.1% and displayed a sustained release manner of PO (86.8 ±6.1%) over 72 h. Moreover, the developed PO/Ns formulation exhibited outstanding stability in terms of size, PDI, ZP, and EE% when stored for 21 days.

SRB assay showed that the IC50 values of PO against ovarian (Skov-3) and breast (MCF-7) cancer cell lines improved from 57.04 to 69.1 µg/mL to 4.88 and 7.39 µg/mL, respectively, when encapsulated in niosomes (PO/Ns). Loading PO in niosomes (PO/Ns) increased its cytotoxicity by 10-folds against Skov-3 and MCF-7 cancer cells. On the other hand, the safety of both PO and PO/Ns was evident by a computed IC50 of >200 µg/mL against the normal breast epithelial cell line (MCF10A). PO/Ns showed enhanced apoptotic effects (combined early and late apoptosis) of 9-fold and 4-fold against Skov-3 and MCF-7 cells, respectively, as compared to unloaded PO treatment. Cell cycle analysis revealed that PO/Ns mainly targets the sub-G1 phase, where it traps the cells, providing further evidence for the induction of apoptosis in both SKOV-3 and MCF-7 cancer cells. Real-time PCR (RT-qPCR) was performed to quantify the gene expression of pro-apoptotic markers as Bak and Bax, as well as the antiapoptotic marker Bcl-2 upon exposure to PO and PO/Ns treatments. Both PO and PO/Ns demonstrated a remarkable ability to upregulate Bak and Bax, and downregulate Bcl-2. As expected, the upregulation of pro-apoptotic genes and induction of mitochondrial death was significantly higher in the case of PO/Ns than free PO treatment among the two investigated cell lines (Skov-3 and MCF-7). Essential oils extracted from Pistacia lentiscus resins and encapsulated into niosomes showed significant cytotoxic and apoptotic effects against breast and ovarian cancer cells." See Figure 1:

Figure 1




IFUS Point 1O-4: Ref(4): Giaginis C, Theocharis S. Current evidence on the anticancer potential of Chios mastic gum. Nutr Cancer. 2011 Nov;63(8):1174-84. doi: 10.1080/01635581.2011.607546. Epub 2011 Nov 1. PMID: 22044444.\

IFUS Point 1O-4a: Abstract: Chios mastic gum derived from the plant Pistacia lentiscus L. variation chia has been shown to exert beneficial effects on a wide range of human disorders. The most comprehensive data so far have indicated that mastic gum provides protection against gastrointestinal malfunctions and bacterial infections. Substantial evidence has also suggested that mastic gum exhibits hepatoprotective and cardioprotective, antiinflammatory/antioxidant, and antiatherogenic properties. In the last decade, an increasing number of studies further evaluated the potential antiproliferative properties of mastic gum against several types of human neoplasia. The present review aims to summarize the current data concerning the anticancer activities of mastic gum and their major constituents, highlighting also the molecular mechanisms through which they exert

anticancer function. Mastic gum constituents that belong to the chemical class of triterpenoids appear to be mainly responsible for its anticancer potential. Thus, a brief discussion is dedicated to the anticancer activity of synthetic and naturally occurring triterpenoid analogues with similar chemical structure to mastic gum constituents. Taking into consideration the available data so far, Chios mastic gum could be considered as a conglomeration of effective anticancer drugs.

IFUS Point 1O-5: Methyl oleanonate is a natural triterpene PPARγ agonist isolated from the species of Pistacia lentiscus var. Chia. Methyl oleanonate is a modified oleanolic acid derivative with anti-cancer effects.
https://www.medchemexpress.com/naturalproducts/pistacia-lentiscus-var-chia.html

IFUS Point 1O-5a: Methyl oleanonate has shown significant efficacy in fighting cancer.It has been found to be as effective against HeLa cancer cell lines as oleanolic acid, with an IC50 value as high as μM. Methyl oleanonate derivatives exhibit strong cytotoxic activity, particularly those with acyloxyimino functions.The mechanisms of its derivatives primarily rely on apoptosis and autophagy, indicating their potential as candidate drugs for cancer treatment. Additionally, methyl oleanonate is recognized as a modified oleanolic acid derivative with anti-cancer effects. These findings suggest that methyl oleanonate could be a promising candidate for further research in cancer therapy. (1,2)

IFUS Point 1O-5a.1: Ref.(1): Bednarczyk-Cwynar B, Ruszkowski P. Acylation of Oleanolic Acid Oximes Effectively Improves Cytotoxic Activity in In Vitro Studies. Pharmaceutics. 2024 Jan 9;16(1):86. doi: 10.3390/pharmaceutics16010086. PMID: 38258097; PMCID: PMC10819243.

