Part 2b: CHIOS MASTIC GUM: Plausible Scientific Evidence of the Efficacy of Nutri-Mastic™, SGP+™, & Supreme AG on or Humans, Animals, and Plants

Added Plausible Scientific Evidence of the Efficacy of SGP+™ in Bovine Herd Performance through Bovine Ration Management

(Continued from IFUS Part 1 White Paper: Lignin, Degraded and Depolymerized Lignin, and Select Synergies from Mastic, Ionic Minerals, and Carob)

(Related to IFUS Part 1a White Paper: CAROB: Plausible Scientific Evidence of the Efficacy of Nutri-Mastic™, SGP+™, & Supreme AG on or Humans, Animals, and Plants)

And

(Related to IFUS Part 3: White Paper: IONIC MINERALS: Plausible Scientific Evidence of the Efficacy of Nutri- Mastic™, SGP+™, & Supreme AG on or Humans, Animals, and Plants)

And

(Related to IFUS Part 4: White Paper: Nutri-Mastic™: Plausible Scientific Evidence of the Efficacy of Nutri- Mastic™, SGP+™, & Supreme AG on or Humans, Animals, and Plants)

NOTE:

Additional White Papers are under construction to consider IFUS' Product Lines to include Nutri-Mastic™, SGP+™, and Supreme(AG) (as well as a somewhat unrelated product produced from IFUS for eco-friendly oil absorption, clean-up, and disposal)

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CONFIDENTIAL

"How vainly mortal men do blame the gods! For of us they say comes evil, whereas they even of themselves through the blindness of their own hearts, have sorrows beyond that which is ordained."—The Odyssey

Henceforth, we begin with the notion that most of humankind's problems have been created by humans. If this be true, then these very problems can be solved by humans. This may require that we suspend what we think we know, and entertain that we don't know what we think we know, and probably don't know what we don't know.

Where “absolutism” may be our enemy, “relativism” becomes our friend. Therefore, in the spirt of inquiry we begin examining one of the ingredients in Nutri-Masic™—that being Chios Mastic Gum, In this “spirit of inquiry” we examine the active chemical nature of this natural substance as we explore each of the key active chemicals independently, interactively, and collectively for its efficacy in human, animal, and plant applications…as well as the “absolute contraindication and relative contraindication” of each chemical compound found within Chios Mastic Gum. In our search for raw truth (a daunting task at best), we further explore the role Chios Mastic Gum (and the various natural chemicals contained within it) act, interact, and relate to the formulation of Nutri-Mastic™, SGP+™, and SupremeAG™ in their application to humans/animals, bovine cattle (and possibly more), and plants, respectively.

Hence, we begin:

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

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. "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: Mastic may also have some value in preventing tooth decay and gingivitis as chewing mastic reduces oral bacteria.

IFUS Point 1I-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: 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 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 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 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** ↓ TNF-a gene expression** ↓ IL-10**	↓ oxLDL ↓ NOX-2 ↑ TAS** ↓ or ↑ Gpx**	↓ SBP ↓ ALT*, AST* ↓ GGT* ↓ pPP	↓ TG, LDL, TC ↓ BF*, VF*, Wt* ↓ Ins, FG, HOMA ↑ HDL, adipo* ↑ Microbiota div. ↓ Lp(a), Apo(B) ↓ LPC, LPE ↓ Cholic acid ↓ Hemoglobin**
Animals	↓ CRP ↓ IL-6		↓ Hepatic steatosis, NAFLD, fibrosis, ALT ↓ SBP, DBP, MAP ↓ Renin ↓ Infarct size Improved cardiac indices	↓ Glucose ↓ TG, LDL, TC ↓ Total lipids ↑ HDL ↑ Microbiota div.
In vitro, In silico	↓ Monocyte attachment ↓ Adhesion molecules ↓ NF-κB, p65 ↓ Cell migration	↓ oxLDL ↓ NRF-2 ↓ CD36 ↑ GSH		↓ PEPCK ↓ GR ↓ PPARα ↓ AMPKα 11β-HSD1 inhibition PPARγ agonist

IFUS Point 1M: 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 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-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

Essential Oil
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

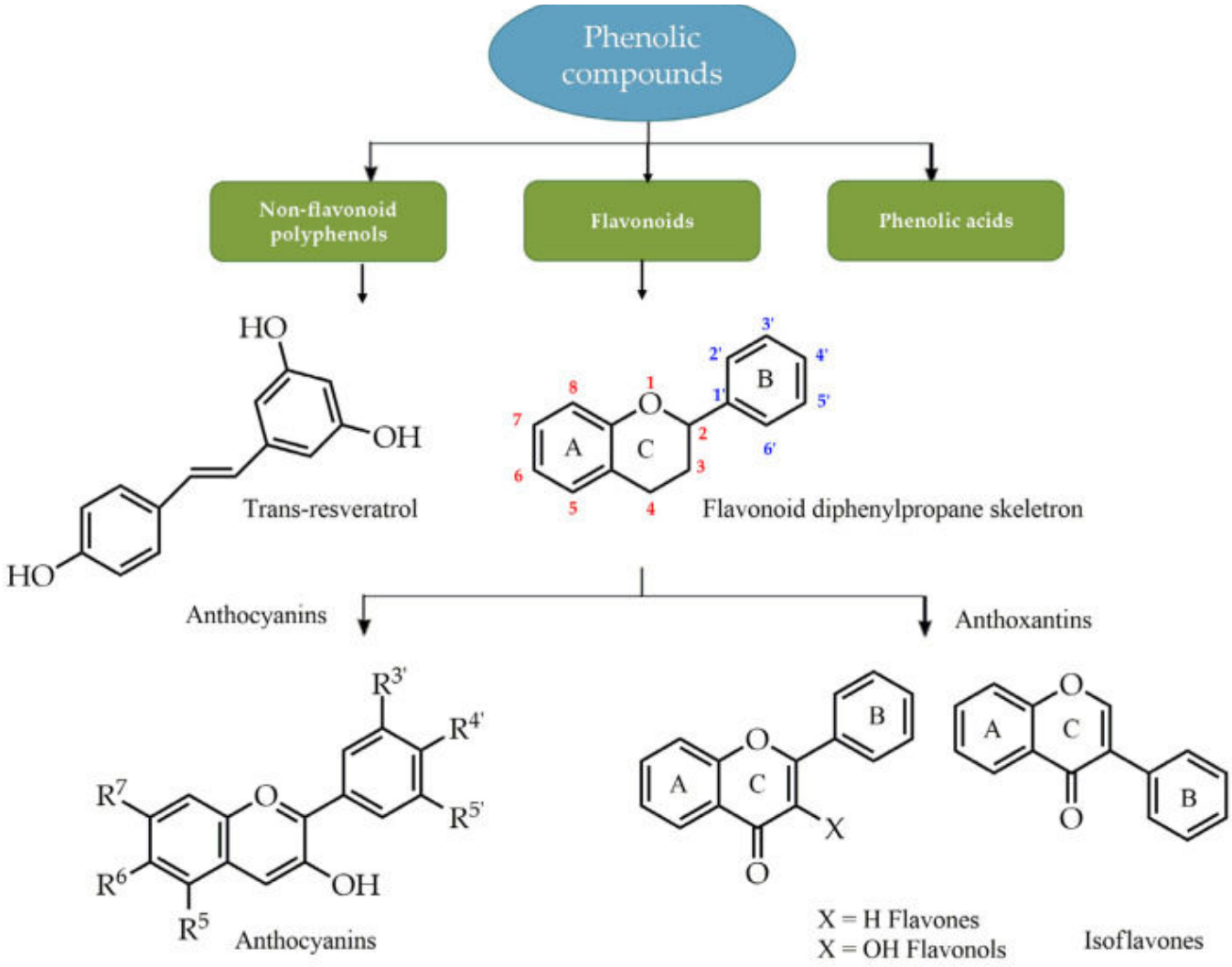
- Gallic acid (and its cousin ellagic acid, which is not on this list, but believe to be contained in some of the ingredients in IFUS' respective product lines)
- cis-1,4-poly-β-myrcene
- members of the "Triterpenes" family
- Other chemical compounds that are revealed in review of scientific studies

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:

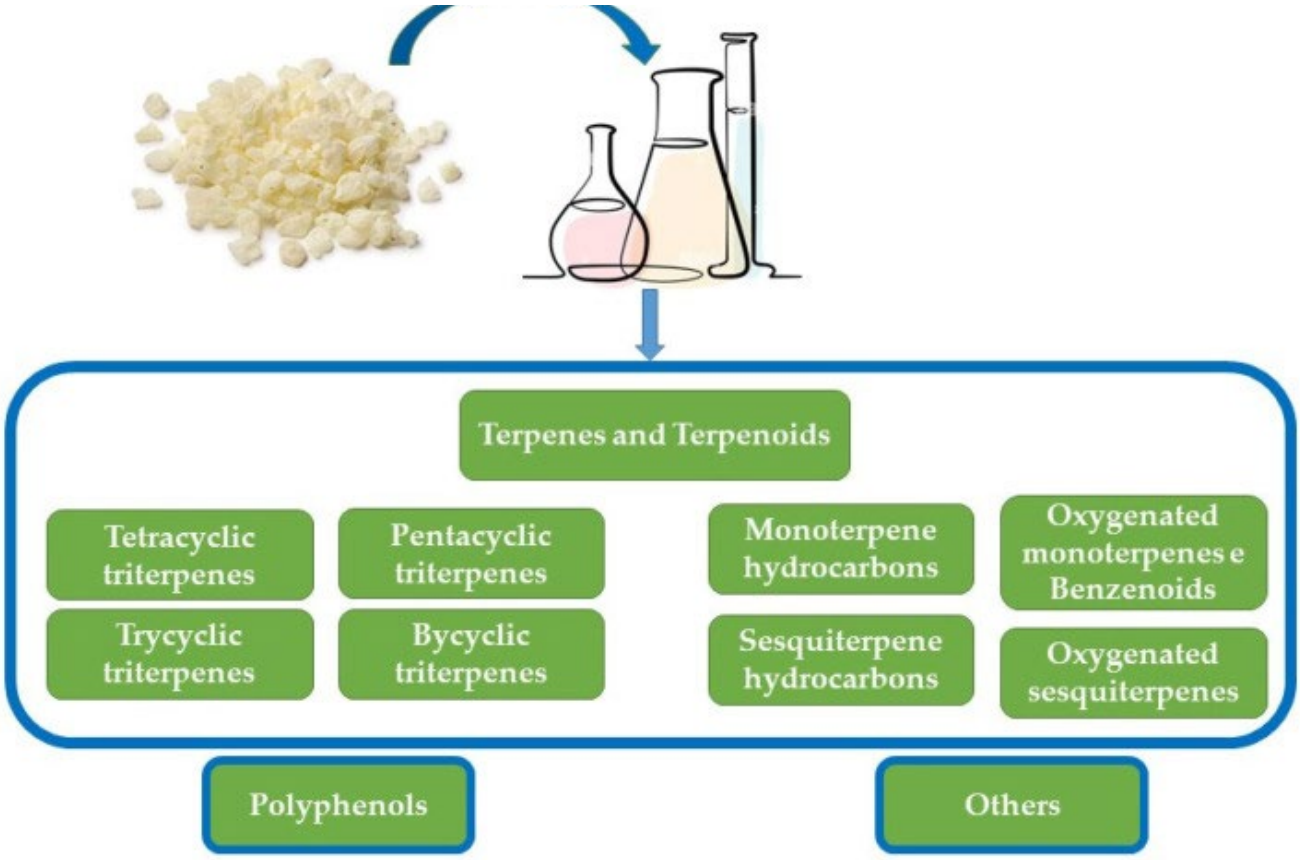
- α-Pinene and β-pinene
- vanillic acid and E-cinnamic acid
- selected members of the "Oxygenated monoterpenes e Benzenoids" family
- expanded members of the "Triterpenes" family

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.

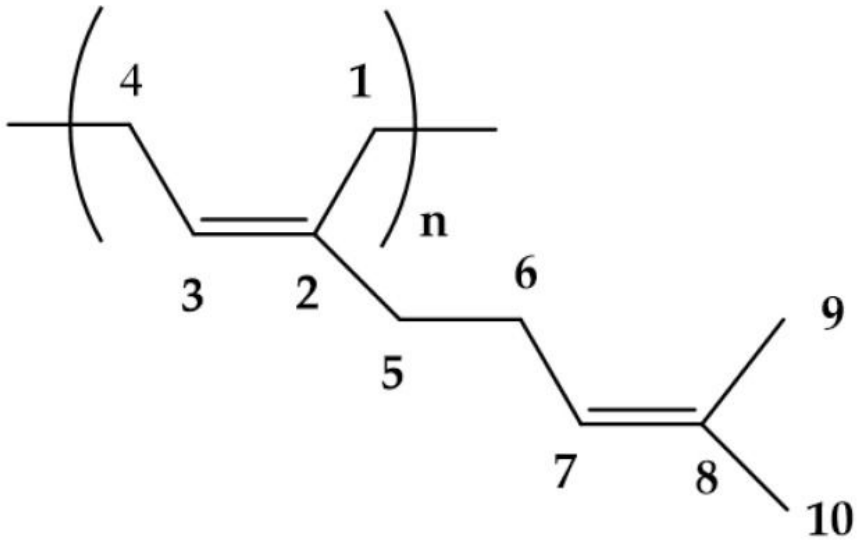
IFUS Point 2b: Figure 4: Classification of Phenolic Compounds from CMG (Int J Mol Sci. 2023 Jul 27;24(15):12038. doi: 10.3390/ijms241512038) (CMG = Chios Mastic Gum)



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 humans could include:

IFUS Point 4: 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 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.

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

6.0 The aforementioned discussion leads us to the question: Do polyphenol oxidases, Gallotannins, and Gallic Acid effect mitochondrial function?

6.1 "Currently, it is clear that part of the antioxidant properties of polyphenols might be due to their capacity to induced mitochondrial biogenesis and improve mitochondrial function, which increases the mitochondrial efficiency leading to reduction in ROS production."

6.1.1 Effects of Polyphenols on Thermogenesis and Mitochondrial Biogenesis, Tanila Wood dos Santos, et.al., Int J Mol Sci. 2018 Sep 13;19(9):2757. doi: 10.3390/ijms19092757

7.0 Gallic Acid and Mitochondria

7.1 Is there fractal modification of mitochondria by gallic acid? "Gallic acid has been shown to modify mitochondria in several ways:

7.1.1 "It acts as a mitochondria-targeting vehicle with antioxidant properties, enhancing its therapeutic potential (1) (Gallic acid-mitochondria targeting sequence-H3R9 induces mitochondria-targeted cytoprotection, Yoonhee Bae, et.al., Korean J Physiol Pharmacol. 2022 Jan 1;26(1):15–24. doi: 10.4196/kjpp.2022.26.1.15)

7.1.2 "Gallic acid and rutin have been associated with increased mitochondrial DNA and enzymatic activities, indicating its beneficial effects on mitochondrial function (2). Rutin and Gallic Acid Regulates Mitochondrial Functions via the SIRT1 Pathway in C2C12 Myotubes, Wei-Tang Chang, et.al., Antioxidants (Basel). 2021 Feb 13;10(2):286. doi: 10.3390/antiox10020286.

7.1.3 “A new mitochondriotropic antioxidant based on gallic acid has been developed, which efficiently transports gallic acid to mitochondria without disrupting their function (3). Discovery of a new mitochondria permeability transition pore (mPTP) inhibitor based on gallic acid, José Teixeira, et.al., J Enzyme Inhib Med Chem. 2018 Mar 7;33(1):567–576. doi: 10.1080/14756366.2018.1442831.

7.1.4 “Gallic acid has protective effects against mitochondrial damage, particularly in response to harmful substances like bisphenol A (4). Gallic acid protects rat liver mitochondria ex vivo from bisphenol A induced oxidative stress mediated damages, Mousumi Dutta, et.al., Toxicol Rep. 2019 Jun 17;6:578–589. doi: 10.1016/j.toxrep.2019.06.011

7.1.5 “Additionally, gallic acid has been synthesized for its mitochondrial targeting, showing preventive effects against oxidative stress (5.) Efficiency of mitochondrially targeted gallic acid in reducing brain mitochondrial oxidative damage, P Parihar, et.al., Cell Mol Biol (Noisy-le-grand). 2014 Jul 3;60(2):35-41.”

7.2 “Gallic acid affects mitochondria by regulating oxidative stress, modulating mitochondrial dysfunction, and targeting mitochondria (1,2,3,4,5). It has antioxidative activity and can mitigate neuroinflammation (2, 5).”

7.2.1 Ref 1: Neuroprotective effects of gallic acid against hypoxia/reoxygenation-induced mitochondrial dysfunctions in vitro and cerebral ischemia/reperfusion injury in vivo, Jing Sun, et.al., Brain Research, Volume 1589, 17 November 2014, Pages 126-139

7.2.2 Ref 2: Gallic Acid and Gallates in Human Health and Disease: Do Mitochondria Hold the Key to Success?, Rekha Yamini Kosuru, et.al., Mol Nutr Food Res. 2018 Jan;62(1). doi: 10.1002/mnfr.201700699. Epub 2017 Dec 19.

7.2.3 Ref 3: Rutin and Gallic Acid Regulates Mitochondrial Functions via the SIRT1 Pathway in C2C12 Myotubes, Wei-

Tang Chang, et.al., Antioxidants (Basel). 2021 Feb 13;10(2):286. doi: 10.3390/antiox10020286.