IFUS Point 1O-5a.1.1: "(4) Conclusions: The introduction of different moieties, particularly the 3,5-dinitro group, resulted in the synthesis of highly potent cytotoxic agents with favorable SI and ADMETox parameters."

IFUS Point 1O-5a.2: Ref.(2) Bednarczyk-Cwynar B, Zaprutko L, Ruszkowski P, Hładoń B. Anticancer effect of A-ring or/and C-ring modified oleanolic acid derivatives on KB, MCF-7 and HeLa cell lines. Org Biomol Chem. 2012 Mar 21;10(11):2201-5. doi: 10.1039/c2ob06923g. Epub 2012 Jan 5. PMID: 22222767.

IFUS Point 1O-5a.2.1: Abstract: New A-ring or/and C-ring modified methyl oleanolate derivatives were prepared. New simple method of synthesis of 3,12-diketone (3) from methyl oleanonate (2) was worked out. The obtained new compounds were tested for cytotoxic activity on KB, MCF-7 and HeLa cell lines. The derivatives had acetoxy, oxo or hydroxyimino function at the C-3 position and in some cases oxo, hydroxyimino or acyloxyimino group at the C-12 position. Almost all of the compounds showed strong cytotoxic activity, higher than unchanged oleanolic acid. The most active substances turned out to be the derivatives with acyloxyimino function, especially 4 and 8d.

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IFUS Point 2: What are the active ingredients of Chios Mastic Gum that could provide efficacy in the improvement of Human, Animal, and Plant Health?

IFUS Point 2a: **Table 1.** Phytoconstituents of CMG. (Int J Mol Sci. 2023 Jul 27;24(15):12038. doi: 10.3390/ijms241512038) (CMG = Chios Mastic Gum)

| **Essential Oil** |
|---|
| *Monoterpene hydrocarbons* |
| α-Pinene, β-pinene, β-myrcene, tricyclene, camphene, verbenene, 2-methylanisole, *p*-cymene, limonene, *trans*-linalool oxide, α-campholene aldehyde, *trans*-pinocarveol, *trans*-verbenol, pinocamphone, pinocarvone, *p*-mentha-1,5-dien-8-ol, myrtenal, myrtenol, verbenone, β-caryophyllene, α-caryophyllene, caryophyllene oxide, 28-nor-12,17-oleanadien-3-ol, lupenone, tirucallone, tirucallol, dammaradienol, 3-methoxy-28-norolean-12-ene, β-amyrone, 28-norolean-17-en-3-ol, 28-norolean-17-en-3-one, 6-methyl-28-norolean-17-en-3-one, olean-18-en-3-one, β-amyrin, 28-nor-12,17-oleanadien-3-one, oleanenone derivative, dammarane derivative, hydroxydammarenone, oleanonic aldehyde, moronic aldehyde, 28-nor-12,18-oleanadien-3-ol, and isomasticadienolic aldehyde |
| *Oxygenated monoterpenes e Benzenoids* |
| Perillene, α-linalool, camphenol, α-campholenal, pinocarveol, *cis*-verbenol, verbenol, verbenone, bornyl acetate, campholene, camphor, 3,6,6-trimethyl norpinan-2-one, pinocarvone, *cis*-3- |

| **Essential Oil** |
| --- |
| pinanone, *cis*-carveol, 1-ethenyl-2,4-dimethylbenzene (or 1-Methyl-4-(2-propenyl)-benzene), *o*-methyl-anisole, *o*-cymene, *m*-cymene, *p*-cymene, β-methyl-cinnamaldehyde, myrtenal, *p*-cymen-8-ol, carvone, and trimethyl-hydroquinone |
| *Sesquiterpene hydrocarbons* |
| β-Caryophyllene, α-humulene, α-longipinene, α-ylangene, α-copaene, β-bourbonene, β-elemene, isocaryophyllene, α-muurolene, and D-germacrene |
| *Oxygenated sesquiterpenes* |
| Caryophyllene oxide, α-humulene epoxide, and 3,8,8-trimethyl-1,2,3,4,5,6,7,8-octahydro-2-naphthalenyl methyl acetate |
| **Triterpenes** |
| *Pentacyclic triterpenes* |
| Oleanonic acid, oleanolic acid, moronic acid, oleanonic aldehyde, oleanolic aldehyde, 28-nor-oleanone, 28-nor-oleanole, β-amyrine, β-amyrone, 28-hydroxy-β-amyrone, germanicol, lupeol, betulonal, lup-20(29)-ene-3-one, 3-oxo-28-norlup-20(29)-ene |
| *Tetracyclic triterpenes* |
| 24*Z*-Masticadienonic acid, 24*Z*-isomasticadienonic acid, 24*Z*-masticadienolic acid, 24*Z*-isomasticadienolic acid, mastichadienolal, isomastichadienolal, tirucallol, dammaradienone, mastichinoic acid, butyrospermol, dipterocarpol, and 20S-3β-acetoxy-20-hydroxydammar-24-ene |
| *Trycyclic triterpenes and bycyclic triterpenes* |
| 3β-Hydroxymalabarica-14(26),17*E*,21-triene, 3-oxomalabarica-14(26),17*E*,21-triene, (8*R*)-3β,8-dihydroxy-polypoda-13*E*,17*E*,21-triene, and (8*R*)-3-oxo-8-hydroxy-polypoda-13*E*,17*E*,21-triene. |
| **Polyphenols** |
| Tyrosol, p-hydroxy-benzoic, p-hydroxy-phenylacetic, vanillic acid, gallic acid, and *E*-cinnamic acid. |
| **Others** |
| 3-Ethylidene-1-methylcyclopentene, methyl-o-cresol, 1-dodecanol, 2,5-dimethoxytoluene, 3,5-dimethoxytoluene, (*E*)-anethole, 2-undecanone, octyl formate, 2-methyl-3-buten-2-ol, pinanediol, *trans*-linalool oxide, *cis*-linalool oxide, 6,7-dihydro-7-hydroxylinalool, 5,5-dimethyl-2(5H)-furanone, α-irone, o-methylanisol, methyleugenol, methylisoeugenol, α-fenchyl acetate, 4-acetyl-1-methylcyclohexene, and 2-undecanone |

IFUS Point 2a-1: To date, IFUS has considered the following chemical compounds from the above list:

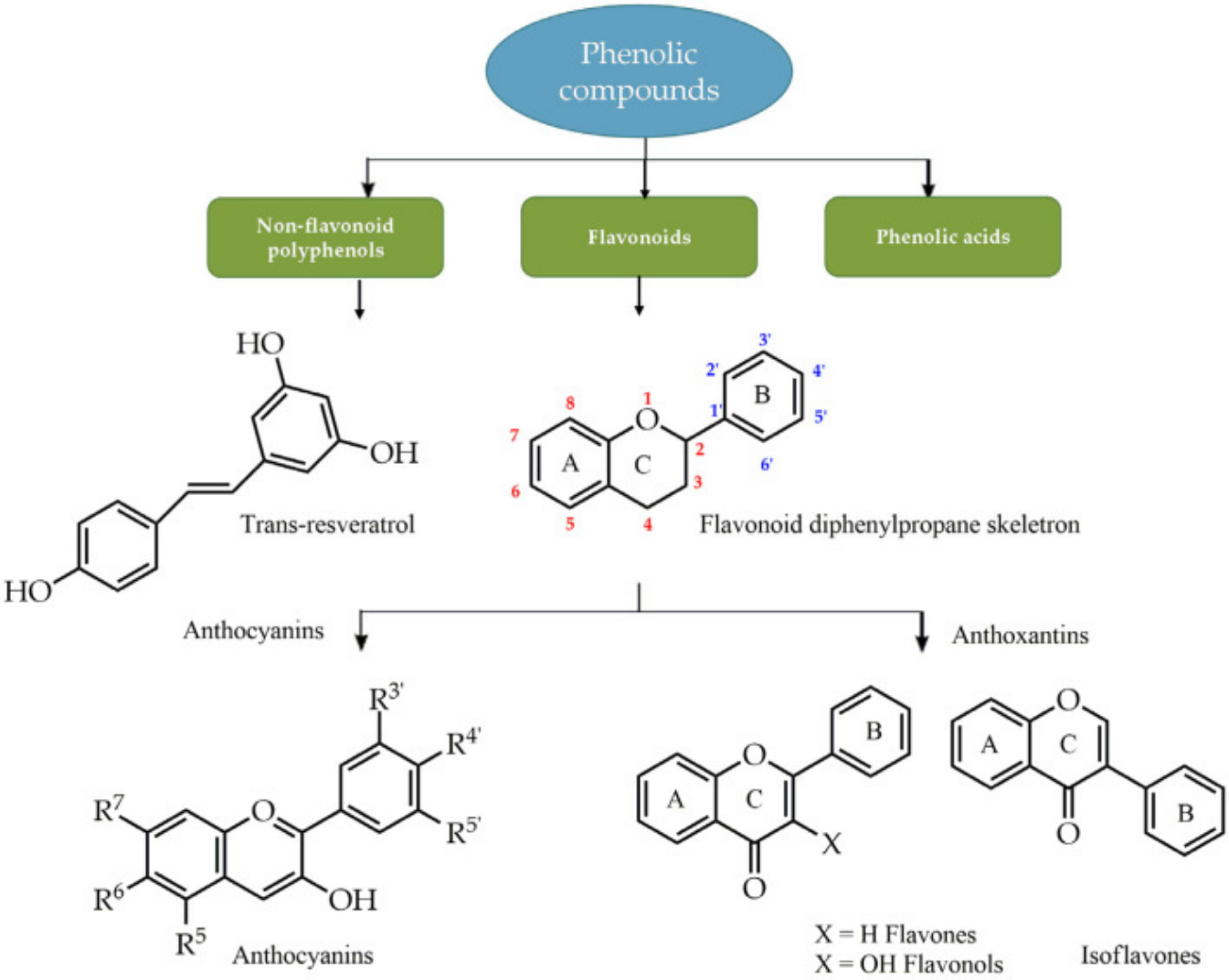
- β-caryophyllene: Fly Management
- Oleanonic acid
- p-hydroxy-benzoic
- Gallic acid (and its cousin ellagic acid, which is not on this list, but believe to be contained in some of the ingredients in IFUS' respective product lines)
- cis-1,4-poly-β-myrcene
- members of the "Triterpenes" family
- Other chemical compounds that are revealed in review of scientific studies