7.2.4 Ref 4: Gallic acid-mitochondria targeting sequence-H3R9 induces mitochondria-targeted cytoprotection, Yoonhee Bae, et.al., Korean J Physiol Pharmacol. 2022 Jan 1;26(1):15–24. doi: 10.4196/kjpp.2022.26.1.15

7.2.5 Ref. 5: Discovery of gallic acid-based mitochondriotropic antioxidant attenuates LPS-induced neuroinflammation, Shubham Garg, et.al., Free Radical Biology and Medicine, Volume 226, January 2025, Pages 302-329

7.3 Gallic acid, along with other mastic compounds, acts as a modulator of peroxisome proliferator-activated receptors (PPARs), which regulate glucose and lipid metabolism, inflammation, and fibrosis progression in the liver (2 & 5). (See Fig. 4 (Lower Right), Page79: "Summary of proposed intracellular catabolic pathways during lignocellulose degradation in WRF")

7.3.1 Ref. 2: "Overview of Chios Mastic Gum (Pistacia lentiscus) Effects on Human Health," Stergios Soulaidopoulos, et.al., rients. 2022 Jan 28;14(3):590. doi: 10.3390/nu14030590

7.3.2 Ref. 5: "Beneficial health effects of Chios Gum Mastic and peroxisome proliferator-activated receptors: indications of common mechanisms," Ioannis Georgiadis, et.al., J Med Food, 2015 Jan;18(1):1-10. doi: 10.1089/jmf.2014.0021.

7.3.2.1 "For thousands of years, Chios Gum Mastic (CGM), the resin produced by the trunk of Pistachia lentiscus var Chia, has been used for culinary and medicinal purposes and several therapeutic properties have been attributed to it. CGM has been used in traditional medicine of various nations in the eastern Mediterranean area. This survey was carried out to identify biological mechanisms that could explain traditional usage and recent pharmacological findings. We reviewed the related scientific literature available from the NCBI PUBMED database on CGM studies and on natural products showing peroxisome

proliferator-activated receptor (PPAR) agonist effects. We investigated whether CGM qualifies as a PPAR modulator. A large number of studies demonstrate that CGM has antioxidant, anti-inflammatory, hypolipidemic, and anticancer properties. Recently, the first evidence of CGM antidiabetic effect became known. CGM chemical composition has been extensively analyzed and the presence of several compounds, especially triterpenoids is well documented. Some of them, oleanonic acid, oleanolic acid, and gallic acid are considered to act as PPAR modulators. PPARs are nuclear receptors functioning as transcription factors and thereby controlling cellular functions at the level of gene expression. PPARs are involved in the pathways of significant diseases, such as metabolic syndrome, diabetes mellitus, dyslipidemia, inflammation, atheromatosis, and neoplasias, constituting a key target for pharmacological interventions. This article proposes that the synergistic action of some constituents of CGM on PPARs and more precisely on both PPARs isotypes-α and -γ, may be one of the major biological mechanisms via which CGM exerts its multiple effects."

8.0 Does this seemingly synergistic science, when overlayed, present evidence of the efficacy of SGP+™ on Bovine Herd Performance?

8.1 "In this study, we showed that gallic acid was active against both Mannheimia haemolytica and Pasteurella multocida, two key BRD associated-pathogens, with the minimum inhibitory concentration (MIC) measured at 250 and 500 μg/mL, respectively." "Gallic Acid Potentiates the Antimicrobial Activity of Tulathromycin Against Two Key Bovine Respiratory Disease (BRD) Causing-Pathogens," Karthic Rajamanickam, et.al., Front Pharmacol. 2019 Jan 4;9:1486. doi: 10.3389/fphar.2018.01486.

8.2 "Bovine mastitis caused by *Staphylococcus aureus* may exacerbate by resulting in significant economic losses and impacting milk quality. To date, the use of gallic acid, a phenolic compound naturally occurring in various plants, holds promise due to its potent

anti-oxidant and anti-inflammatory effects in many pieces of literature, thus, making it a subject of interest in bovine innate immunity research. Here we used gallic acid to assess its potential immunomodulation on milk phagocytes *in vitro* challenges with mastitis-causing bacteria. Our findings indicated that cells exposed to gallic acid showed no harm to cell viability but might maintain the longevity of cells during the bacterial infection. Gallic acid-treated cells displayed reduced cell migration, phagocytosis, and bacterial killing ability, while showing an increase in ROS production, all of which are undoubtedly linked to the intracellular killing abilities of the cells. Nonetheless, the extracellular structure called neutrophil extracellular traps (NETs) was significantly released after receiving gallic acid, representing extracellular killing. We also reported that gallic acid neutralizes inflammation by regulating specific pro-inflammatory genes (*IL1B*, *IL6*, *TNF*) and ROS-generating genes (*CYBA*, *LAMP1*, *RAC1*), subsequently preventing tissue damage. Regarding apoptosis-related genes and proteins, the increased production of caspase-3 and Bcl-2 family proteins could potentially promote the longevity of cells, implicated in the mechanism of combating bacterial invasion during udder inflammation and infection. The novel role of gallic acid on milk phagocytes highlights its potential immunomodulatory properties and contributes to our understanding of its effects on bacterial-host interactions, and provides valuable molecular insights." Gallic acid as a key substance to inhibit proliferation and adipogenesis in bovine subcutaneous adipocyte, Qing Jin, Anim Biotechnol. 2022 Aug;33(4):657-663. doi: 10.1080/10495398.2020.1822370. Epub 2020 Sep 18.

8.3 "Gallic acid (GA) has inhibitive effects on bovine subcutaneous adipocyte proliferation and adipogenesis. It activates the metabolic master factor AMP-activated protein kinase alpha (AMPKα) to promote programmed cell death and lipolysis. GA also reduces triglyceride levels and induces autophagy in bovine subcutaneous adipocytes." Gallic acid as a key substance to inhibit proliferation and adipogenesis in bovine subcutaneous adipocyte, Qing Jin, Anim Biotechnol. 2022 Aug;33(4):657-663. doi: 10.1080/10495398.2020.1822370. Epub 2020 Sep 18.

8.4 Gallic acid: a dietary metabolite's therapeutic potential in the management of atherosclerotic cardiovascular disease, Front. Pharmacol., Xiao-Lan Zhao, et.al., 06 January 2025, Cardiovascular

and Smooth Muscle Pharmacology, Volume 15 – 2024, https://doi.org/10.3389/fphar.2024.1515172

8.5 Recently, we reported that GA exerts protective effects against inflammation. To test our hypothesis that the anti-inflammatory effect of GA partially contributes to the improvement of metabolic diseases, we examined the effect of GA on inflammation caused by adipocyte-macrophage crosstalk in obesity. We showed that GA enhanced adipocyte differentiation in 3 T3-L1 adipocytes. Consistent with the enhancement of adipogenesis, GA decreased the gene expression of monocyte chemoattractant protein-1 and increased that of adiponectin and the upstream mediator peroxisome proliferator-activated receptor gamma. GA also reduced inflammatory mediator expression induced by the co-culture of 3 T3-L1 adipocytes with RAW 264 macrophages.

8.5.1 Gallic acid regulates adipocyte hypertrophy and suppresses inflammatory gene expression induced by the paracrine interaction between adipocytes and macrophages in vitro and in vivo, Miori Tanaka, Nutr Res. 2020 Jan:73:58-66. doi: 10.1016/j.nutres.2019.09.007. Epub 2019 Oct 24.

8.5.2 Gallic acid has been studied for its effects on liver function in bovines. Some studies suggest that it may reduce liver steatosis, hepatic lipogenesis, and serum lipid levels. However, more research is needed to confirm its effectiveness and mechanisms of action."

8.5.2.1 Oral gallic acid improve liver steatosis and metabolism modulating hepatic lipogenic markers in obese mice, Jaciara Neves Sousa, et.al., Experimental Gerontology, Volume 134, June 2020, 110881

8.5.3 Gallic acid reduces hepatic inflammation by modulating NF-κB pathway. Gallic acid boosts hepatic antioxidant enzymes in HFD-fed diabetic mice. Gallic acid inhibits NF-κB pathway, lowering lipid accumulation in steatohepatitis.

8.5.3.1 Gallic acid ameliorates diabetic steatohepatitis in db/db mice fed with a high-fat diet, Chi-Chih Wang,

et.al., Journal of Agriculture and Food Research, Volume 19, March 2025, 101553

8.5.4 In conclusion, GA counteracted the progression of hepatic fibrosis through reduction of HSCs proliferation/activation mutually with their apoptosis induction.

8.5.4.1 Antifibrotic effects of gallic acid on hepatic stellate cells: In vitro and in vivo mechanistic study, Naglaa M El-Lakkany, et.al., J Tradit Complement Med. 2018 Apr 27;9(1):45–53. doi: 10.1016/j.jtcme.2018.01.010

8.5.5 "Diseases of the liver produce clinical signs of depression, anorexia, icterus, and sometimes photosensitization. Chronic liver disease is often accompanied by weight loss. Abdominal ultrasonography enables documentation of liver enlargement (more common in acute disease) or atrophy (more common in chronic disease), and it provides guidance for liver biopsy as a diagnostic and prognostic test. Depending on the specific cause, treatment often includes the administration of antimicrobials (if an infectious process is suspected), anti-inflammatories, and intravenous fluids with glucose, as well as sedation if neurologic signs are present from hepatic encephalopathy."

8.5.5.1 Overview of Hepatic Disease in Large Animals, Jonathan H. Foreman, DVM, DACVIM, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Reviewed/Revised May 2023 | Modified Sept 2024

9.0 In yet another study (van Vliet, Stephan & Provenza, Frederick & Kronberg, Scott. (2021). "Health-Promoting Phytonutrients Are Higher in Grass-Fed Meat and Milk," Frontiers in Sustainable Food Systems. 4. 555426. 10.3389/fsufs.2020.555426.), "Emerging data indicate that when livestock are eating a diverse array of plants on pasture, additional health-promoting phytonutrients—terpenoids, phenols, carotenoids, and anti-oxidants—become concentrated in their meat and milk. Several phytochemicals found in grass-fed meat and milk are in quantities comparable to those found in plant foods known to have anti-inflammatory,

anti-carcinogenic, and cardioprotective effects. As meat and milk are often not considered as sources of phytochemicals, their presence has remained largely underappreciated in discussions of nutritional differences between feedlot-fed (grain-fed) and pasture-finished (grass-fed) meat and dairy, which have predominantly centered around the ω-3 fatty acids and conjugated linoleic acid. Grazing livestock on plant-species diverse pastures concentrates a wider variety and higher amounts of phytochemicals in meat and milk compared to grazing monoculture pastures, while phytochemicals are further reduced or absent in meat and milk of grain-fed animals. The co-evolution of plants and herbivores has led to plants/crops being more productive when grazed in accordance with agroecological principles. The increased phytochemical richness of productive vegetation has potential to improve the health of animals and upscale these nutrients to also benefit human health. Several studies have found increased anti-oxidant activity in meat and milk of grass-fed vs. grain-fed animals."

10.0 POLYPHENOLS: Phenolic Acids: Hydroxybenzoic acid

10.1 In the study be Gasaly, Naschla & Gotteland, Martin in (2022), "Interference of dietary polyphenols with potentially toxic amino acid metabolites derived from the colonic microbiota," Amino Acids. 54. 3. 10.1007/s00726-021-03034-3, itr was demonstrated that "Domesticated ruminants do not express proline-rich proteins in their saliva (Austin et al. 1989) and it has been proposed that tannins could bind dietary plant proteins in the rumen (pH 5.5–7.0), preventing their microbial degrada tion by the commensal microbiota. In the abomasum where pH is lower (2.5–3.5), the non-covalent linkages between proteins and tannins are broken, releasing free proteins that can be absorbed in the distal small intestine (Bunglavan et al. 2013). Therefore tannin-bound proteins should be more resistant to bacterial degradation in the rumen, and tannin supplementation would improve protein utilization and nitrogen balance and decreases the emission of gas and other amino acid-derived microbial metabolites in ruminants (Jayanegara et al. 2012; Min et al. 2015). Though the pres ence of bacterial populations capable of

degrading in vitro the tannin/protein complexes has been reported in wild and domesticated ruminants (Goel et al. 2005), their physiologi cal importance in situ remains unclear."

10.2 The study continues with, "The effect of tannin supplementation was also evaluated in ruminants. As described above, in these animals, dietary proteins are rapidly degraded by the rumen microbiota to peptides and amino acids that are used by these microorganisms as nitrogen and energy source. Accordingly, pro tein bioavailability in the small intestine decreases, while the production of ammonia and other metabolites such as indole and skatole is enhanced, deteriorating milk and meat quality, and increasing manure emissions. The daily administration of quebracho tannins (8.93% in food, 40.3 g of tannic acid equivalent per kg DM.) in male lambs for 60d did not affect indole levels in the rumen and fat tissue but decreased skatole concentrations and improved meat odor (Priolo et al. 2009)

10.3 The dietary supplementation (0, 4.5, 9, and 18 g/kg) of lactating Holstein dairy cows with a mixture of red quebracho (2/3) and chestnut (1/3) tannin extracts contain ing 79% tannins resulted in a decrease in ammonia emission by 16 and 8% in the animals fed a diet with low (155 g/kg) or high (168 g/kg) level of protein, respectively, compared with the controls (Powell et al. 2011). Fecal urease activity was attenuated in the supplemented animals and direct spread ing of tannins on barn floors also lowered NH3 emissions. Similar results were reported after the direct application of different types of tannins to ready-to-spread farm-made manure slurry from cattle, which reduced pH and ammonia emissions by more than 95% (Sepperer et al. 2020).

10.4 Tannin impact has also been evaluated in vitro. The addi tion of PACs from the forage legume, Dorycnium rectum, to rumen inocula reduced by 75% the formation of skatole and indole, and specifically inhibited by 85% the transforma tion of indoleacetic acid to skatole (Tavendale et al. 2005). Similar diminutions of skatole, indole, and ammonia were reported by Schreurs et al. in in vitro rumen fermentation of fresh white clovers (which does not contain PACs) alone or with growing concentrations of the PAC-containing for age Lotus pedunculatus, or PACs extracted from this forage (Schreurs et al. 2007). Such decrease was inhibited in pres ence of polyethylene glycol, a compound that binds PACs with high affinity, preventing them to bind proteins. The addition of tryptophan to the incubation medium increased indole and skatole

production, indicating that the bacteria implicated in their formation were not affected by the PACs.

11.0 The Topic: Phenolic Acids: Hydroxybenzoic acids: 4-Hydroxybenzoic acid contained and/or produced by Carob, Chios Mastic Gum, and Depolymerized Lignin from Sugarcane Bagasse. (Of Note: 4-Hydroxybenzoic acid is isomeric with 2-hydroxybenzoic acid, known as salicylic acid, a precursor to aspirin, and with 3-hydroxybenzoic acid.)

11.1 This is but one more ingredient, which when considered in the context of the biochemical processes of the metabolic pathways associated with mammalian physiology, presents to us another group of plausible explanations as to the efficacy of Nutri-Mastic™, SGP+™, and SupremeAG™.

11.1.1 Like all things, too much of anything is a problem. Conversely, not enough to that thing is also a problem. Hence, striking a balance so as to create and maintain a level of healthy stasis in a multi-dimensional dynamic environment is both the challenge and the task at hand. (Remember the Map of Metabolic Pathways in IFUS Part 1 White Paper.) Such is the case with 4-Hydroxybenzoic acid.

11.1.2 Hence, we consider another thread seemingly (1) impacting gut microbiota, (2) their respective individual and collective functions, (3) the health benefits (if these functions are in statis) and (4) health consequences (if these functions are not).

11.1.3 As a reminder, it is the Health and Wellness of the person, animal, and/or the plant that is the absolute Performance Measure. All other things are simply indicators and a guidance system to provides data, information, knowledge, and/or actual intelligence (NOT AI; i.e., Artificial Ignorance).

11.1.4

11.2 All the ingredients in both Nutri-Mastic™, SGP+™, and SupremeAG™ contain 4-Hydroxybenzoic acid:

11.2.1 "4-Hydroxybenzoic acid can be found naturally in coconut.[3] It is one of the main catechins metabolites found in humans after consumption of green tea infusions.[4] It is also found in wine,[5] in vanilla, in Macrotyloma uniflorum (horse gram), carob [6] and in Phyllanthus acidus (Otaheite gooseberry)."

11.2.1.1 Ref.(6) Components of Carob Fruit: Linking the Chemical and Biological Space". International Journal of Molecular Sciences. 17 (11): 1875. doi:10.3390/ijms17111875. PMC 5133875. PMID 27834921.

11.2.2 Pistacia lentiscus (Chios Mastic Gum) contains 4-Hydroxybenzoic acid (1, 2, 5)

11.2.2.1 Ref.(1) Aghiles Karim Aissat, Nassima Chaher-Bazizi, Tristan Richard, Dina Kilani-Atmani, Eric Pedrot, Elodie Renouf, Djebbar Atmani, Josep Valls Fonayet, Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in Pistacia lentiscus L. fruits during ripening, Journal of Food Composition and Analysis, Volume 106,2022,104286, ISSN 0889-1575, https://doi.org/10.1016/j.jfca.2021.104286. (https://www.sciencedirect.com/science/article/pii/S0889157521004865)

11.2.2.2 Ref. (2) Ouahabi, S.; Loukili, E.H.; Elbouzidi, A.; Taibi, M.; Bouslamti, M.; Nafidi, H.-A.; Salamatullah, A.M.; Saidi, N.; Bellaouchi, R.; Addi, M.; et al. Pharmacological Properties of Chemically Characterized Extracts from Mastic Tree: In Vitro and In Silico Assays. Life 2023, 13, 1393. https://doi.org/10.3390/life13061393

11.2.2.3 Ref. (5) Phenolic Compounds Characterization from Pistacia lentiscus (lentisc) Fruit , Hajer Trabelsi et al, J. Chem. Pharm. Res., 2016, 8(8):1-8, ISSN : 0975-7384, CODEN(USA) : JCPRC5

11.2.3 Depolymerized lignin from sugarcane bagasse can produce 4-Hydroxybenzoic acid (1, 3)

11.2.3.1 Ref.(1) Saleh Al Arni, Extraction and isolation methods for lignin separation from sugarcane bagasse: A review, Industrial Crops and Products, Volume 115, 2018, Pages 330-339, ISSN 0926-6690, https://doi.org/10.1016/j.indcrop.2018.02.012.

11.2.3.2 Ref.(3) A Review: Depolymerization of Lignin to Generate High-Value Bio-Products: Opportunities, Challenges, and Prospects, Ningning Zhou, et.al., Front. Energy Res., 10 January 2022, Sec. Bioenergy and Biofuels, Volume 9 - 2021 | https://doi.org/10.3389/fenrg.2021.758744

11.3 The Good: 4-Hydroxybenzoic acid (4-HBA and also known as p-hydroxybenzoic acid (PHBA)) has several beneficial health effects, including anticancer, neuroprotective, and cardioprotective effects. It is also a precursor of coenzyme Q10 (CoQ10). Sources of 4-HBA include coconut, green tea, olive products, berries, wine, coriander, almonds, and other plants, with production also occurring through bacterial strains in the microbiome (1, 2)

11.3.1 Ref.(1) https://www.rupahealth.com/biomarkers/4-hydroxybenzoic-acid

11.3.2 Ref. (2) Chaudhary, Jasmine, et.al., 2013/09/01, A Comprehensive Review on Biological activities of p-hydroxy benzoic acid and its derivatives, Vol. 2, International Journal of Pharmaceutical Sciences Review and Research

11.4 The Good 1: For Humans, Nutri-Mastic™ (specifically the Mastic) contains 4-Hydroxybenzoic Acid. In a study conducted by Herebian D, Seibt A, Smits SHJ, Rodenburg RJ, Mayatepek E, Distelmaier F. 4-Hydroxybenzoic acid restores CoQ10 biosynthesis in human COQ2 deficiency. Ann Clin Transl Neurol. 2017 Oct 17;4(12):902-908. doi: 10.1002/acn3.486. PMID: 29296619; PMCID: PMC5740244.

11.4.1 "The clinical phenotypes of human CoQ10-deficiency caused by COQ2 mutations range from fatal neonatal disease to adult-onset multisystem atrophy. So far, treatment options for these diseases are unsatisfactory. Here, we demonstrate that supplementation of 4-hydroxybenzoic acid (4-HBA) fully restores endogenous

CoQ10-biosynthesis in COQ2-deficient cell lines. This was accompanied by increased protein expression of CoQ10-biosynthesis-enzymes as well as a rescue of cell viability during stress conditions. In silico analysis suggested a ligand transportation path for 4-HBA through the COQ2 protein towards the mitochondrial matrix side. This process is apparently hindered by disease-causing mutations, which can be overcome by increasing 4-HBA concentrations."

11.5 The Good 2: For Humans, Nutri-Mastic™ (specifically the Mastic) contains 4-Hydroxybenzoic Acid . In a study "4-Hydroxybenzoic acid for multiple system atrophy?, Parkinsonism & Related Disorders," Volume 50, 2018, Pages 119-120, ISSN 1353-8020, https://doi.org/10.1016/j.parkreldis.2018.01.019. (https://www.sciencedirect.com/science/article/pii/S1353802018300348)

11.5.1 Abstract: Increasing evidence supports a link between multiple system atrophy and coenzyme Q10 (CoQ10) biosynthesis. However, so far this knowledge was not translated into tangible benefits for affected patients. Poor bioavailability of oral CoQ10 might constitute a major problem. Current research suggests that 4-hydroxybenzoic acid might constitute an interesting alternative treatment option.

11.6 The Good 3: For both humans and animals another study shows, "4-Hydroxybenzoic acid has several benefits for animals per a study, 4-Hydroxybenzoic acid rescues multisystemic disease and perinatal lethality in a mouse model of mitochondrial disease," (Julia Corral-Sarasa, et.al., Cell Reports, Volume 43, Issue 5114148, May 28, 2024, https://doi.org/10.1016/j.celrep.2024.114148)

11.6.1 The Good 4: For both humans and animals further study shows, "A Comprehensive Review on Biological activities of p-hydroxy benzoic acid and its derivatives,"(Jasmine Chaudhary, et.al., September 2013International Journal of Pharmaceutical Sciences Review and Research 22(2)):

11.6.1.1 It rescues multisystemic disease and perinatal lethality in a mouse model of CoQ deficiency.

11.6.1.2 It stimulates endogenous CoQ biosynthesis in tissues of Coq2 mutant mice.

11.6.1.3 It shows antifungal, antimutagenic, antisickling, estrogenic, and antimicrobial activities.

11.6.1.4 It has a growth stimulation effect on freshwater green alga.

11.6.1.5 Some derivatives of 4-hydroxybenzoic acid inhibit acetic acid-induced edema and are used in the management of sickle cell disease.

11.6.1.6 The alkyl esters of para-hydroxybenzoic acid are commonly used to preserve cosmetics, toiletries, drugs, and food products

11.7 The Good 5: For Humans, "Increasing evidence supports a link between multiple system atrophy and coenzyme Q10 (CoQ10) biosynthesis. However, so far this knowledge was not translated into tangible benefits for affected patients. Poor bioavailability of oral CoQ10 might constitute a major problem. Current research suggests that 4-hydroxybenzoic acid might constitute an interesting alternative treatment option." Felix Distelmaier, 4-Hydroxybenzoic acid for multiple system atrophy?, Parkinsonism & Related Disorders, Volume 50, 2018, Pages 119-120, ISSN 1353-8020, https://doi.org/10.1016/j.parkreldis.2018.01.019. (https://www.sciencedirect.com/science/article/pii/S1353802018300348)

11.8 The Good 6: "4-Hydroxybenzoic acid (HBA) has been found to be neuroprotective against oxidative stress. It is a metabolite of pelargonidin-based anthocyanins and may possess significant antioxidant abilities in vitro. HBA supplementation has been shown to rescue multisystemic disease and perinatal lethality in a mouse model of CoQ deficiency." (Hence, when IFUS reports impacts of customers taking Nutri-Mastic™ as it applies to Autism, ADHD, improved cognitive function, or other such improvements, here is plausible science that provides insight into "How and Why?"

11.8.1 Winter AN, Brenner MC, Punessen N, Snodgrass M, Byars C, Arora Y, Linseman DA. Comparison of the Neuroprotective and Anti-Inflammatory Effects of the Anthocyanin Metabolites,

Protocatechuic Acid and 4-Hydroxybenzoic Acid. Oxid Med Cell Longev. 2017;2017:6297080. doi: 10.1155/2017/6297080. Epub 2017 Jun 27. PMID: 28740571; PMCID: PMC5504963.

11.8.2 Winter AN, Bickford PC. Anthocyanins and Their Metabolites as Therapeutic Agents for Neurodegenerative Disease. Antioxidants (Basel). 2019 Aug 22;8(9):333. doi: 10.3390/antiox8090333. PMID: 31443476; PMCID: PMC6770078.

11.9 The Not So Good…when in excess or not properly metabolized: 4-Hydroxybenzoic acid has estrogenic activity both in vitro and in vivo,[15] and stimulates the growth of human breast cancer cell lines.[16][17] It is a common metabolite of paraben esters, such as methylparaben.[15][16][17] The compound is a relatively weak estrogen, but can produce uterotrophy with sufficient doses to an equivalent extent relative to estradiol, which is unusual for a weakly estrogenic compound and indicates that it may be a full agonist of the estrogen receptor with relatively low binding affinity for the receptor.[16][18][19] It is about 0.2% to 1% as potent as an estrogen as estradiol.[18] (As a note, there is NO evidence to date to indicate that the application of Nutri-Mastic™, SGP+™, and SupremeAG™ have produced negative impacts when applied as recommended.

11.9.1 Ref. (15) https://hmdb.ca/metabolites/HMDB0000500

11.9.2 Ref. (16) Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. Oestrogenic activity of parabens in MCF7 human breast cancer cells. J Steroid Biochem Mol Biol. 2002 Jan;80(1):49-60. doi: 10.1016/s0960-0760(01)00174-1. PMID: 11867263.

11.9.3 Ref. (17) Lemini C, Jaimez R, Avila ME, Franco Y, Larrea F, Lemus AE. In vivo and in vitro estrogen bioactivities of alkyl parabens. Toxicol Ind Health. 2003 Jul;19(2-6):69-79. doi: 10.1191/0748233703th177oa. PMID: 15697177.

11.9.4 Ref.(18) Jasmine Chaudhary, et.al., ,A Comprehensive Review on Biological activities of p-hydroxy benzoic acid and its derivatives, September 2013International Journal of Pharmaceutical Sciences Review and Research 22(2)

11.9.5 Ref.(19) Nguyen, Q.N.; Lee, S.R.; Kim, B.; Hong, J.-H.; Jang, Y.S.; Lee, D.E.; Pang, C.; Kang, K.S.; Kim, K.H. Estrogenic Activity of 4-Hydroxy-Benzoic Acid from Acer tegmentosum via Estrogen Receptor α-Dependent Signaling Pathways. Plants 2022, 11, 3387. https://doi.org/10.3390/plants11233387

11.10 Based on reports from Beef Ranchers and Dairymen applying SGP+™ to respective herds as part of their respective Ration Management Strategy, (1) milk bags, (2) colostrum, (3) infant mortality, (4) calf health and well-being, and (5) increased calving. This in turn begs the question once again as to "Why and How?"

11.10.1 The "Why and How? may be explained by the interplay of 4-Hydroxybenzoic acid and White Rot Fungi. As a reminder, "White-rot fungi (WRF) are efficient organisms for lignin degradation in nature. Recent studies have shown that WRF can utilize lignin-related aromatic compounds, such as 4-hydroxybenzoic acid (4HBA), as carbon sources."

11.10.1.1 In the study performed by Monteiro, L.M.O., del Cerro, C., Kijpornyongpan, T. et al. "Metabolic profiling of two white-rot fungi during 4-hydroxybenzoate conversion reveals biotechnologically relevant biosynthetic pathways," (Commun Biol 8, 224 (2025). https://doi.org/10.1038/s42003-025-07640-9), it was shown that:

11.10.1.1.1 "White-rot fungi are efficient organisms for the mineralization of lignin and polysaccharides into CO2 and H2O. Despite their biotechnological potential, WRF metabolism remains underexplored. Building on recent findings regarding the utilization of lignin-related aromatic compounds as carbon sources by WRF, we aimed to gain further insights into these catabolic processes. For this purpose, Trametes versicolor and Gelatoporia subvermispora were incubated in varying conditions – in static and agitation modes and different antioxidant levels – during the conversion of 4-hydroxybenzoic acid (a lignin-related compound) and cellobiose. Their

metabolic responses were assessed via transcriptomics, proteomics, lipidomics, metabolomics, and microscopy analyses. These analyses reveal the significant impact of cultivation conditions on sugar and aromatic catabolic pathways, as well as lipid composition of the fungal mycelia. Additionally, this study identifies biosynthetic pathways for the production of extracellular fatty acids and phenylpropanoids – both products with relevance in biotechnological applications – and provides insights into carbon fate in nature."

11.10.1.2 As an added note, 4-Hydroxybenzoic acid (4-HBA) can be naturally converted into 4-hydroxybenzoate (and vice versa) in the following ways: (1) In prokaryotes, 4-HBA forms from chorismate via the shikimate pathway and (2) In microbes, chorismate can be converted into 4-hydroxybenzoate and pyruvate by the chorismate-pyruvate-lyase enzyme. (https://www.chemicalbook.com/article/4-hydroxybenzoic-acid-synthesis-method-and-biological-activity.htm)

11.11 Hence, one might offer a plausible theory that the Mastic and the Carob added to Sugarcane Bagasse improves the efficacy of White Rot Fungi (and possibly other components in the biome of Sugarcane Bagasse) to both degrade and depolymerize the lignin; hence, putting into motion the interplay of 4-Hydroxybenzoic acid CoQ10-biosynthesis in COQ2-deficient cell lines.

11.12 Carob fruit contains benzoic acids, including syringic acid, 4-hydrobenzoic acid, and gentisic acid. (Goulas V, Stylos E, Chatziathanasiadou MV, Mavromoustakos T, Tzakos AG. Functional Components of Carob Fruit: Linking the Chemical and Biological Space. Int J Mol Sci. 2016 Nov 10;17(11):1875. doi: 10.3390/ijms17111875. PMID: 27834921; PMCID: PMC5133875.)

11.12.1 Pistacia lentiscus contains benzoic acid. (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.)

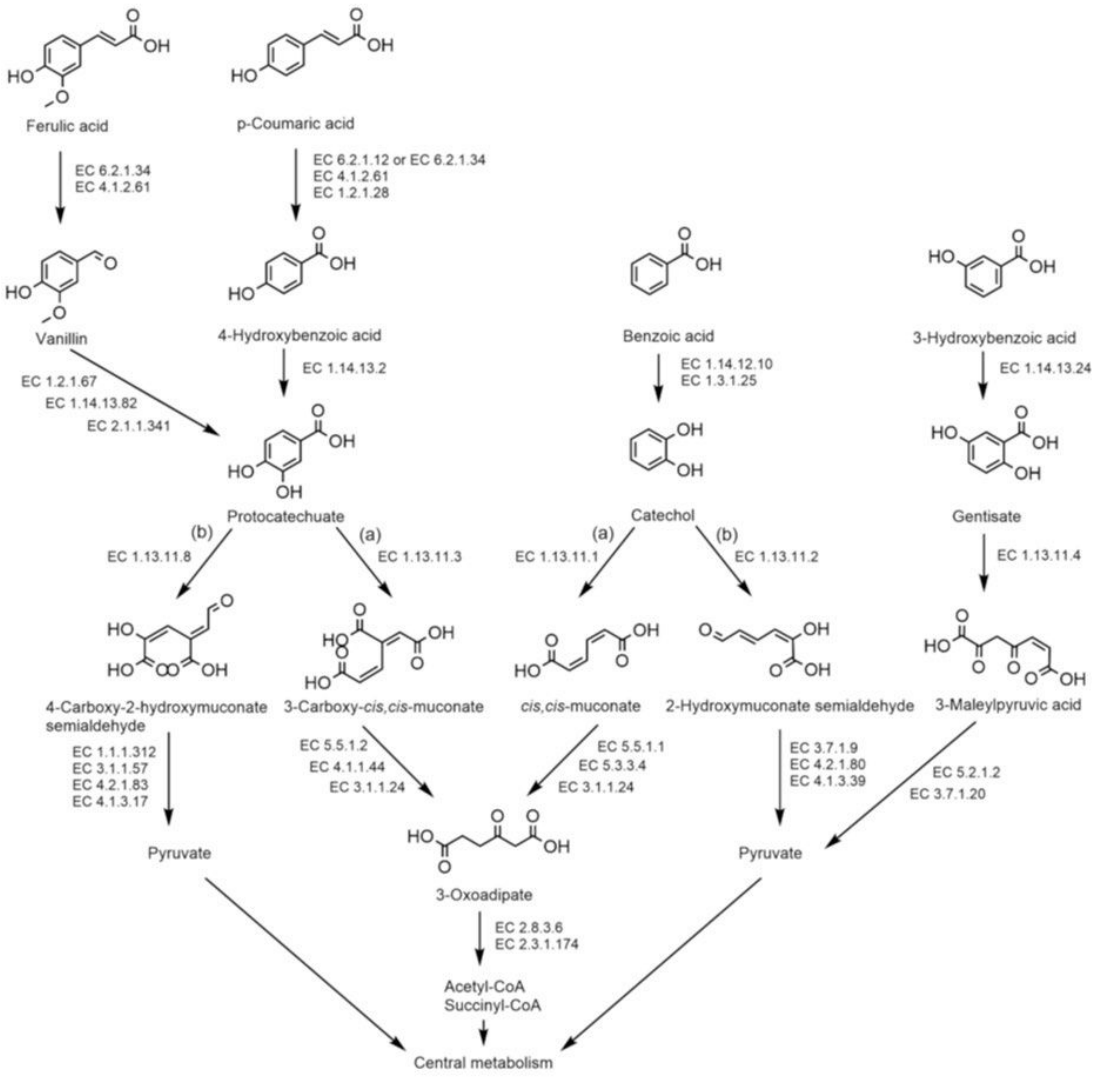
11.12.2 As a note, Benzoic acid was offered as a supplement to beef and day cattle. The following study suggests no improvements noted other than impacts to DMI and ruminal pH. As a rule, IFUS does not believe that individual supplements work per se as these must be introduced as part of integrated ration management systems.

11.12.2.1 Williams MS, Mandell IB, Bohrer BM, Wood KM. The effects of feeding benzoic acid and/or live active yeast (Saccharomyces cerevisiae) on beef cattle performance, feeding behavior, and carcass characteristics. Transl Anim Sci. 2021 Oct 1;5(4):txab143. doi: 10.1093/tas/txab143. PMID: 34877478; PMCID: PMC8643465.

11.12.2.1.1 "Implications: This preliminary research on benzoic acid in high-grain finishing diets may indicate potential as an antibiotic alternative for feedlot cattle. Results also show that the supplementation of benzoic acid, active live Saccharomyces cerevisiae, or in a combination in finisher diets with monensin did not impact carcass characteristics or performance, but BA supplementation increased DMI relative to control and yeast, suggesting no negative impacts on feed intake."

11.12.3 Furthermore, per the biochemical pathway illustrated below, the interplay between 4-Hydroxybenzoic acid, Benzoic Acid, and other compounds contained in the natural ingredients found in SGP+™ (e.g., Ferulic Acid, p-Coumaric Acid, Vanillin, etc.)