IFUS Point 2a-2: On the IFUS short-list of additional chemical compounds found in CMG to be studied (as these are coming up consistently in scientific studies being reviewed) include:

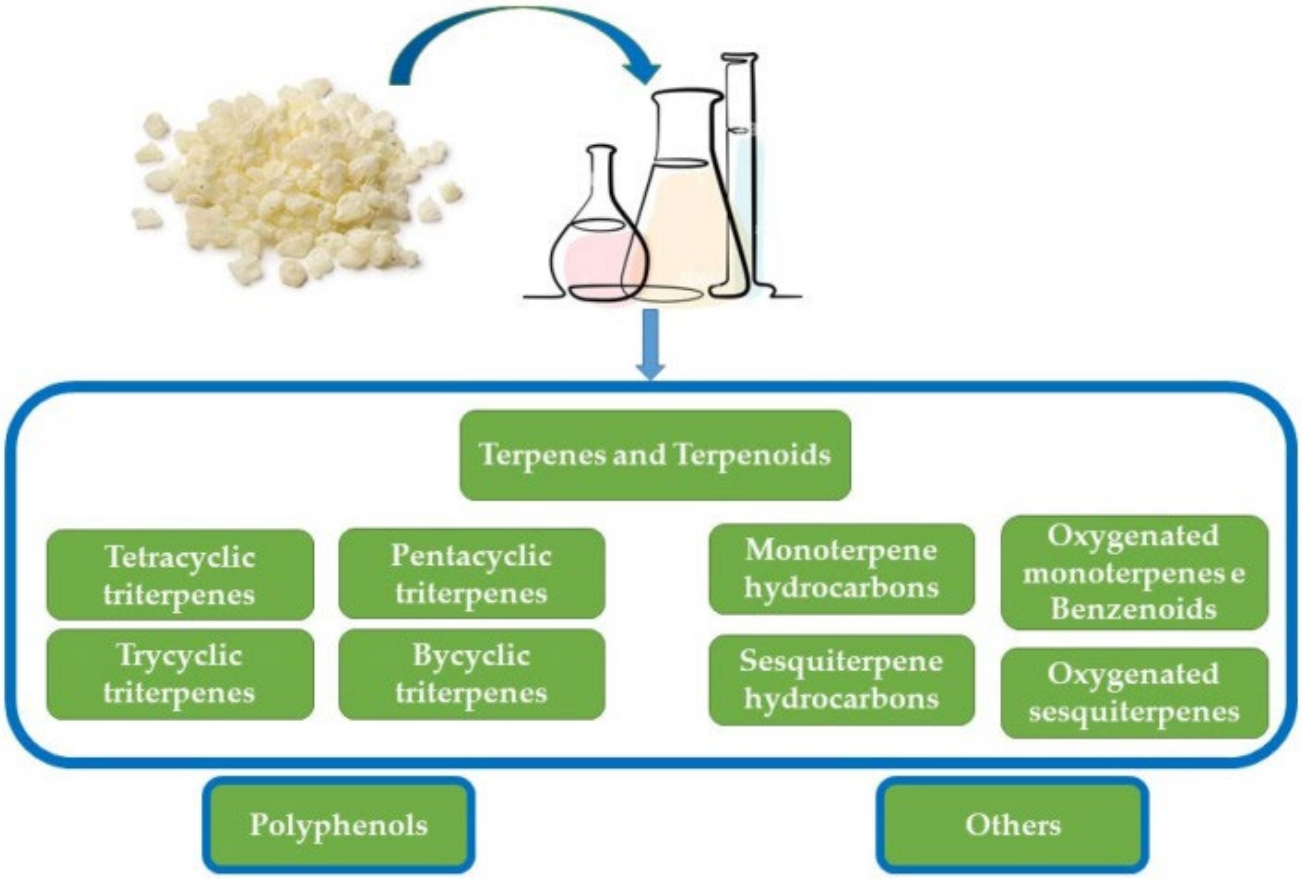
- α-Pinene and β-pinene
- vanillic acid and E-cinnamic acid
- selected members of the "Oxygenated monoterpenes e Benzenoids" family
- expanded members of the "Triterpenes" family