11.12.4

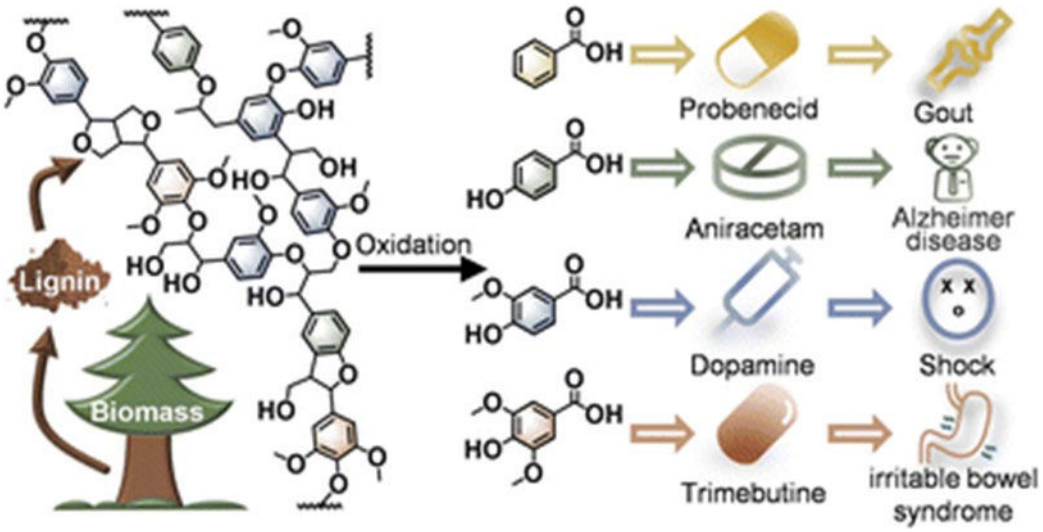


11.12.4.1 The diagram above is further supported by "Sustainable production of active pharmaceutical ingredients from lignin-based benzoic acid derivatives via "demand orientation"," Yuguo Dong, et.al., Green Chemistry, Issue 10, 2023 https://pubs.rsc.org/en/content/articlelanding/2023/gc/d3gc00241a

11.12.4.1.1 Abstract: Research related to lignin valorization into fine chemicals is an extremely demanding task due to the wide distribution and low economic value of depolymerization products. Benzoic acid derivatives (BADs) are the final products of the selective oxidation of the lignin

side chain (i.e., p-hydroxybenzoic acid, vanillic acid, syringic acid, and benzoic acid), with favorable processibility functionality, showing great potential in the synthesis of active pharmaceutical ingredients (APIs) containing ester and carbonyl groups. Herein, this tutorial review presents our views on lignin utilization, especially using lignin-based benzoic acid derivatives (LBADs) as raw materials for the synthesis of APIs, with the aim of providing a greener, more eco-friendly approach for providing a sustainable route for the production of APIs. We first introduce the conversion of lignin or lignin platform compounds via the oxidative cleavage of C–C and C–O bonds to LBADs. Subsequently, focusing on the functional group modification strategy for the conversion of LBADs, we raise a "Demand Orientation" concept and several typical API synthesis routes. This tutorial review provides green production routes from LBADs in six instances (i.e., trimebutine, aniracetam, diethylstilbestrol, dopamine, acetaminophen, oxybuprocaine). Finally, several others APIs from LBADs via theoretically feasible and sustainable routes are proposed. Some personal perspectives are provided to highlight the opportunities within this attractive field.

11.12.4.1.2 Graphical abstract: Sustainable production of active pharmaceutical ingredients from lignin-based benzoic acid derivatives via "demand orientation"



11.12.4.2 Also, the diagram below illustrates a view of Central Metabolic Pathways driven in this view by Pyruvate (and as a continuation from the diagram in 11f:

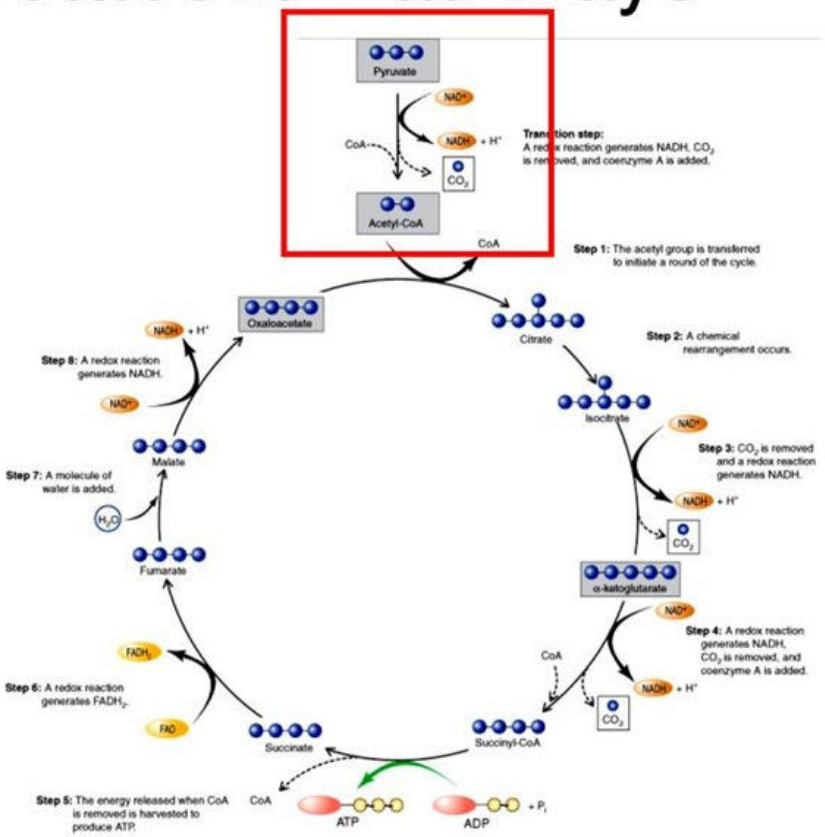
Central Metabolic Pathways

Transition step

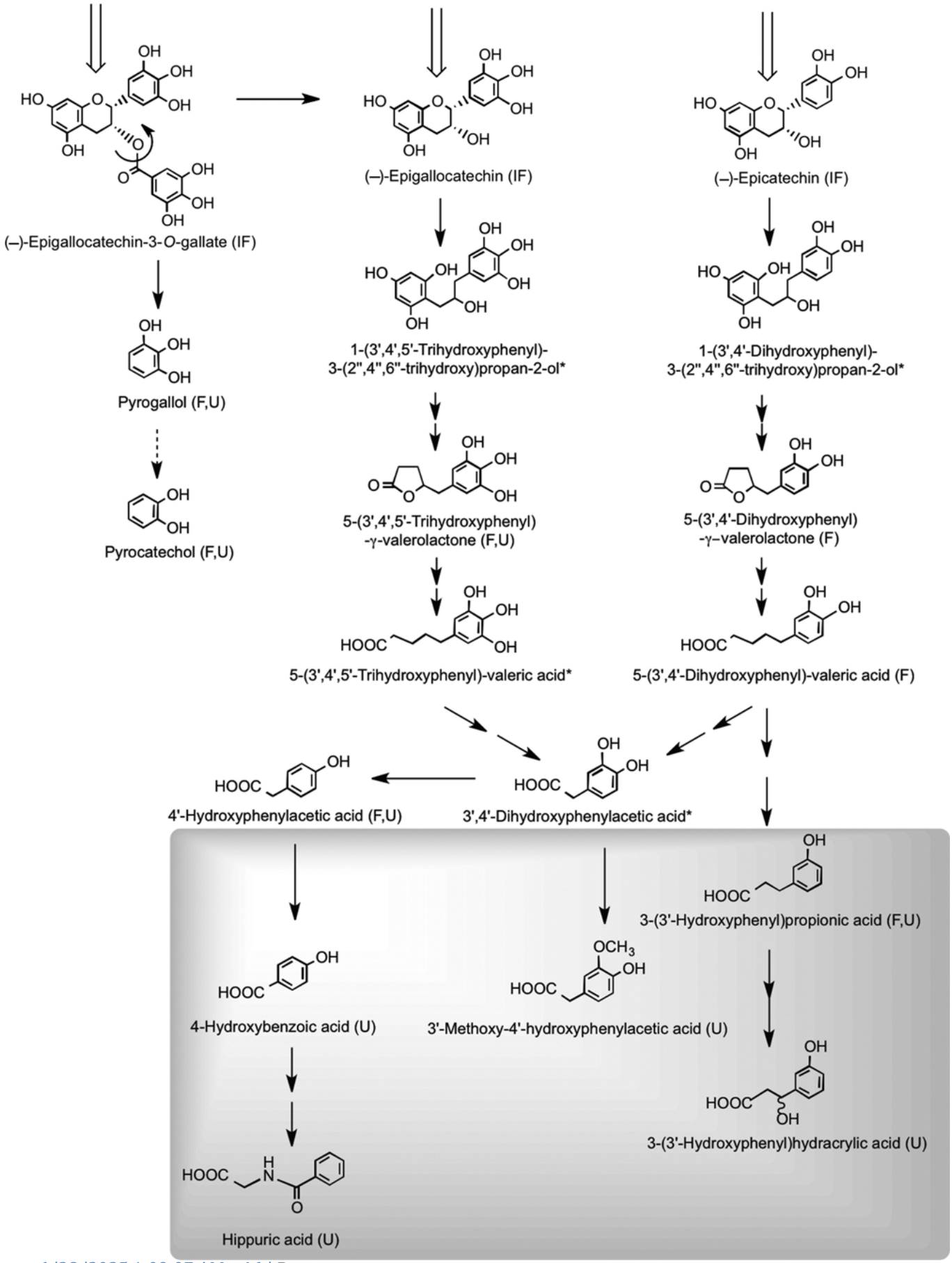
pyruvate (3 C) → acetyl CoA (2 C) + CO_2

(twice per glucose)

- •NADH
- •precursor metabolite



11.13 Another pathway involving 4-Hydroxybenzoic acid results in the production of Hippuric acid. “Hippuric acid is a carboxylic acid and organic compound formed from the combination of benzoic acid and glycine. It is primarily found in urine and its levels can increase with the consumption of phenolic compounds, such as those found in fruit juice, tea, and wine. Hippuric acid is also significant in various fields, including cell biology and nutrition, and is used to study metabolism and urinary excretion. Additionally, it is derived from herbivorous animals and plays a role in the body's detoxification processes.”


(–)-Epigallocatechin-3-O-gallate (IF)
(–)-Epigallocatechin (IF)
(–)-Epicatechin (IF)
Pyrogallol (F,U)
1-(3',4',5'-Trihydroxyphenyl)-
3-(2",4",6"-trihydroxy)propan-2-ol*
1-(3',4'-Dihydroxyphenyl)-
3-(2",4",6"-trihydroxy)propan-2-ol*
Pyrocatechol (F,U)
5-(3',4',5'-Trihydroxyphenyl)
-γ-valerolactone (F,U)
5-(3',4'-Dihydroxyphenyl)
-γ–valerolactone (F)
5-(3',4',5'-Trihydroxyphenyl)-valeric acid*
5-(3',4'-Dihydroxyphenyl)-valeric acid (F)
4'-Hydroxyphenylacetic acid (F,U)
3',4'-Dihydroxyphenylacetic acid*
3-(3'-Hydroxyphenyl)propionic acid (F,U)
4-Hydroxybenzoic acid (U)
3'-Methoxy-4'-hydroxyphenylacetic acid (U)
3-(3'-Hydroxyphenyl)hydracrylic acid (U)
Hippuric acid (U)

11.14 “Biochemically, hippuric acid is produced from benzoic acid and glycine, which occurs in the liver, intestine, and kidneys.[5] Hippuric acid has been reported to be a marker for Parkinson's disease.[9]”

11.14.1 Ref. 5: Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G (March 2009). "Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites". Proc. Natl. Acad. Sci. U.S.A. 106 (10): 3698–3703. Bibcode:2009PNAS..106.3698W. doi:10.1073/pnas.0812874106. PMC 2656143. PMID 19234110.

11.14.2 Ref. 9: "Parkinson's smell test explained by science". BBC News. BBC. 20 March 2019. Retrieved 11 March 2023.

11.14.3 As a note, a relationship cycle is known as “4-Hydroxybenzoic acid is an aromatic organic acid derived from benzoic acid. It can be produced endogenously through the metabolism of tyrosine, an aromatic amino acid.” (https://www.rupahealth.com/biomarkers/4-hydroxybenzoic-acid)

11.15 “Hippuric acid, or N-benzoylglycine, is an amino acid derivative found in the urine of herbivorous animals. In 1879, Loew wrote about the source of the acid in herbivores’ urine. According to an account of the article in the first volume of the Journal of the American Chemical Society (1879), quinic acid in hay turns into hippuric acid during digestion. (https://www.acs.org/molecule-of-the-week/archive/h/hippuric-acid.html)

11.15.1 “Hippuric acid is present in beef cattle urine and acts as a natural inhibitor of N2O emissions (1,3,5). It reduces the N2O emissions from cattle urine by inhibiting the activities of soil nitrifiers (1).”

11.15.1.1 Ref.(1) Gao J, Zhao G. Potentials of using dietary plant secondary metabolites to mitigate nitrous oxide emissions from excreta of cattle: Impacts, mechanisms and perspectives. Anim Nutr. 2022 Jan 23;9:327-334. doi: 10.1016/j.aninu.2021.12.006. PMID: 35647327; PMCID: PMC9118128.

11.15.1.1.1 Abstract: Nitrous oxide (N2O) is a potent greenhouse gas as well as the key component depleting the ozone sphere of the earth. Cattle have high feed and water intakes and excrete large amounts of urine and feces. N2O can be produced from cattle excreta during storage and use as fertilizer. Mitigating the N2O emissions from cattle excreta during production is important for protecting the environment and the sustainable development of the cattle industry. Feeding cattle with low-protein diets increases N utilization rates, decreases N excretion and consequently reduces N2O emissions. However, this approach cannot be applied in the long term because of its negative impact on animal performance. Recent studies showed that dietary inclusion of some plant secondary metabolites such as tannins, anthocyanins, glucosinolates and aucubin could manipulate the N excretion and the urinary components and consequently regulate N2O emissions from cattle excreta. This review summarized the recent developments in the effects of dietary tannins, anthocyanins and glucosinolates on the metabolism of cattle and the N2O emissions from cattle excreta and concluded that dietary inclusion of tannins or anthocyanins could considerably reduce N2O emissions from cattle excreta.

11.15.1.1.2 Ref.(3) J. Dijkstra, O. Oenema, J.W. van Groenigen, J.W. Spek, A.M. van Vuuren, A. Bannink, Diet effects on urine composition of cattle and N2O emissions, Animal, Volume 7, Supplement 2, 2013, Pages 292-302, ISSN 1751-7311, https://doi.org/10.1017/S1751731113000578. (https://www.sciencedirect.com/science/article/pii/S1751731113000578)

11.15.1.1.2.1 Abstract: Ruminant production contributes to emissions of nitrogen (N)

to the environment, principally ammonia (NH_3), nitrous oxide (N_2O) and di-nitrogen (N_2) to air, nitrate (NO_3^-) to groundwater and particulate N to surface waters. Variation in dietary N intake will particularly affect excretion of urinary N, which is much more vulnerable to losses than is faecal N. Our objective is to review dietary effects on the level and form of N excreted in cattle urine, as well as its consequences for emissions of N_2O. The quantity of N excreted in urine varies widely. Urinary N excretion, in particular that of urea N, is decreased upon reduction of dietary N intake or an increase in the supply of energy to the rumen microorganisms and to the host animal itself. Most of the N in urine (from 50% to well over 90%) is present in the form of urea. Other nitrogenous components include purine derivatives (PD), hippuric acid, creatine and creatinine. Excretion of PD is related to rumen microbial protein synthesis, and that of hippuric acid to dietary concentration of degradable phenolic acids. The N concentration of cattle urine ranges from 3 to 20 g/l. High-dietary mineral levels increase urine volume and lead to reduced urinary N concentration as well as reduced urea concentration in plasma and milk. In lactating dairy cattle, variation in urine volume affects the relationship between milk urea and urinary N excretion, which hampers the use of milk urea as an accurate indicator of urinary N excretion. Following its deposition in pastures or in animal houses, ubiquitous microorganisms in soil and waters

transform urinary N components into ammonium (NH_4^+), and thereafter into NO_3^- and ultimately in N_2 accompanied with the release of N_2O. Urinary hippuric acid, creatine and creatinine decompose more slowly than urea. Hippuric acid may act as a natural inhibitor of N_2O emissions, but inhibition conditions have not been defined properly yet. Environmental and soil conditions at the site of urine deposition or manure application strongly influence N_2O release. Major dietary strategies to mitigating N_2O emission from cattle operations include reducing dietary N content or increasing energy content, and increasing dietary mineral content to increase urine volume. For further reduction of N_2O emission, an integrated animal nutrition and excreta management approach is required.

11.15.1.2 Ref.(5) Jian Gao, Bingbing Cheng, Yufeng Liu, Meng M. Li, Guangyong Zhao, Dietary supplementation with red cabbage extract rich in anthocyanins increases urinary hippuric acid excretion and consequently decreases nitrous oxide emissions in beef bulls, Animal Feed Science and Technology, Volume 281, 2021, 115075, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2021.115075. (https://www.sciencedirect.com/science/article/pii/S0377840121002613)

11.15.1.2.1 Abstract: Two consecutive experiments were conducted to investigate the effects of dietary supplementation with red cabbage extract (RCE) rich in anthocyanins on the nitrogen (N) metabolism in beef bulls and the nitrous oxide (N_2O) emissions from the soil applied with the urine of beef bulls. In Experiment 1, 8 Simmental

beef bulls (body weight 387.9 ± 40.7 kg) were used as experimental animals. Two levels of RCE, i.e. 0 and 114 g dry matter per day, were supplemented to a basal ration as experimental treatments. The animals and the treatments were allocated in a 2 × 2 crossover design. In Experiment 2, the static incubation technique was used to determine the N2O-N emissions from the soil applied with the urine samples collected from Experiment 1. The results of Experiment 1 indicated that RCE supplementation did not affect the N excretion, N retention, and N retention rate in beef bulls ($P > 0.10$). However, RCE supplementation increased the ratio of hippuric acid-N/urinary N ($P < 0.05$) and tended to increase the ratio of creatinine-N/urinary N ($P = 0.081$) without changing the excretion of other urinary nitrogenous components ($P > 0.10$). Plasma metabolome analysis indicated that RCE supplementation upregulated 12 metabolites while downregulated 18 metabolites ($P < 0.05$). The results of Experiment 2 indicated that RCE supplementation reduced the estimated urine N2O-N emissions by 33.1% through decreasing the ratio of N2O-N/urine-N ($P < 0.05$). In conclusion, RCE supplementation did not affect the N retention and N excretion of beef bulls, whereas it attenuated the urine N2O-N emissions of beef bulls through increasing urinary hippuric acid excretions.

11.15.2 Hence, IFUS finds another plausible biochemical pathway supporting claims by ranchers and dairymen applying SGP+™ as part of their Ration Management Strategy, whereby the claims of pastures devoid of smells of ammonia, manure, and the like replaced with a “somewhat spicy and fresh earth smell,” whereas dilute concentrations of N2O can gave a distinct sweet smell.

11.15.2.1 And, the reduction of N2O by Hippuric Acid is linked to 4-Hydroxybenzoic acid, which in turn is found in and/or produced by the active ingredients in SGP+™.

11.15.2.2 “4-Hydroxybenzoic acid supplementation in dairy cow diets may improve milk quality by modifying the fatty acid profile and increasing the bioavailability of polyphenolic compounds. Troubleshooting milk quality issues requires a whole farm approach, including nutrition and feed management, environmental cleanliness, milking parlor prep procedures and maintenance, genetics, and culling decisions.”

11.15.2.3 “Since the (poly)phenols are highly reactive, to overcome these problems, the formulation of a complex of polyphenolic compounds with natural biopolymers is an effective approach. Besides, to increase the bioavailability and bioaccessibility of polyphenolic compounds, milk proteins such as whey protein concentrate, sodium caseinate, and milk protein concentrate act as natural vehicles, due to their specific structural and functional properties with high nutritional value. Therefore, milk proteins are suitable for the delivery of polyphenols to parts of the gastrointestinal tract. Therefore, this review reports on types of (poly)phenols, methods for the analysis of binding interactions between (poly)phenols–milk proteins, and structural changes that occur during the interaction.” (1) Lignin would be one such natural biopolymer as would be other natural biopolymer-like compounds (e.g., waxes, ISP’s, etc.) found in Sugarcane Bagasse. Also, “Pistacia lentiscus, also known as Ash, contains natural polymers such as resin, which is a natural polymer found in the plant. The resin has been characterized and found to contain bioactive compounds like flavonoids and phenolics. It has been recognized for its antioxidant activity and potential use in food and cosmetic industries.” (2)

11.15.2.3.1 Ref.(1) Tosif MM, Najda A, Bains A, Krishna TC, Chawla P, Dyduch-Siemińska M, Klepacka J, Kaushik R. A Comprehensive Review on the Interaction of Milk Protein Concentrates with Plant-Based Polyphenolics. Int J Mol Sci. 2021 Dec 17;22(24):13548. doi:

10.3390/ijms222413548. PMID: 34948345; PMCID: PMC8709213.

11.15.2.3.2 Ref.(2) Vasiliki K. Pachi, Eleni V. Mikropoulou, Petros Gkiouvetidis, Konstantinos Siafakas, Aikaterini Argyropoulou, Apostolis Angelis, Sofia Mitakou, Maria Halabalaki,

11.15.2.3.3 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)

11.15.2.3.3.1 "Abstract: Ethnopharmacological relevance: Chios mastic gum constitutes a unique Greek product, produced exclusively in the southern part of the island of Chios. References about its use from local populations for the treatment of gastrointestinal disorders or as a cosmetic agent can even be encountered in ancient texts of Galen, Theophrastus and Dioscorides. Nowadays, this versatile resin has been rediscovered, not only as a traditional remedy and aromatic agent, but as a potent phytotherapeutic product with various biological properties."

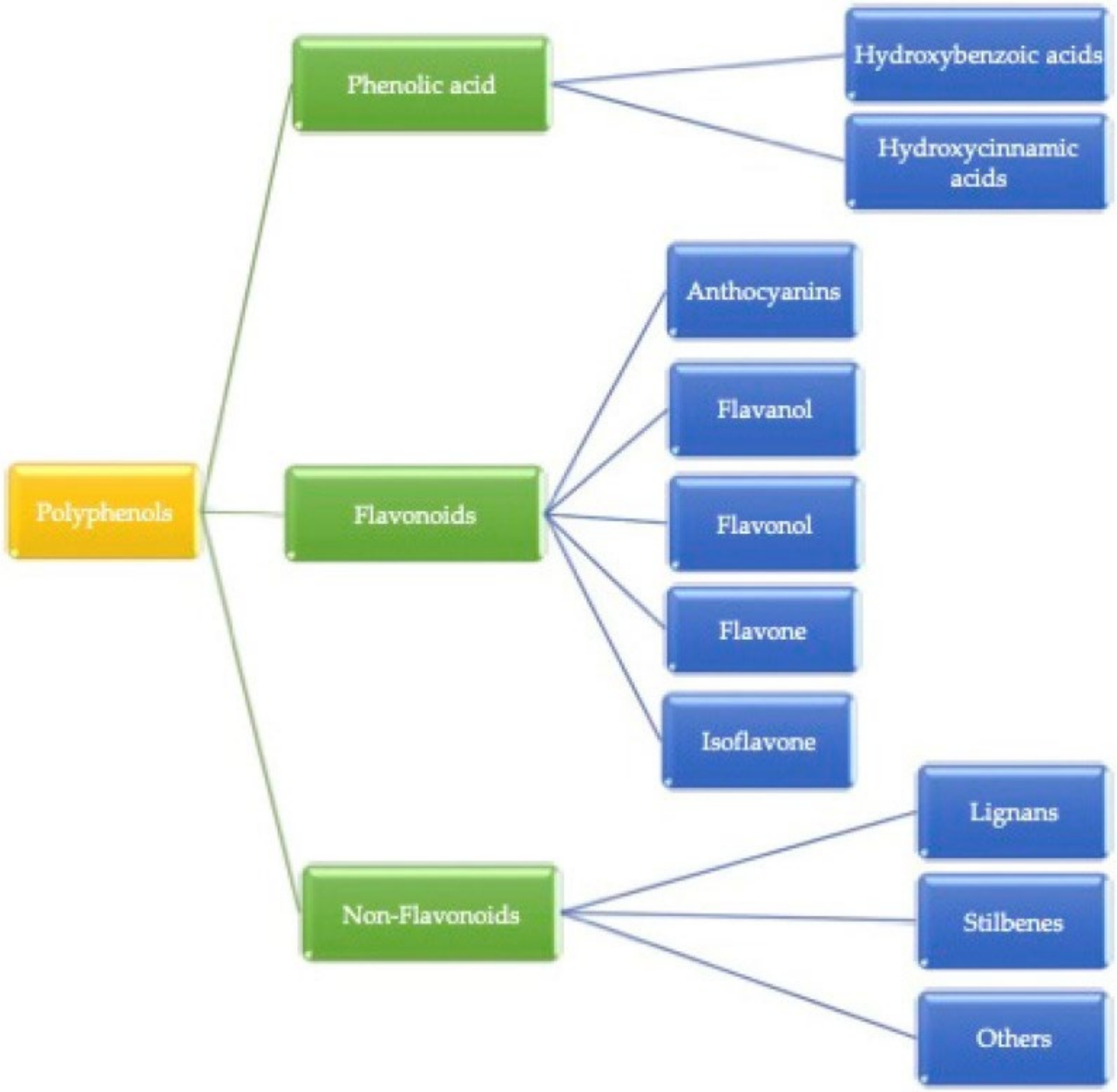
11.15.2.3.4 "4-Hydroxybenzoic acid can affect dairy cows in the following ways (1, 2,3,4): (a) It can increase the solubility of milk proteins. (b) It may cause dysfunction, sickness, or death in animals. (c) It can pass into milk or animal tissues and enter the human food supply. (d) It may cause liver damage and decreased milk production at high levels."

11.15.2.3.4.1 Where Items (a)and (b) produce beneficial effects in the dairy cow, Items (c) and (d) reflect the imbalance or improper metabolism of 4-Hydroxybenzoic acid. This is true for virtually any natural or synthetic ingredient. Hence, rather than throwing a supplement or some synthetic ingredient at the cow, why not attempt to create and integrated ration mix with natural ingredients that works with forage and some minimal level of grain (e.g., cracker corn) to produced eco-friendly, cost-effective, and healthy Herd Performance

11.15.2.3.4.1.1 Ref.(1): In a study published by Tosif MM, et.al., "A Comprehensive Review on the Interaction of Milk Protein Concentrates with Plant-Based Polyphenolics." (Int J Mol Sci. 2021 Dec 17;22(24):13548. doi: 10.3390/ijms222413548. PMID: 34948345; PMCID: PMC8709213.), an exert from the "Abstract" states, "Besides, to increase the bioavailability and bioaccessibility of polyphenolic compounds, milk proteins such as whey protein concentrate, sodium caseinate, and milk protein concentrate act as natural vehicles, due to their specific structural and functional properties with high nutritional value. Therefore, milk proteins are suitable for the delivery of polyphenols to parts of the gastrointestinal tract. Therefore, this review

reports on types of (poly)phenols, methods for the analysis of binding interactions between (poly)phenols–milk proteins, and structural changes that occur during the interaction.)

11.15.2.3.5 In “FIGURE 1: Classification of (poly)phenols”, and the subsequent discussions to follow,, we find value-added information like “Moreover, the binding of (poly)phenolic compounds and milk proteins greatly influence the digestibility of proteins, the bioavailability of (poly)phenols, and essential amino acids. Besides, the positive aspects of milk protein (poly)phenolic complexes and commercialization of the food product are important.”



11.15.2.3.6 (Ref.2) We find added support for this information in work published by Zeb, A. (2021). "Phenolic Antioxidants in Dairy Products. In: Phenolic Antioxidants in Foods: Chemistry," (Biochemistry and Analysis. Springer, Cham. https://doi.org/10.1007/978-3-030-74768-8_10). Again, an exert from the "Abstract" states. "Milk obtained from goats, cows, buffaloes, and camels is considered a complete diet of having all the required nutrients. Phenolic compounds in milk

originated from the animal feed, whereas supplementation is carried out in other dairy products for attaining the desired taste, odor, and stability. The phenolic compounds present in dairy products include simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, and anthocyanidins. The applications of these phenolic compounds have been discussed."

11.15.2.3.7 In Ref.(3), we find guidance on plants and specific compounds that when improperly used, digested, and absorbed prove detrimental to both beef and dairy cows. ("Nutrient Requirements of Dairy Cattle: Eighth Revised Edition." National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Agriculture and Natural Resources; Committee on Nutrient Requirements of Dairy Cattle.) Washington (DC): National Academies Press (US); 2021 Aug 30. To date, not a single negative effect of SGP+™ has been reported in application to beef and dairy heifers, calves, and steers. At this time SGP+™ is NOT recommended for breeding bulls.

11.15.2.3.8 In Ref.(4), we find. "Mycotoxin Effects on Dairy Cattle," by Bill Seglar, DVM, PAS, Nutritional Sciences Manager, Pioneer Hi-Bred Intl., Inc., Box 1150, Johnston, IA 50131-1150, Team Forage, Division of Extension, University of Wisconsin-Madison. To date, not a single negative effect of SGP+™ has been reported in application to beef and dairy heifers, calves, and steers. At this time SGP+™ is NOT recommended for breeding bulls.

11.16 In two studies, certain biting flies have been linked to bovine mastitis (1, 2).

11.16.1 Ref.(1) Biting Flies on Dairy Farms Can Spread Bovine Mastitis, Press Release from American Society of Microbiology, June 26, 2024.

11.16.2 Highlights: Bovine mastitis is a potentially fatal condition with myriad known causes, including bacteria.

1. Biting flies may help cause mastitis, but the mechanisms are not well elucidated.

2. Researchers characterized microbial diversity in biting flies and manure to look for connections.

3. The flies carried relevant bacterial strains, also found in the manure, associated with mastitis.

4. The research may point to new strategies for protecting cows from disease-causing pathogens.

11.16.2.1 Ref.(2) Genetic analysis confirms stable flies as mastitis vector: Research shows flies can carry the bacteria from manure breeding sites, Stew Slater, July 22, 2024, Farmtario: Cow health, Dairy, https://farmtario.com/dairy/cow-health/genetic-analysis-confirms-stable-flies-as-mastitis-vector/

11.17 Natural compounds found and/or metabolized from plants in healthy concentrations in the beef and dairy cows (like 4-Hydroxybenzoic acid) have been linked in numerous studies to fly and overall insect repellency. This would seem to be particularly true if these compounds are found in milk. While creating healthy benefits for humans, they might as well offer a level of protection to the cow.

11.17.1 ADD SOURCES

12.0 Furthermore, 4-Hydroxybenzoic acid demonstrates the capacity to prevent bad fungi from impacting healthy plants:

12.1 Maniak H, Matyja K, Pląskowska E, Jarosz J, Majewska P, Wietrzyk J, Gołębiowska H, Trusek A, Giurg M. 4-Hydroxybenzoic Acid-Based Hydrazide-Hydrazones as Potent Growth Inhibition Agents of Laccase-

Producing Phytopathogenic Fungi That Are Useful in the Protection of Oilseed Crops. Molecules. 2024 May 8;29(10):2212.

12.2 The aforementioned study goes on to demonstrate that "4-Hydroxybenzoic acid can be used to:

12.2.1 Promote plant growth and development.

12.2.2 Increase abiotic stress tolerance, such as drought and freezing tolerance.

12.2.3 Improve the economics of biorefineries when accumulated in sorghum biomass.

12.2.4 Accumulate in phloem fluids and be involved in plant defense."

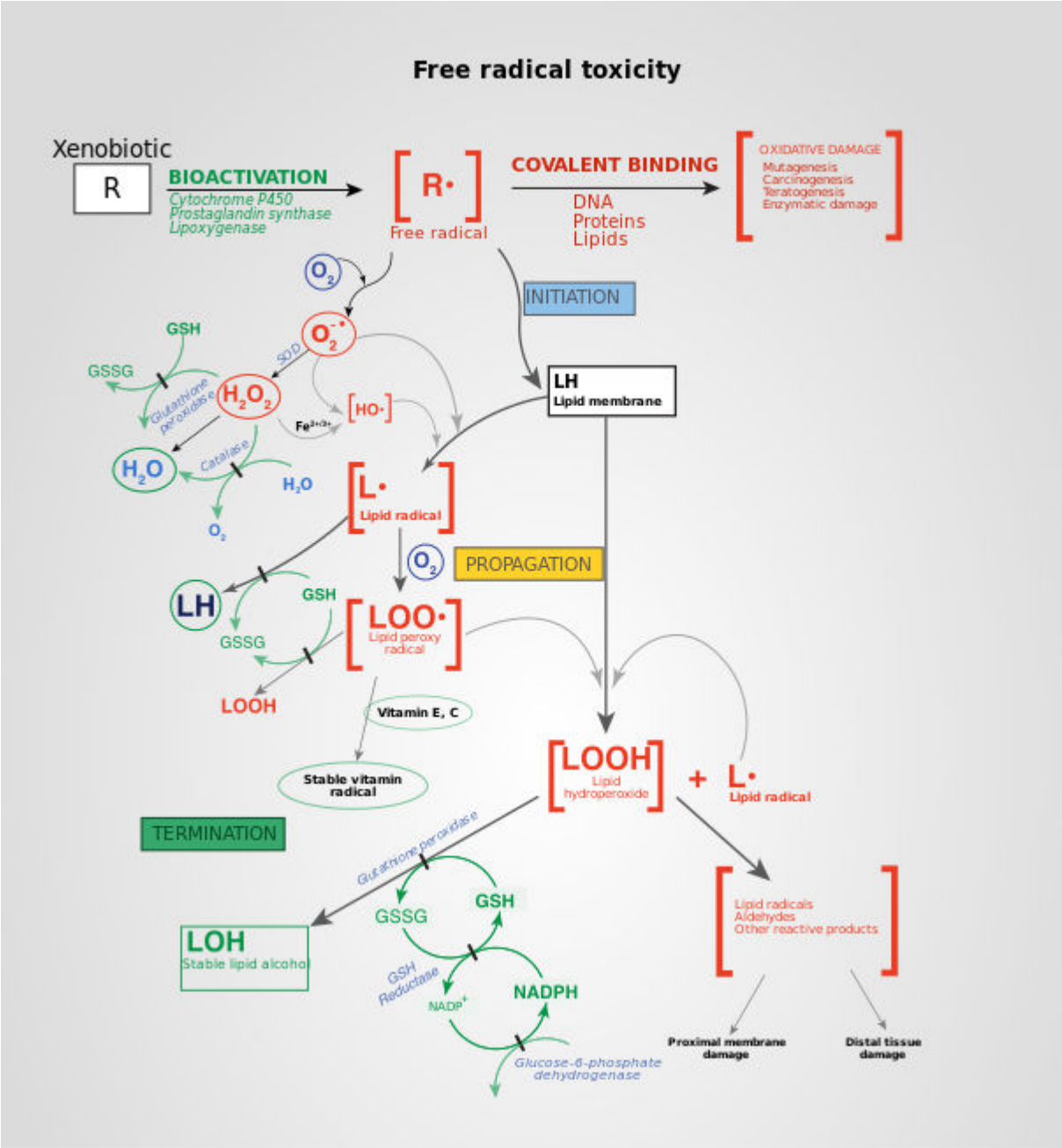
13.0 Flavonoids:

13.1 The results obtained in the present meta-analysis indicate that FLAs can be used as natural growth promoters in beef cattle and, at the same time, improve feed conversion. The best result for daily weight gain is obtained with FLAs supplementation periods up to 75 days and diets high in concentrate (>700 g/kg DM). Likewise, including FLAs in bovine diets improves dry matter intake and nutrient digestibility. The best dry matter intake is obtained with periods up to 75 days and when the FLAs used are puerarin, anthocyanin, and daidzein. Furthermore, supplementation with FLAs improves total antioxidant status and immune response in cattle by reducing serum concentration of malondialdehyde and increasing serum levels of antioxidant enzymes and immunoglobulins. The best results for serum concentration of superoxide dismutase are obtained with FLAs extracts and when the FLAs used are puerarin or daidzein. At the same time, FLAs supplementation improves meat quality by reducing shear force and malondialdehyde content. In addition, FLAs improve milk production and composition. The highest milk production is obtained when FLAs extracts are used, with daidzein or mixtures of FLAs, and low doses of FLAs (≤600 mg/kg DM). The best results for milk protein content are obtained with supplementation periods longer than 75 days, diets with moderate levels of concentrate (400–700 g/kg DM), and daidzein or mixtures of FLAs. Likewise, the best fat content in milk is achieved with daidzein or mixtures of FLAs and using cows with more than 100 days

in milk. Finally, FLAs supplementation improves ruminal fermentation in cattle through increased ruminal propionate concentration and reduced total rumen protozoa. The best rumen propionate concentration is obtained with supplementation periods of up to 75 days.