IFUS Point 2a-3: It is the intention of the IFUS Scientific Team to ultimately research each of the chemicals listed in "Table 1. Phytoconstituents of CMG" and to add to this list should other chemicals be found in Chios Mastic Gum.

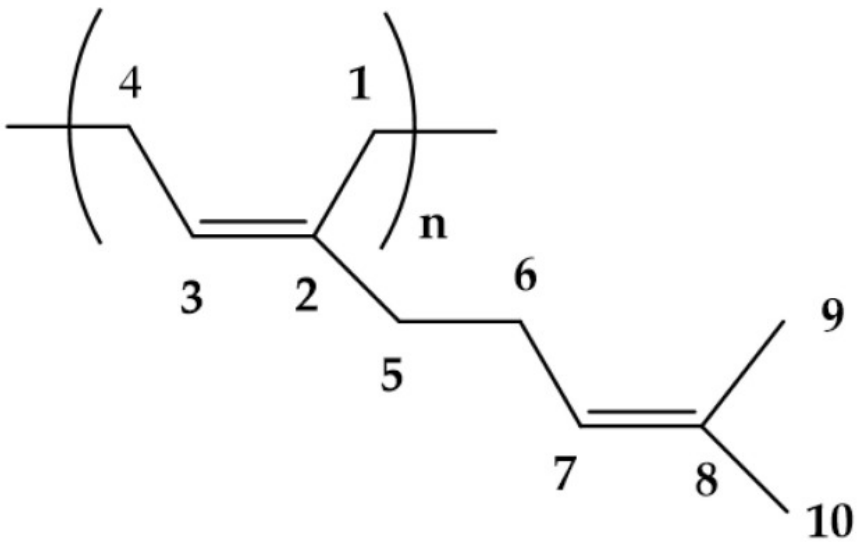
IFUS Point 2b: Figure 4: Classification of Phenolic Compounds from CMG (Int J Mol Sci. 2023 Jul 27;24(15):12038. doi: 10.3390/ijms241512038) (CMG = Chios Mastic Gum)




IFUS Point 2c: Figure 5: Majors and minor components of CMG.




IFUS Point 2d: Figure 6: The monomeric base unit of CMG's polymer: cis-1,4-poly-β-myrcene



IFUS Point 2d-1: “Reducing inflammation and proinflammatory cytokines: Poly-β-myrcene has been studied for its efficacy in reducing inflammation and proinflammatory cytokines in conditions such as colitis and pain. It has been shown to reduce key inflammatory responses and may serve as a complementary treatment for patients with inflammatory bowel disease (IBD).”

IFUS Point 2d-1a: “In conclusion, β-myrcene administration suppresses colon inflammation by inhibiting MAP kinases and NF-κB pathways.” (Almarzooqi S, Venkataraman B, Raj V, Alkuwaiti SAA, Das KM, Collin PD, Adrian TE, Subramanya SB. β-Myrcene Mitigates Colon Inflammation by Inhibiting MAP Kinase and NF-κB Signaling Pathways. Molecules. 2022 Dec 9;27(24):8744. doi: 10.3390/molecules27248744. PMID: 36557879; PMCID: PMC9782154.)