13.1.1 Orzuna-Orzuna JF, Dorantes-Iturbide G, Lara-Bueno A, Chay-Canul AJ, Miranda-Romero LA, Mendoza-Martínez GD. Meta-analysis of flavonoids use into beef and dairy cattle diet: Performance, antioxidant status, ruminal fermentation, meat quality, and milk composition. Front Vet Sci. 2023 Feb 15;10:1134925. doi: 10.3389/fvets.2023.1134925. PMID: 36876000; PMCID: PMC9975267.

Free Radical Toxicity & The Role of Antioxidants:



Free radical mechanisms in tissue injury. Lipid peroxidation induced by xenobiotics and the subsequent detoxification by cellular enzymes (termination).

Fractals 1: A fractal is a complex geometric shape that exhibits self-similarity across different scales, meaning that its structure is repeated at various levels of magnification. Fractals are often described as never-ending patterns created by repeating a simple process in a recursive manner, resulting in infinitely complex designs. Mathematically, fractals can have a fractal dimension that exceeds their topological dimension, distinguishing them from traditional geometric shapes like squares and circles. They are used in various fields, including mathematics, nature, and art, to model complex structures and phenomena.

Fractals 2: Fractals in mitochondria are characterized by their multifractal structure, which is influenced by features such as loops, hairpins, and inverted palindromes in the mitochondrial DNA genome1. This self-similarity is linked to the function of subsequences, establishing a relationship between their fractal dimension and structure1. Additionally, dynamic fractals in mitochondria express long-term correlation and intrinsic coordination, indicating a broad range of oscillatory frequencies3. Techniques like MitoMorF combine automated mitochondrial morphology with fractal analysis to discern subtle changes in mitochondrial dynamics4.

-

To date, we have focused on Gallic Acid (and its relatives to include Ellagic Acid, 4-Hydroxybenzoic acid), B- caryophyllene, and selected terpinenes. As we move forward, we will continue to consider these compounds, but will add α-pinene, limonene, myrcene, and more. At this time, one could conclude that there is a worthy supposition, that being, the efficacy of these added chemical compounds found in Pistacia lentiscus will show “overlayed” synergies through a series of individual, collective, and/or interrelated Metabolic Pathways (as reflected in “The Map of Metabolic Pathways” in the IFUS Part 1 White Paper). As these pathways are elucidated and reconciled at the biochemical level, out studies to date suggest that added evidence of the

efficacy of Nutri-Mastic™, SGP+™, SupremeAG™, and other IFUS products will be reinforced at a minimum.

Hence, for purposes of this topic, the focus will be Gallic Acid as this alone gives some insight into the potential Efficacy of Nutri-Mastic(tm) in combating Human Granulocytic Anaplasmosis (from Tick Bite) as well as similar situations in other mammals (dogs, cats, bovines, etc.)

A. Anaplasma phagocytophilum is "(formerly Ehrlichia phagocytophilum)[2] is a Gram-negative bacterium that is unusual in its tropism to neutrophils. It causes anaplasmosis in sheep and cattle, also known as tick-borne fever and pasture fever, and also causes the zoonotic disease human granulocytic anaplasmosis.[3] It causes human granulocytic anaplasmosis, which is a tick-borne rickettsial disease. Because this bacterium invades neutrophils, it has a unique adaptation and pathogenetic mechanism.[4]"
    a. Ref.(2) Dumler JS, Barbet AF, Bekker CP, et al. (2001). "Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila". Int. J. Syst. Evol. Microbiol. 51 (Pt 6): 2145–65. doi:10.1099/00207713-51-6-2145. PMID 11760958.
    b. Ref.(3) TickBorne Fever reviewed and published by WikiVet, accessed 12 October 2011.
    c. Ref.(4) Dumler JS, Choi KS, Garcia-Garcia JC, et al. (December 2005). "Human granulocytic anaplasmosis and Anaplasma phagocytophilum". Emerging Infect. Dis. 11 (12): 1828–34. doi:10.3201/eid1112.050898. PMC 3367650. PMID 16485466.

B. Treatment of Human Granulocytic Anaplasmosis (from Tick Bite): "Doxycycline: The treatment for Human Granulocytic Anaplasmosis (HGA) involves the use of antibiotics to eliminate the bacteria causing the infection. The most common antibiotic used is doxycycline, which is an oral antibiotic effective against the bacteria Anaplasma phagocytophilum. Patients with HGA should seek medical attention as soon as symptoms appear, especially if they have been exposed to ticks. Early treatment is crucial to prevent the

progression of the disease and reduce the risk of severe complications. In cases where the infection is more severe, intravenous antibiotics may be required, and hospitalization might be necessary. It is important to note that doxycycline is not recommended for pregnant women or children without a high risk of Lyme disease. In such cases, alternative treatments may be considered. For more detailed information on the treatment of HGA, including the use of doxycycline and the importance of early treatment, please refer to the resources provided by the Cleveland Clinic and other medical authorities.(2,4,6)

a. Ref.(2) https://www.webmd.com/a-to-z-guides/what-is-human-granulocytic-anaplasmosis
b. Ref.(4) https://www.aafp.org/pubs/afp/issues/2020/0501/p530.html
c. Ref.(6) https://www.health.ny.gov/diseases/communicable/ehrlichiosis/fact_sheet.htm

C. "**Doxycycline** is an antibiotic used to treat bacterial infections. **Pistacia lentiscus** is a plant with traditional use and rich in antimicrobial biomolecules. The resin of **Pistacia lentiscus** has been recognized as a herbal medicinal product with therapeutic indications for mild dyspeptic disorders and skin inflammation/healing of minor wounds[2] [3]. The plant contains terpenoids, including α-pinene, terpinene, caryophyllene, limonene, and myrcene[5]."

a. Ref.(2) There is an increasing interest in revisiting plants for drug discovery proving scientifically their role as remedies. Pistacia lentiscus (PL) is a wild-growing shrub rich in terpenoids, which are pharmacological appealing. The more recurrent components in the oil are represented by α-pinene, terpinene, caryophyllene, limonene, and myrcene. High concentration of polyphenols enriches the extracts. PL-extracts showed in vitro and in animal model strong anti-inflammatory and anti-oxidative activities. The anti-inflammatory activity mainly occurs due to inhibition of NF-kB pathway or directly toward the proinflammatory cytokines, or arachidonic acid cascade against COX-2 and LOX. The antimicrobial activity of PL essential oil and extracts includes among others Staphylococcus aureus, Escherichia coli, periodontal bacteria and Candida sp.. In conclusion, the biological properties, and particularly the anti-inflammatory and anti-microbial capacity, propose PL as a new safe pharmaceutical agent. Milia, E.; Bullitta, S.M.; Mastandrea, G.; Szotáková, B.; Schoubben, A.; Langhansová, L.; Quartu, M.; Bortone, A.; Eick, S. Leaves and Fruits Preparations of Pistacia lentiscus L.: A Review on the

Ethnopharmacological Uses and Implications in Inflammation and Infection. Antibiotics 2021, 10, 425.

b. Ref.(3) Pistacia lentiscus L. (PlL) has been used for centuries in traditional medicine. The richness in antimicrobial biomolecules of Pll derivates can represent an alternative to chemically formulated agents used against oral infections. This review summarizes the knowledge on the antimicrobial activity of PlL essential oil (EO), extracts, and mastic resin against microorganisms being of relevance in oral biofilm-associated diseases. Results demonstrated that the potential of PlL polyphenol extracts has led to increasing scientific interest. In fact, the extracts are a significantly more effective agent than the other PlL derivates. The positive findings regarding the inhibition of periodontal pathogens and C. albicans, together with the antioxidant activity and the reduction of the inflammatory responses, suggest the use of the extracts in the prevention and/or reversal of intraoral dysbiosis. Toothpaste, mouthwashes, and local delivery devices could be effective in the clinical management of these oral diseases. Milia EP, Sardellitti L, Eick S. Antimicrobial Efficiency of Pistacia lentiscus L. Derivates against Oral Biofilm-Associated Diseases-A Narrative Review. Microorganisms. 2023 May 24;11(6):1378. doi: 10.3390/microorganisms11061378. PMID: 37374880; PMCID: PMC10305426.

c. Ref.(5) Abstract - Ethnopharmacological relevance: Chios mastic gum constitutes a unique Greek product, produced exclusively in the southern part of the island of Chios. References about its use from local populations for the treatment of gastrointestinal disorders or as a cosmetic agent can even be encountered in ancient texts of Galen, Theophrastus and Dioscorides. Nowadays, this versatile resin has been rediscovered, not only as a traditional remedy and aromatic agent, but as a potent phytotherapeutic product with various biological properties. 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)

D. Chios Mastic Gum: "Pistacia lentiscus may have potential effects in treating Anaplasma phagocytophilum, as it is being explored for ethnopharmacological

uses. However, no studies can be found under this short search of Mastic Gum's efficacy on the prevention and/or treatment from infections caused by Anaplasma phagocytophilum. There is "interest in revisiting plants for drug discovery, indicating that Pistacia lentiscus could be a subject of further research in this area."

E. Interaction Details: There were no interactions found between Doxycycline and Chios Mastic Gum. This does not mean the potential for an interaction does not exist, however. There is often a lack of studies and data surrounding traditional medicine, especially concerning drug interactions, so it is important to always consult your provider before making any changes to your medication regimen. https://hellopharmacist.com/drug-supplement-interactions/drug-herbal/mastic-with-doxycycline

F. HOWEVER, "The presence of Anaplasma phagocytophilum in antigen-presenting cells (APCs) may lead to dysfunctional recognition by immune CD8 T cells, NKT cells, and NK cells (4). Ref.(4) "Anaplasma phagocytophilum-Related Defects in CD8, NKT, and NK Lymphocyte Cytotoxicity," Diana G. Scorpio, et.al., Front. Immunol., 08 April 2018, Sec. Microbial Immunology, Volume 9 - 2018 | https://doi.org/10.3389/fimmu.2018.00710

G. "Gallic acid (GA) has been shown to enhance T-cell-mediated GVL effects without exacerbating GVHD. GA has been found to increase the ratio of CD3 + T cells and CD3 + CD8 + T cells, elevate the expression level of activation marker CD25, and significantly increase the secretion levels of cytotoxic cytokines such as TNF-α and IFN-γ by CD3 + CD4 + T cells and CD8 + T cells. These findings suggest that GA could bolster T cell cytotoxic function, which in turn promotes the anti-leukemia efficacy against AML cell.(Ref.1 701.Experimental Transplantation: Basic and Translational| November 5, 2024, "Gallic Acid Enhances GVL Effects of T Cells without Exacerbating Gvhd after Hematopoietic Stem Cell Transplantation," Qianqian Huang, et.al., Blood (2024) 144 (Supplement 1): 3396. https://doi.org/10.1182/blood-2024-202861

H. This begs the question: Are the effected metabolic pathways of leukemia similar to Human granulocytic anaplasmosis?
    a. "Yes, the metabolic pathways affected in leukemia are similar to human granulocytic anaplasmosis;

i. Both conditions involve aberrant metabolism that is central to leukemia proliferation and survival (1) (Rashkovan M, Ferrando A. Metabolic dependencies and vulnerabilities in leukemia. Genes Dev. 2019 Nov 1;33(21-22):1460-1474. doi: 10.1101/gad.326470.119. PMID: 31676734; PMCID: PMC6824464.)
ii. Myeloblasts from acute granulocytic leukemia exhibit similar metabolic properties to more mature leukemic cells (2) John Laszlo, Energy Metabolism of Human Leukemic Lymphocytes and Granulocytes, Blood, Volume 30, Issue 2, 1967, Pages 151-167, ISSN 0006-4971, https://doi.org/10.1182/blood.V30.2.151.151. (https://www.sciencedirect.com/science/article/pii/S0006497120827074)
iii. The glycerophospholipid metabolism is one of the most affected pathways in leukemia (3). Bolkun, L., Pienkowski, T., Sieminska, J. et al. Metabolomic profile of acute myeloid leukaemia parallels of prognosis and response to therapy. Sci Rep 13, 21809 (2023). https://doi.org/10.1038/s41598-023-48970-0
iv. Altered metabolism is a hallmark of both leukemia and chronic lymphocytic leukemia, indicating shared metabolic pathways (4). Lymphoid Neoplasia| August 11, 2022, Characterization of metabolic alterations of chronic lymphocytic leukemia in the lymph node microenvironment
v. Zhenghao Chen, Helga Simon-Molas, Gaspard Cretenet, Beatriz Valle-Argos, Lindsay D. Smith, Francesco Forconi, Bauke V. Schomakers, Michel van Weeghel, Dean J. Bryant, Jaco A. C. van Bruggen, Fleur S. Peters, Jeffrey C. Rathmell, Gerritje J. W. van der Windt, Arnon P. Kater, Graham Packham, Eric Eldering, Blood (2022) Volume 140, Issue 6 August 11 2022140: 630–643., https://doi.org/10.1182/blood.2021013990
vi. Leukemia remodels its metabolism, which parallels the metabolic changes observed in granulocytic anaplasmosis (5). Rattigan KM, Zarou MM, Helgason GV. Metabolism in stem cell-driven leukemia: parallels between hematopoiesis and immunity. Blood. 2023 May 25;141(21):2553-2565. doi: 10.1182/blood.2022018258. PMID: 36634302; PMCID: PMC10646800.”

I. Hence, a focus on one pathway, that being that "Glycerophospholipid metabolism is impacted by anaplasmosis through several mechanisms:
    a. Anaplasmosis can alter the chemical activity of glycerophospholipids, leading to increased efflux from membranes and susceptibility to hydrolysis by phospholipases (1). Somerharju P, Virtanen JA, Hermansson M. Hypothesis: Chemical activity regulates and coordinates the processes maintaining glycerophospholipid homeostasis in mammalian cells. FASEB Bioadv. 2020 Jan 27;2(3):182-187. doi: 10.1096/fba.2019-00058. PMID: 32161907; PMCID: PMC7059623.
    b. Macrophage lipid metabolism plays a crucial role in immune responses, and infections like anaplasmosis can affect this process, contributing to the overall immune response (2). Zhang, C., Wang, Y., Wang, F. et al. Quantitative profiling of glycerophospholipids during mouse and human macrophage differentiation using targeted mass spectrometry. Sci Rep 7, 412 (2017). https://doi.org/10.1038/s41598-017-00341-2
    c. The regulation and coordination of glycerophospholipid biosynthesis and degradation are essential for maintaining cellular homeostasis, which can be disrupted by pathogens like anaplasmosis (3). Martin Hermansson, Kati Hokynar, Pentti Somerharju, Mechanisms of glycerophospholipid homeostasis in mammalian cells, Progress in Lipid Research, Volume 50, Issue 3, 2011, Pages 240-257, ISSN 0163-7827, https://doi.org/10.1016/j.plipres.2011.02.004. (https://www.sciencedirect.com/science/article/pii/S0163782711000063) "

J. This raises the question as to the impact of Gallic Acid on Glycerophospholipid metabolism. "Gallic acid (GA) has been shown to have a significant impact on glycerophospholipid metabolism through its various pharmacological activities. Here are some key points regarding its effects on glycerophospholipid metabolism:
    a. Anti-inflammatory properties: GA has been shown to reduce the release of inflammatory cytokines and chemokines, which can be beneficial for glycerophospholipid metabolism by reducing inflammation in the liver and other tissues (1):
        i. Jinrong Bai, Yunsen Zhang, Ce Tang, Ya Hou, Xiaopeng Ai, Xiaorui Chen, Yi Zhang, Xiaobo Wang, Xianli Meng, Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases, Biomedicine & Pharmacotherapy, Volume 133, 2021, 110985, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2020.110985.

(https://www.sciencedirect.com/science/article/pii/S07533322203 1177X)

1. Abstract: Gallic acid (GA), also known as 3,4,5-trihydroxybenzoic acid, is a natural secondary metabolite and widely isolated from various fruits, plants and nuts. In recent years, GA has received increasing attention for its powerful anti-inflammatory properties. The purpose of this review is to clearly illuminate the pharmacological activities and related molecular mechanisms of GA in inflammatory diseases. After consulting a large number of literatures, we made a comprehensive exposition on the chemical characteristics, plant origins, pharmacokinetics and toxicity of GA, especially its pharmacological activities and mechanisms of action. Although the plant source of GA is very rich, its lower extraction rate limits the application of GA in development. It is worth mentioning that GA can not only be separated from many plants, but also be produced in large quantities through biological and chemical synthesis. According to pharmacokinetic studies, the absorption and elimination of GA after oral administration are fast, while the structural optimization or dosage form adjustment of GA is beneficial to increase its bioavailability. Promisingly, toxicity studies have shown that GA scarcely has obvious toxicity or side effects in a variety of animal experiments and clinical trials. The results show that the anti-inflammatory mechanisms of GA mainly involved MAPK and NF-κB signaling pathways. It thus weakens the inflammatory response by reducing the release of inflammatory cytokines, chemokines, adhesion molecule and cell infiltration. Due to its excellent pharmacological activities, GA is expected to be a potential candidate for the treatment of various inflammation-related diseases. This paper will provide theoretical basis for the clinical application of GA and guide the future research and medicinal development of GA.

b. Hepatoprotective effects: GA has been found to protect against liver damage and improve lipid peroxidation, which are important for glycerophospholipid metabolism. It does this by scavenging free radicals and inactivating the ACC-PPARα axis signaling (2).

i. Jiaxin Zhang, Wenxin Zhang, Li Yang, Wenjing Zhao, Zuojia Liu, Erkang Wang, Jin Wang,, Phytochemical gallic acid alleviates nonalcoholic fatty liver disease via AMPK-ACC-PPARa axis through dual regulation of lipid metabolism and mitochondrial function, Phytomedicine, Volume 109, 2023,154589, ISSN 0944-7113,

ii. https://doi.org/10.1016/j.phymed.2022.154589. (https://www.sciencedirect.com/science/article/pii/S0944711322006778)

1. Abstract: Background: Nonalcoholic fatty liver disease (NAFLD) usually includes NAFL called simple hepatosteatosis and nonalcoholic steatohepatitis (NASH) called more steatohepatitis. The latter is a leading pathogenic promotor of hepatocellular carcinoma (HCC). Phytochemical gallic acid (GA) has been proved to exert positive efficacy in HCC in our work, but it remains unclear whether its hepatoprotective effect attributes to the controlled transition from simple steatosis to steatohepatitis.

c. Antioxidant activity: GA's antioxidant properties help to scavenge free radicals and improve the antioxidant potential of the liver, which is crucial for glycerophospholipid metabolism (3). Bhattacharyya, S., Ahammed, S.M., Saha, B.P. et al. The Gallic Acid–Phospholipid Complex Improved the Antioxidant Potential of Gallic Acid by Enhancing Its Bioavailability. AAPS PharmSciTech 14, 1025–1033 (2013). https://doi.org/10.1208/s12249-013-9991-8

d. Metabolic pathway modulation: GA has been shown to modulate metabolic pathways such as lipid metabolism, glucose metabolism, and amino acids metabolism, which are all important for glycerophospholipid metabolism (4). Gallic Acid Ameliorated Impaired Glucose and Lipid Homeostasis in High Fat Diet-Induced NAFLD Mice, Jung Chao,Teh-Ia Huo,Hao-Yuan Cheng,Jen-Chieh Tsai,Jiunn-Wang Liao,Meng-Shiou Lee,Xue-Mei Qin,Ming-Tsuen Hsieh,Li-Heng Pao ,Wen-Huang Peng, Published: June 11, 2014, https://doi.org/10.1371/journal.pone.0096969

e. These findings suggest that GA may play a significant role in maintaining glycerophospholipid metabolism and supporting overall liver health. However, further research is needed to fully understand its impact on glycerophospholipid metabolism and to explore its potential therapeutic applications."

K. Hence, as GA (Gallic Acid) is a significant ingredient in Chios Mastic Gum (Pistacia lentiscus), one could extrapolate the potential Efficacy of Nutri-Mastic™ in combating Human Granulocytic Anaplasmosis (from Tick Bite) as well as other related (or commonly shared) metabolic pathways.. This CERTAINLY warrants further study on both Humans, Bovines, and other mammalian species.

L. Bovine Granulocytic Anaplasmosis:
   a. "Bovine Granulocytic Anaplasmosis, also known as tick-borne fever, is caused by the bacterium Anaplasma phagocytophilium (1). It is transmitted by hard ticks belonging to the Ixodes persulcatus complex1. Another form of bovine anaplasmosis is caused by Anaplasma marginale, which affects erythrocytes and is transmitted by at least 20 tick species worldwide, including Rhipicephalus spp., Dermacentor spp., and Ixodes Ricinus (2)."
      i. Ref.(1) https://www.vetlexicon.com/bovis/internal-medicine/articles/tick-borne-fever/
      ii. Ref.(2) Tick Infestation and Disease transmission in livestock and human beings, B Deepika and M Dehuri, International Journal of Veterinary Sciences and Animal Husbandry 2024; 9(6): 709-712, ISSN: 2456-2912, VET 2024; 9(6): 709-712, © 2024 VET, www.veterinarypaper.com
   b. "Bovine anaplasmosis, caused by the rickettsia Anaplasma marginale, is an economically important tick-borne disease of cattle that is found worldwide. Its clinical effects of severe anemia, decreased growth, weight loss, and death negatively impact cattle welfare and create a significant economic burden for cattle producers. Despite availability of highly sensitive and specific assays for anti-A. marginale antibodies (competitive ELISA) and A. marginale genetic material (real-time PCR), the interpretation of test results for the diagnosis of clinical anaplasmosis in cattle remains challenging. Treatment and control usually consist of administration of oral and/or injectable tetracyclines; however, this approach is unlikely to be sustainable in the face of increasing scrutiny of antimicrobial usage in livestock. Statistically robust prospective studies are needed to characterize the prevalence, distribution, and transmission of bovine anaplasmosis under field conditions, as the epidemiology of this disease remains incompletely understood."
      i. Ierardi RA. A review of bovine anaplasmosis (Anaplasma marginale) with emphasis on epidemiology and diagnostic testing. J Vet Diagn Invest. 2025 Mar 28:10406387251324180. doi:

10.1177/10406387251324180. Epub ahead of print. PMID: 40156087; PMCID: PMC11955989.

IFUS Point 20: The effect of Gallic Acid on T-cells:

IFUS Point 20a: "Enhances T-cell-mediated gut-vascular leakage (GVL) effects: Gallic Acid (GA), a dietary polyphenolic compound found in fruits, vegetables, gallnuts, sumac, tea leaves, and herbal medicines, enhances T-cell-mediated gut-vascular leakage (GVL) effects without exacerbating gut-vascular hemorrhage (GVHD). GA treatment increases T-cell activation and tumor necrosis factor-α secretion, and it augments T-cell-mediated GVL effects through the activation of the MAPK and NF-κB pathways (1,2)

IFUS Point 20b-1: Ref.(1) Gallic acid enhances GVL effects of T cells without exacerbating GVHD after haematopoietic stem cell transplantation. Abstract: Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for acute myeloid leukaemia (AML), predominantly due to its potent graft-versus-leukaemia (GVL) effect. However, leukaemia relapse remains a major obstacle to the success of allo-HSCT. In this study, we demonstrated that gallic acid (GA), a natural dietary compound, can enhance T-cell-mediated GVL effects both in vitro and in vivo. GA-treated T cells exhibited increased activation and elevated secretion of cytotoxic cytokines, leading to the apoptosis of AML cells in co-culture systems in vitro. In a non-irradiated leukaemia mouse model, we showed that GA treatment prolonged the survival of leukaemic mice and reduced leukaemia cell infiltration. Further analysis revealed that GA treatment increased T-cell activation and tumour necrosis factor-α secretion. Moreover, integrated transcriptomic and proteomic analyses indicated that GA augments T-cell-mediated GVL effects through the activation of the MAPK and NF-κB pathways. Blocking these pathways individually diminished the protective effect of GA in AML model mice. Importantly, GA administration did not accelerate graft-versus-host disease (GVHD) progression in a mouse model. In conclusion, our study revealed that GA can enhance the GVL effects of T cells without exacerbating GVHD, offering insights into its potential to improve outcomes for patients after HSCT. (Huang Q, Jiang X, Wang B, Wu Z, Zhang F, Huang XJ, Guo H. Gallic acid enhances GVL effects of T cells without exacerbating GVHD after haematopoietic stem cell transplantation. Br J Haematol. 2025 Jan;206(1):120-132. doi: 10.1111/bjh.19863. Epub 2024 Oct 29. PMID: 39472128.)

IFUS Point 20b-2: (Ref.2) Gallic acid induces T-helper-1-like Treg cells and strengthens immune checkpoint blockade efficacy. Background: Foxp3+ regulatory T (Treg) cells facilitate tumor immune evasion by forming a suppressive tumor microenvironment. Therefore, immune therapies promoting Treg fragility may greatly enhance immune checkpoint blockade (ICB) efficacy in cancers. Results: Mechanistically, gallic acid prevents STAT3 phosphorylation and the binding of phosphorylated STAT3 to Usp21 gene promoter. The deubiquitinated Usp21 and stabilized PD-L1 proteins boost the function of Treg cells. Combination of gallic acid and anti-PD-1 antibody, in colorectal cancer (CRC) treatment, not only significantly dampen Treg cell function by impairing PD-L1/PD-1 signaling and downregulating Foxp3 stability, but also promote CD8+ T cells' production of IFN-γ and limited tumor growth. Conclusion: Our findings have implications for improving the efficacy of ICB therapy in CRC by inducing T-helper-1-like Foxp3lo Treg cells. (Deng B, Yang B, Chen J, Wang S, Zhang W, Guo Y, Han Y, Li H, Dang Y, Yuan Y, Dai X, Zang Y, Li Y, Li B. Gallic acid induces T-helper-1-like Treg cells and strengthens immune checkpoint blockade efficacy. J Immunother Cancer. 2022 Jul;10(7):e004037. doi: 10.1136/jitc-2021-004037. Erratum in: J Immunother Cancer. 2022 Oct;10(10):e004037corr1. doi: 10.1136/jitc-2021-004037corr1. PMID: 35817479; PMCID: PMC9274539.)

IFUS Point 20c: Gallic acid enhances anti-lymphoma function of anti-CD19 CAR-T cells in vitro and in vivo. Chimeric antigen receptor T (CAR-T) cell targeting CD19 antigen has achieved exhilarative clinical efficacy in B-cell malignancies. However, challenges still remain for the currently approved anti-CD19 CAR-T therapies, including high recurrence rates, side effects and resistance. Herein, we aim to explore combinatorial therapy by use of anti-CD19 CAR-T immunotherapy and gallic acid (GA, an immunomodulatory natural product) for improving treatment efficacy. We assessed the combinatorial effect of anti-CD19 CAR-T immunotherapy with GA in cell models and a tumor-bearing mice model. Then, the underlying mechanism of GA on CAR-T cells were investigated by integrating network pharmacology, RNA-seq analysis and experimental validation. Furthermore, the potential direct targets of GA on CAR-T cells were explored by integrating molecular docking analysis with surface plasmon resonance (SPR) assay. The results showed that GA significantly enhanced the anti-tumor effects, cytokine production as well as the expansion of anti-CD19 CAR-T cells, which may be mainly through the activation of IL4/JAK3-STAT3 signaling pathway. Furthermore, GA may directly target and activate STAT3, which may, at least in part, contribute to STAT3 activation. Overall, the findings reported here suggested

that the combination of anti-CD19 CAR-T immunotherapy with GA would be a promising approach to increase the anti-lymphoma efficacy. (Luo Z, Shi J, Jiang Q, Yu G, Li X, Yu Z, Wang J, Shi Y. Gallic acid enhances anti-lymphoma function of anti-CD19 CAR-T cells in vitro and in vivo. Mol Biomed. 2023 Mar 5;4(1):8. doi: 10.1186/s43556-023-00122-6. PMID: 36871129; PMCID: PMC9985527.)

IFUS Point 20d: Effect of gallic acid derivatives on secretion of Th1 cytokines and Th2 cytokines from anti CD3-stimulated spleen cells. Abstract: As reported previously (Kosuge et al., Yakugaku Zasshi, 120, 408 (2000)), methyl gallate, a gallic acid derivative, which has been one of compounds isolated from extracts of Psidium geneus Myrtaceae, selectively suppresses Th2 cytokine secretion. In the present study, to examine more effective compounds than methyl gallate, the effects of various gallic acid derivatives on the secretion of helper T cell subtype specific cytokines from anti CD3-stimulated spleen cells were investigated. Ten micrograms/ml of methyl gallate and ethyl gallate remarkably suppressed the secretion of IL-4 and IL-5, Th2 cytokines, but did not suppress meaningfully the secretion of IFN-gamma, a Th1 cytokine. On the other hand, the other gallic acid derivatives suppressed the secretion of both IL-4 and IFN-gamma. Ten micrograms/ml of methyl gallate suppressed the secretion of IL-2, a Th1 cytokine, but the same concentration of ethyl gallate did not suppress it. In conclusion, it seemed that ethyl gallate was the most selective inhibitor for the secretion of Th2 cytokines among gallic acid derivatives used in this study. (Kato K, Yamashita S, Kitanaka S, Toyoshima S. [Effect of gallic acid derivatives on secretion of Th1 cytokines and Th2 cytokines from anti CD3-stimulated spleen cells]. Yakugaku Zasshi. 2001 Jun;121(6):451-7. Japanese. doi: 10.1248/yakushi.121.451. PMID: 11433779.)

IFUS Point 20e: Delphinidin Chloride and Its Hydrolytic Metabolite Gallic Acid Promote Differentiation of Regulatory T cells and Have an Anti-inflammatory Effect on the Allograft Model. Abstract
Regulatory T cells (Tregs) control the reactivity of other T cells to prevent excessive inflammatory responses. They also plays a role in preventing autoimmune diseases; but when they are overproduced, they decreased vital immunity, which can lead to invasion of external pathogens. Therefore, it is most important in preventing the development of immune diseases to maintain the homeostasis of these cells. Delphinidin chloride is an anthocyanidin and known to have anti-oxidant activities. However, its structure is very unstable and easily decomposed. One of these degradation products is gallic acid, which also has anti-oxidant effects. In this study, we examined the effect of these materials on Tregs

in controlling immune response. It was found that these materials further promote differentiation into Tregs, and TGF-β and IL-2 related signals are involved in this process. Furthermore, it was verified that a variety of immunosuppressive proteins were secreted more, and the function of induced Tregs was also increased. Finally, in the allograft model, we could find a decrease in activated T cells when these materials were treated because they increased differentiation into Tregs. Therefore, these two materials are expected to become new candidates for the treatment of diseases caused by excessive activation of immune cells, such as autoimmune diseases. PRACTICAL APPLICATION: Delphinidin, a kind of anthocyanin rich in pigmented fruits, and its hydrolytic metabolite, gallic acid, are known to have antimicrobial and anti-oxidant properties. In this experiment, it was shown that delphinidin and gallic acid had an effect of increasing the differentiation of regulatory T cells, and the effect of suppressing the function of memory T cells was also observed. Due to these functions, delphinidin and gallic acid might have the potential to be used as immune suppressive agents in organ transplant and autoimmune disease patients or be a model for food development associated with the immune system. (Hyun KH, Gil KC, Kim SG, Park SY, Hwang KW. Delphinidin Chloride and Its Hydrolytic Metabolite Gallic Acid Promote Differentiation of Regulatory T cells and Have an Anti-inflammatory Effect on the Allograft Model. J Food Sci. 2019 Apr;84(4):920-930. doi: 10.1111/1750-3841.14490. PMID: 30977922.)

IFUS Point 21: Does Gallic Acid show efficacy against cancer?

IFUS Point 21a: Gallic acid: Molecular rival of cancer:

Abstract: Gallic acid, a predominant polyphenol, has been shown to inhibit carcinogenesis in animal models and in vitro cancerous cell lines. The inhibitory effect of gallic acid on cancer cell growth is mediated via the modulation of genes which encodes for cell cycle, metastasis, angiogenesis and apoptosis. Gallic acid inhibits activation of NF-κB and Akt signaling pathways along with the activity of COX, ribonucleotide reductase and GSH. Moreover, gallic acid activates ATM kinase signaling pathways to prevent the processes of carcinogenesis. The data so far available, both from in vivo and in vitro studies, indicate that this dietary polyphenol could be promising agent in the field of cancer chemoprevention. Highlights: (A.) The inhibitory effects exerted by gallic acid (GA) on cancerous cells. Inhibition of COX-2, Ribonucleotide Reductase, GSH and UGDH. (B.) The molecular mechanisms of apoptosis induction and angiogenesis inhibition by GA. (C.) Anticancerous effect of gallic acid derivatives. (Sharad Verma, Amit Singh, Abha Mishra, Gallic acid: Molecular rival of cancer, Environmental Toxicology

and Pharmacology, Volume 35, Issue 3, 2013, Pages 473-485, ISSN 1382-6689, https://doi.org/10.1016/j.etap.2013.02.011. (https://www.sciencedirect.com/science/article/pii/S1382668913000276)

IFUS Point 21b: Effects of Gallic Acid on Endometrial Cancer Cells in Two and Three Dimensional Cell Culture Models: Abstract

Background and Aim: Cell culture studies are an indispensable tools used to understand basic physiological, biophysical and biomolecular mechanisms. Although traditional two-dimensional (2D) cell culture models are more preferred in experimental studies, three-dimensional (3D) cell culture models, attract more attention due to several advantages including mimicking tumor physiology, biochemistry and biomechanics. We aimed to investigate the effects of Gallic Acid, an antimutagenic, antioxidant and anticarcinogenic agent, on both 2D and 3D cultured endometrial cancer cells for the first time.

Methods: IC50 values were determined in 2D and 3D cultured endometrial cancer cells exposed to different doses of GA. In the 2D culture model exposed to GA, Caspase 3 expression levels were analyzed. In addition, the effect of GA on the migration of 2D cultured endometrium cancer cells was investigated.

Results: IC50 value in the 3D model was found significantly higher than the 2D model. In 2D culture model, Caspase 3 expression and apoptosis was increased significantly in cells of GA exposed group compared to the control group. GA did not have a significant effect on the migration profile of cells.

Conclusion: Gallic Acid is shown to induce apoptosis in Ishikawa cells via Caspase 3 activation. We demonstrated a significantly higher IC50 level in 3D model which provide evidence to prefer 3D models in drug-test trials. The data obtained in the current study will provide a basis for further experiments to analyze the effects of GA on endometrial cancer and to develop strategies for clinical treatment.