IFUS Point 2d.1b: “Anti-inflammatory & anti-nociceptive properties of β-myrcene: B-myrcene is a natural compound with a stellar safety profile which could significantly improve immune functions, as well as decrease pain sensation in patients suffering from inflammatory as well as chronic pain. Conclusions: (A) Given the known anti-inflammatory and antinociceptive effects of cannabis, it is important to discern the qualities that can provide the correct terpene synergy to manage different disorders such as ostheoarthritis, neuropathic pain or dermatitis. (2,21,22), (B) Some cannabis strain more than others contain high levels of myrcene, usually those with more "sedative" effects, which, by the recreational world, have been called "indica plants". (C) Although the mechanism of function of myrcene is not fully understood yet, it is evident its promising role in the treatment of inflammation and pain. (D) Better understanding of myrcene interactions within the cannabis plant and its clinical effect in humans may contribute to the developing of specific cannabis genetics to target at best inflammatory disorders. (https://www.fundacion-canna.es/en/anti-inflammatory-anti-nociceptive-properties-v-myrcene)

IFUS Point 2d.1b-1: Ref.(2) Russo, E. (2011) Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British Journal of Pharmacology 163: 1344-1364

IFUS Point 2d.1b-2: Ref.(21) Tavares, Ana Cristina, et al. "Essential oils from Distichoselinum tenuifolium: chemical composition, cytotoxicity, antifungal and anti-inflammatory properties." Journal of ethnopharmacology 130.3 (2010): 593-598.

IFUS Point 2d.1b-3: Ref.(22) Rufino, Ana Teresa, et al. "Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis." European journal of pharmacology 750 (2015): 141-150.

IFUS Point 2e: Hence, the information in IFUS Point 2 as well as information contained in IFUS Point 1 strongly suggests that when applied properly Chios Mastic demonstrates promising efficacy in the treatment and prevention of any number of ailments and diseases. Where the complexity of the activity and interactivity of phytochemical components of Chios Mastic Gum affecting human, animal, and plant life is daunting, the story is oft reflected in the results produced. To date, very promising results are being produced to include the impacts of the application of Chios Mastic Gum in the form of Nutri-Mastic™ taken as recommended for human and animal health and well-being as well as a key ingredient in SGP+™ (a bovine ration management formulation) and SupremeAG™ (a natural plant mulch and fertilizer system).

IFUS Point 3: Beneficial effects of Mastic on the health of the bovine should include:

- Healing of lesions and ulcerations on the surfaces of the mouth, the reticulum, the rumen, the omasum, the abomasum, the intestines, the liver, the kidney and the pancreas.
- Reduction and / or elimination of the Helicobacter pylori bacteria in the mouth, the reticulum, the rumen, the omasum, the abomasum, the intestines, the liver, the kidney and the pancreas.
- Improved reproductive performance
    - "Data reviewed shows that supplementation with different sources of lipids and fatty acids improve reproductive performance of the female ruminant. However, it is important to consider that the optimum response will be achieved when under-nutrition status of the female is not extremely severe. A nutrient balance (protein: energy) in the ration consumed by the animal is fundamental to obtain maximum benefit from supplementation with fat, since fatty acids do not supply nitrogen for amino acid synthesis and consequently for the correct functioning of the hypothalamus-hypophysis axis. Improvements in reproductive performance may be a result of increased energy density of the ration or of the direct effects of specific fatty acids on reproductive processes. As is the case for any technology or management strategy that improves specific aspects of ovarian physiology and cyclic activity, actual improvements in pregnancy rate or total weight of calf weaned are dependent on a variety of management practices and environmental conditions.
    - Until these interrelationships are better understood, livestock producers are recommended to attempt to formulate low cost/balanced rations. If a source of supplemental fat is available locally and can be incorporated with little or no change

in the cost of the ration, it would be wise for farmers to do so. Research studying the role of fat supplementation on reproductive responses has not been that consistent, therefore, adding fat to the ration would be advised when the risk of low reproductive performance (young, growing animals and limiting nutrients [protein, energy] in the basal ration) is the greatest.

  - Effect of Fatty Acids on Reproductive Performance of Ruminants, Herrera-Camacho, José1, Soberano-Martínez, Alejandra1, Orozco Durán, Karlos Edmundo2, Aguilar-Pérez, Carlos2 and Ku-Vera, Juan Carlos2, *1Instituto de Investigaciones Agropecuarias y Forestales Universidad Michoacana de San Nicolás de Hidalgo, 2Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Artificial Insemination in Farm Animals,* www.intechopen.com

- Improved lactation and estrogenic balance have also been suggested by cattle fed Mastic.

IFUS Point 4: Affect on Plants?

IFUS Point 5: What are the major active ingredients (chemical compunds) found in Chios Mastic Gum?

- Gallic Acid
- ADD

IFUS Point 5a: One of the key ingredients of Mastic is Gallic acid ($C_6H_2(OH)_3COOH$). It is:

- a trihydroxybenzoic acid
- a type of phenolic acid
- a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid.
- found both free and as part of hydrolysable tannins.

IFUS Point 5a-1: Gallic Acid is an active ingredient in mastic and is thought to have positive impacts on protein digestibility and nitrogen utilization in the rumen:

IFUS Point 5a-1a: Pyrogallol, a hepatotoxin and nephrotoxin, is a product of hydrolyzable tannins (HT) degradation by ruminal microbes.

IFUS Point 5a-1b: Proanthocyanidins (PA) (condensed tannins) are considered to be non-toxic because they are not absorbed, but they are associated with lesions of the gut mucosa.

IFUS Point 5a-1b.1: Research on tannins in forage legumes has determined their effects on protein digestion and metabolism but more research on tannin structure in relation to digestion of specific proteins is needed.

IFUS Point 5a-1b.2: The widely accepted explanation for positive effects of PA on protein digestion and metabolism is that PA-protein complexes escape ruminal degradation and the protein is available in the lower tract.

IFUS Point 5a-1b.3: This proposed mechanism may be incorrect because PA also complex carbohydrates, endogenous proteins, and microbial products and the degradability of PA-protein complexes by ruminal microbes has not been adequately studied.

IFUS Point 5a-1b.4: Several alternative hypotheses (to escape protein) that explain the effect of PA on protein digestion and metabolism in ruminants are also consistent with experimental results on forage legumes.

IFUS Point 5a-1b.5: These include increased microbial protein synthesis, increased use of endogenous nitrogen in the rumen, and increased secretion of salivary glycoproteins.

IFUS Point 5a-1b.6: Research on manipulating the content and type of PA in forage legumes is justified because they are associated with non-bloating legumes, lower soluble non-protein nitrogen in silage, and improved efficiency of protein utilization. (Jess D Reed, Nutritional Toxicology Polyphenols in Tannins and Related Forage Legumes, Volume: 73, Issue: 5, Pages: 1516-1528, Journal of Animal Science (1995)

IFUS Point 5a-1b.7: As a note, Carob is also considered to be a legume.

IFUS Point 5a-1: Gallic acid is commonly used in the pharmaceutical industry

- S. M. Fiuza. "Phenolic acid derivatives with potential anticancer properties—a structure–activity relationship study. Part 1: Methyl, propyl and octyl esters of caffeic and gallic acids". Elsevier. doi:10.1016/j.bmc.2004.04.026.

- a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay
  - Andrew Waterhouse. "Folin-Ciocalteau Micro Method for Total Phenol in Wine". UC Davis.
- a starting material in the synthesis of the psychedelic alkaloid mescaline
  - Tsao, Makepeasce (July 1951). "A New Synthesis Of Mescaline". *Journal of the American Chemical Society* **73** (11): 5495–5496. doi:10.1021/ja01155a562. ISSN 0002-7863.

UNDER DEVELOPMENT

Commonly held benefits of mastic include:

- Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil have been identified: verbenone, alpha-terpineol, and linalool. The sensitivity to these compounds was different for different bacteria tested (Escherichia coli, Staphylococcus aureus, and Bacillus subtilis), which suggests that the antibacterial efficacy of mastic oil is due to a number of its components working synergistically.
    - J Agric Food Chem. 2005 Oct 5; 53(20):7681-5. Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia., Koutsoudaki C, Krsek M, Rodger A., Department of Chemistry and Department of Biological Sciences, University of Warwick, Warwick, Coventry CV4 7AL, United Kingdom.
- Gum of Chios mastic (Pistacia lentiscus var. chia) is a natural antimicrobial agent that has found extensive use in pharmaceutical products and as a

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IFUS Point 3: Ionic Minerals

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IFUS Point 4: Intact Digest™

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IFUS Point 5: Intact Endurance

IFUS Point ???: "Using Prime Directives of the Unconscious Mind to Increase Your Client's Performance" (https://spencerinstitute.com/using-prime-directives-of-the-unconscious-mind-to-increase-your-clients-performance/)


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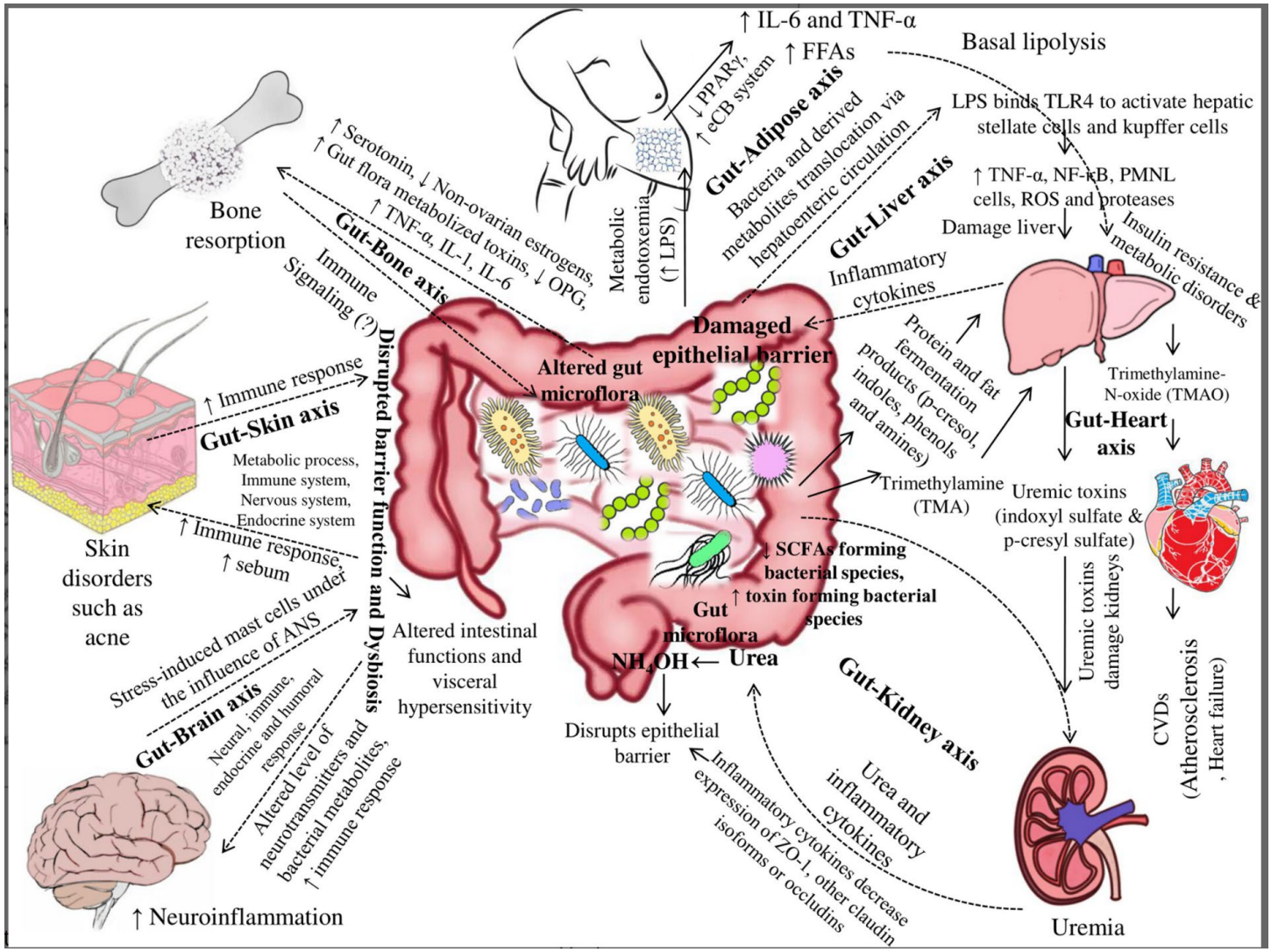
IFUS Point 6: Practical Guidance

## IFUS Point 7: Testimonials and Scientific Trials

## IFUS Point 8: Summary and Conclusions

Figure 1: Representation of a bi- or multidirectional communication link or 'axis' between gut, associated microbiota and various organs. [Colour figure can be viewed at wileyonlinelibrary.com]



Ahlawat, S., Asha, & Sharma, K. K. (2021, June 1). Gut–organ axis: a microbial outreach and networking. Letters in Applied Microbiology. John Wiley and Sons Inc. https://doi.org/10.1111/lam.13333