IFUS Point 21c: Antiproliferative Effect of Gallic Acid is Mediated via Mitochondrial- or ER-Stress-Induced Apoptosis and Canonical Autophagy in HT-29 Cells

Abstract: Cancer is a noncommunicable disease burden and the second-leading cause of death worldwide. Novel medications and drug possibilities are sought everyday throughout the world in pursuit of cancer therapy. Colorectal cancer as one of the leading cancers across the globe can be treated effectively using TCM

via decreasing the incidence and increasing the survival of patients. Gallic acid (GA) is an effective polyphenol used in TCM to treat several disorders including cancer. Our hypothesis was set to determine that GA possesses cytotoxic effects on CRC cells and that apoptosis and/or autophagy could be the rationale behind the mechanism. Therefore, in the current study, we investigated the cytotoxic effects of GA on human colorectal adenocarcinoma HT-29 cells and the mechanisms of such effects. The IC50 for the cytotoxic effects of GA as evidenced by the MTT assay was 37.08 μg/mL. The outcomes indicated that GA can cause changes in MMP and result in the formation or accumulation of exogenous ROS at the IC50 dose. Consequently, the polyphenol can induce mitochondrial- or ER-stress-induced apoptosis and canonical autophagy other than contributing to the induction of ferroptosis in HT-29 cells as evidenced by quantitative PCR and western blotting. Our results indicate that GA is an effective cytotoxic agent for the management of CRC at the preclinical level and is a valuable candidate for clinical trials. (Antiproliferative Effect of Gallic Acid is Mediated via Mitochondrial- or ER-Stress-Induced Apoptosis and Canonical Autophagy in HT-29 Cells, Jie Shao, Lin Yang, Zhichao Jin, Yanmin Bao, Jiawen He, Q. H. Le, Ponnurengam Malliappan Sivakumar, Mao Wang, Ruiping Wang First published: 05 June 2024 https://doi.org/10.1155/2024/7139556

IFUS Point 21d: Gallic acid enhances anti-lymphoma function of anti-CD19 CAR-T cells in vitro and in vivo

Abstract: Chimeric antigen receptor T (CAR-T) cell targeting CD19 antigen has achieved exhilarative clinical efficacy in B-cell malignancies. However, challenges still remain for the currently approved anti-CD19 CAR-T therapies, including high recurrence rates, side effects and resistance. Herein, we aim to explore combinatorial therapy by use of anti-CD19 CAR-T immunotherapy and gallic acid (GA, an immunomodulatory natural product) for improving treatment efficacy. We assessed the combinatorial effect of anti-CD19 CAR-T immunotherapy with GA in cell models and a tumor-bearing mice model. Then, the underlying mechanism of GA on CAR-T cells were investigated by integrating network pharmacology, RNA-seq analysis and experimental validation. Furthermore, the potential direct targets of GA on CAR-T cells were explored by integrating molecular docking analysis with surface plasmon resonance (SPR) assay. The results showed that GA significantly enhanced the anti-tumor effects, cytokine production as well as the expansion of anti-CD19 CAR-T cells, which may be mainly through the activation of IL4/JAK3-STAT3 signaling pathway. Furthermore, GA may directly target and activate STAT3, which may, at least in part, contribute to STAT3 activation. Overall, the findings reported here suggested that the combination of anti-CD19 CAR-T immunotherapy with GA would be a

promising approach to increase the anti-lymphoma efficacy. (Luo, Z., Shi, J., Jiang, Q. et al. Gallic acid enhances anti-lymphoma function of anti-CD19 CAR-T cells in vitro and in vivo. Mol Biomed 4, 8 (2023). https://doi.org/10.1186/s43556-023-00122-6)

IFUS Point 21e: Immunomodulatory Effects of Gallic Acid against Cyclophosphamide- and Cisplatin-induced Immunosuppression in Swiss Albino Mice

Abstract: Gallic acid is a triphenolic acid, widely distributed in fruits, vegetables and plants and is reported to produce antioxidant, antiinflammatory, antifungal, antiviral and antitumor effects. In the present study, immunomoduatory effect of gallic acid was tested against cyclophosphamide and cisplatin; two widely used anticancer agents induced immunosuppression in Swiss albino mice. Cyclophosphamide and cisplatin are known immunosuppressive agents, which elicit variety of immune responses. In recent years much attention is given for the identification of plants or their bioactive compounds as immunomodulators. Three different doses of gallic acid i.e., 100, 200 and 400 mg/kg weight were administered orally for 7 consecutive days. Cyclophosphamide (50 mg/kg) and cisplatin (10 mg/kg) were administered intraperitoneally as single dose. Levamisole 50 mg/kg was used as standard immunomodulatory drug. 0.5 % carboxymethyl cellulose was used as solvent control. Evaluation of immunomodulatory property of gallic acid was done by using haemagglutination antibody titre response and haematological parameters such as white blood cells, red blood cells, platelet counts and haemoglobin levels. Relative weight of thymus an important lymphoid organ was also determined. Augmentation of antibody titre values and haematological end points clearly indicated immunomodulatory effect of gallic acid against cyclophosphamide and cisplatin-induced myelosuppression in Swiss albino mice. Results indicate that, gallic acid could be used as an adjuvant with immunosuppressive drugs to reduce their adverse effects on immune system. (S, Shruthi & Vijayalaxmi, K & Shenoy, K Bhasker. (2018). Immunomodulatory Effects of Gallic Acid against Cyclophosphamide- and Cisplatin-induced Immunosuppression in Swiss Albino Mice. Indian Journal of Pharmaceutical Sciences. 80. 10.4172/pharmaceutical-sciences.1000340.)

IFUS Point 21f: (Note: This is an intense study and needs several reads and a deep exploration into the citations so as to extract valuable content into the efficacy of Gallic Acid found in Mastic Gum) Effects of NAC and Gallic Acid on the Proliferation Inhibition and Induced Death of Lung Cancer Cells with Different Antioxidant Capacities (Liao CY, Wu TC, Yang SF, Chang JT. Effects of NAC and Gallic Acid on the Proliferation Inhibition and Induced Death of Lung Cancer

Cells with Different Antioxidant Capacities. Molecules. 2021 Dec 23;27(1):75. doi: 10.3390/molecules27010075. PMID: 35011309; PMCID: PMC8746925.)

Abstract and Figures: N-acetylcysteine (NAC) is a recognized antioxidant in culture studies and treatments for oxidative stress-related diseases, but in some cases, NAC is a pro-oxidant. To study the effect of NAC on cell proliferation in the presence or absence of ROS stress, we used the stable ROS generator gallic acid (GA) to treat CL1-0 lung cancer cell models with different antioxidant activities. Different antioxidant activities were achieved through the ectopic expression of different PERP-428 single nucleotide polymorphisms. GA increased ROS levels in CL1-0/PERP-428C cells and caused cell death but had no effect on CL1-0/PERP-428G cells within 24 h. We found that 0.1 mM NAC eliminated GA-induced growth inhibition, but 0.5 mM NAC enhanced GA-induced CL1-0/PERP-428C cell death. However, in the absence of GA, NAC exceeding 2 mM inhibited the growth of CL1-0/PERP-428G cells more significantly than that of CL1-0/PERP-428C cells. Without GA, NAC has an antioxidant effect. Under GA-induced ROS stress, NAC may have pro-oxidant effects. Each cell type has a unique range of ROS levels for survival. The levels of ROS in the cell determines the sensitivity of the cell to an antioxidant or pro-oxidant. Cells with different antioxidant capacities were used to show that the intracellular ROS level affects NAC function and provides valuable information for the adjuvant clinical application of NAC.

Discussion: GA (3,4,5-trihydroxybenzoic acid) is a natural plant phenol that can be used as an antioxidant at a concentration of 5μg/mL [16], but at high concentrations, GA is an ROS producer [17]. GA induces cell death in cancer cells but has less cytotoxicity to fibroblasts and endothelial cells [18,19]. GA-induced death of lung cancer cells and HeLacells is associated with increased ROS and glutathione (GSH) depletion [14,15]. Our previous studies have shown that the intracellular ROS level of CL1-0/PERP-428C cells is approximately two times higher than that of the CL1-0/PERP-428G cells. GA treatment significantly increased ROS and induced necrosis of CL1-0/PERP-428C cells in 8 h. In the CL1-0/PERP-428G cells that did not undergo GA-induced cell necrosis, GA treatment only slightly increased the ROS level. This phenomenon occurred because cells expressing PERP-428G have higher level of antioxidant enzymes, catalase and glutathione reductase than cells expressing PERP-428C [12]. To show that GA-induced ROS were responsible for the death of cells expressing PERP-428C, we used NAC as an antioxidant. Unexpectedly, we observed that NAC concentrations higher than 0.25 mM dose-dependently enhanced GA-induced inhibition of proliferation in CL1-0 cells expressing PERP-428 variants.

However, when the NAC level was below 0.1 mM, GA-induced proliferation inhibition was eliminated in CL1-0 cells expressing PERP-428C. Each cell type has a specific range of viable ROS levels that are required to maintain physiological functions, including proliferation, survival, differentiation, metabolism and angiogenesis. This is known as redox biology [20]. Oxidative stress involves high levels of ROS that cause damage to DNA, protein and lipids. Therefore, ROS levels that are too high are cytotoxic, and ROS levels that are too low are cytostatic [21]. We observed that treatment of A549 or CL1-0 lung cancer cells with NAC concentrations below 2 mM did not affect cell proliferation, but higher concentrations of NAC reduced cell proliferation. Interestingly, NAC showed greater dose-dependent inhibition of the proliferation of CL1-0 Molecules 2022,27, 75 8 of 12cells expressing PERP-428G than cells expressing PERP-428C. Our previous results indicate that cells expressing PERP-428C have higher intracellular ROS levels than cells expressingPERP-428G. NAC treatment may reduce the levels of ROS in cells expressing PERP-428G, exceeding the lower limit of ROS levels required for redox biology to support cell proliferation. Since the cells expressing PERP-428C have a higher basal ROS level, the reduction in ROS induced by a high concentration of NAC will not cause the intracellular ROS level to drop below the lower limit, so the final ROS level of cells expressing PERP-428C is still sufficient to support cell proliferation. Hypothetical intracellular ROS levels related to cell proliferation or cell death are illustrated in Figure 7. We found that 0.05–0.1 mM NAC abolished GA-induced proliferation inhibition, but when CL1-0/PERP-428 SNPs cells were cotreated with 0.5 mM or higher NAC and 50μg/mL GA, cell proliferation was completely inhibited, and many cells died. This phenomenon can be explained by NAC at a high concentration becoming a pro-oxidant in an environment containing GA. We and others have observed that GA produces H2O2, and H2O2generates hydroxyl radicals through the Fenton reaction, thereby stimulating the pro-oxidant activity of NAC [8]. The free radicals generated by GA and NAC in three days will exceed the tolerance limit of CL1-0/PERP-428SNP cells, leading to inhibition of cell proliferation and cell death. In fact, CL1-0/PERP-428G cells were well tolerated in the cotreatment with 1mM NAC and GA. They survived well after 24 h of treatment but started to die after 48 h of treatment. On the other hand, in 0.25 mM or higher NAC and GA treatments, a high proportion of CL1-0/PERP-428Ccells died within 24 h. Therefore, depending on the concentration of NAC, environment (presence of H2O2), and reaction time, NAC may become a pro-oxidant. It has been shown that NAC enhances GA-induced cell death by increasing ROS levels and depleting GSH [13]. However, the authors found that the ROS levels increased within24 h after treatment, which was not observed in our system. By 24 h, we found that a large number of CL1-0/PERP-428C cells had died, and ROS

levels were very low. One possible explanation for the low ROS levels is that the DCF fluorescence diffused outside of the dead cells. We further observed that live cells (PI-positive cells) in the groups with GA and high levels of NAC cotreatment had reduced ROS levels before membrane integrity failed. We speculate that cotreatment with higher concentrations of NAC and GA may increase the level of ROS to a threshold, which greatly triggers the activity of antioxidant systems (such as GSH), thereby reducing the level of ROS in living cells. However, this may deplete GSH and eventually lead to cell death. Although we speculate that high concentrations of NAC in GA-treated cells act as a pro-oxidant, thereby enhancing GA-induced cell death, there may be other mechanisms by which NAC may induce GA-induced cell death. However, the mechanism of NAC's pleiotropic function is still elusive. NAC enhances the growth inhibitory activity of EGCG in lung cancer cells by forming an EGCG-20-NAC adduct, thereby stabilizing EGCG and enhancing EGCG-mediated cell death [22]. In addition, NAC (5 mM) has been shown to enhance imatinib-induced apoptosis of Bcr-Abl+chronicmyeloid leukemia cells by enhancing the production of endothelial nitric oxide (NO) [23]. This study revealed that NAC concentrations lower than 2 mM do not affect the proliferation of A549 and CL1-0 lung cancer cells. However, in A549 cells, 2 mM NAC significantly enhanced GA-induced growth inhibition. In CL1-0/PERP-428C cells, 0.25 mMNAC was sufficient to enhance GA-induced growth inhibition, and over 0.5 mM NAC and GA treatment induced cell death and completely inhibited cell proliferation. NAC has been proposed for cancer prevention or treatment [24–27] and as an adjuvant in treating many chronic diseases, such as liver and bowel diseases, metabolic syndrome, infectious disease, and neurodegenerative disorders [14,28]. In clinical trials, the oral administration dose of NAC ranges from 600 to 6000 mg/day, and the maximum plasma NAC concentration was observed to range from 0.012 to 0.123 mM [29–31]. Some clinical trials used intravenous NAC at doses of 12.5–25 mg/kg/day, resulting in a maximum NAC concentration range of0.406–1.22 mM [32,33]. These clinical trials showed that NAC has no obvious side effects. However, a recent paper demonstrated that NAC promotes intestinal tumor progression in mice [33]. Thus, it is important to understand the characteristics of NAC in terms of antioxidant/pro-oxidant activity. Our research suggests that intracellular redox status may affect the biological action of NAC, shifting from antioxidant to pro-oxidant, which in turn impacts cellular functions and survival. This study provides information about the safe antioxidant dose of NAC in cells not attacked by H2O2and the pro-oxidant dose of NAC in cells attacked by H2O2. This information may be valuable for the development of anti-lung cancer treatments using NAC and other polyphenols. In addition, for high-dose intravenous NAC, it

may be important to consider the patient's ROS level. (Note: See original article for citation list.)

IFUS Point 21g: Anti-leukemic effects of gallic acid on human leukemia K562 cells: downregulation of COX-2, inhibition of BCR/ABL kinase and NF-κB inactivation (Chandramohan Reddy T, Bharat Reddy D, Aparna A, Arunasree KM, Gupta G, Achari C, Reddy GV, Lakshmipathi V, Subramanyam A, Reddanna P. Toxicol In Vitro. 2012 Apr;26(3):396-405. doi: 10.1016/j.tiv.2011.12.018. Epub 2012 Jan 8. PMID: 22245431.)
Abstract: Gallic acid (GA) induces apoptosis in various cancer cell lines. In this study, we investigated the apoptotic activity induced by GA on chronic myeloid leukemia (CML) cell line-K562 and the underlying mechanism. GA reduced the viability of K562 cells in a dose and time dependent manner. GA led to G0/G1 phase arrest in K562 cells by promoting p21 and p27 and inhibiting the levels of cyclin D and cyclin E. Further studies indicated apoptosis with impaired mitochondrial function as a result of deranged Bcl-2/Bax ratio, leakage of cytochrome c and PARP cleavage along with DNA fragmentation and by up-regulating the expression of caspase-3. GA also activated the protein expressions of fatty acid synthase ligand and caspase-8. GA is more effective in imatinib resistant-K562 (IR-K562) cells (IC50 4 μM) than on K562 cells (IC50 33 μM). GA inhibited cyclooxygenase-2 (COX-2) in K562 as well as IR-K562 cells appears to be COX-2 involved in the suppression of growth. Interestingly, GA also inhibited BCR/ABL tyrosine kinase and NF-κB. In conclusion, GA induced apoptosis in K562 cells involves death receptor and mitochondrial-mediated pathways by inhibiting BCR/ABL kinase, NF-κB activity and COX-2.

IFUS Point 21h: Gallic Acid Enhances the Anti-Cancer Effect of Temozolomide in Human Glioma Cell Line via Inhibition of Akt and p38-MAPK Pathway
Abstract: (1) Background: Temozolomide (TMZ), an oral alkylating agent, is used to treat malignant gliomas and other difficult-to-treat tumors. TMZ can enter the cerebrospinal fluid p.o. (per os) and does not need hepatic metabolism for activation of its use as a standard chemotherapeutic regimen after surgical resection of malignant glioma of the brain. However, the prognosis remains poor for most patients, and the survival rate is still unsatisfactory. Gallic acid (Ga) is a secondary metabolite existent in numerous plants. Ga shows various bioactivities, including antioxidant, anti-inflammatory, anticancer and antimicrobial effects. In this study, the latent enhanced anti-cancer efficacy of Ga in TMZ-treated U87MG cells (a human glioma line) was evaluated.
(2) Methods: The U87MG cell line was cultured for 24 h. The cells were incubated with Ga alone, TMZ alone, or their combination for various time points.

Cell viability and the drug combination index were evaluated by an XTT-based analysis and isobologram analysis, respectively. DNA destruction and intracellular reactive oxygen species (ROS) generation were analyzed by flow cytometer. The expression of various proteins was assessed via Western blotting.
(3) Results: Compared with the action of TMZ alone or Ga alone, TMZ/Ga combination augmented the inhibition of cellular viability and apoptotic level in the U87MG glioma cell line. This enhanced anti-cancer effect correlated with the decreased expression of Bcl-2 and p-Akt, and corresponded with the activation of the p38 mitogen-activated protein kinase (MAPK) pathway. In addition, Ga suppressed the TMZ-promoted ROS generation.
(4) Conclusions: Ga can augment the anti-cancer effect of TMZ via the repression of Bcl-2 expression and Akt activation and the enhancement of the p38 MAPK pathway. Our results offer a novel probable approach for the medical treatment of malignant glioma.

IFUS Point 21g: Gallic Acid Inhibits Proliferation and Induces Apoptosis in Lymphoblastic Leukemia Cell Line (C121)

Abstract: Leukemia is known as the world's fifth most prevalent cancer. New cytotoxic drugs have created considerable progress in the treatment, but side effects are still the important cause of mortality. Plant derivatives have been recently considered as important sources for the treatment of various diseases, including cancer. Gallic acid (GA) is a polyhydroxyphenolic compound with a wide range of biological functions. The aim of the present study was to evaluate the effect of GA on proliferation inhibition and apoptosis induction of a lymphoblastic leukemia cell line. Jurkat cell (C121) line was cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) with different concentrations of GA (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µM) for 24, 48 and 72 hours. The effect of GA on cell viability was measured using MTS assay. Induction of apoptosis was evaluated with Annexin V-FITC/PI kit and flow cytometry. Data were analyzed by SPSS version 20 using Kruskal-Wallis and Dunn's multiple comparison tests. Decline of cell viability to less than 50% was observed at 60.3±1.6, 50.9±1.5, and 30.9±2.8 µM concentration after 24, 48, and 72 hours incubation, respectively. All concentrations of GA (10, 30, 50 and 80 µM) enhanced apoptosis compared to the control ($P<0.05$). The results demonstrate that the polyphenolic compound, GA, is effective in inhibition of proliferation and induction of apoptosis in Jurkat cell line. It is recommended to study the mechanism of apoptosis induction in future investigations.

IFUS Point 21h:

IFUS Point 22: Does Gallic Acid show efficacy against cardiovascular disease?

IFUS Point 22b: Molecular mechanisms underlying gallic acid effects against cardiovascular diseases: An update review (Akbari G. Molecular mechanisms underlying gallic acid effects against cardiovascular diseases: An update review. Avicenna J Phytomed. 2020 Jan-Feb;10(1):11-23. PMID: 31921604; PMCID: PMC6941690.)

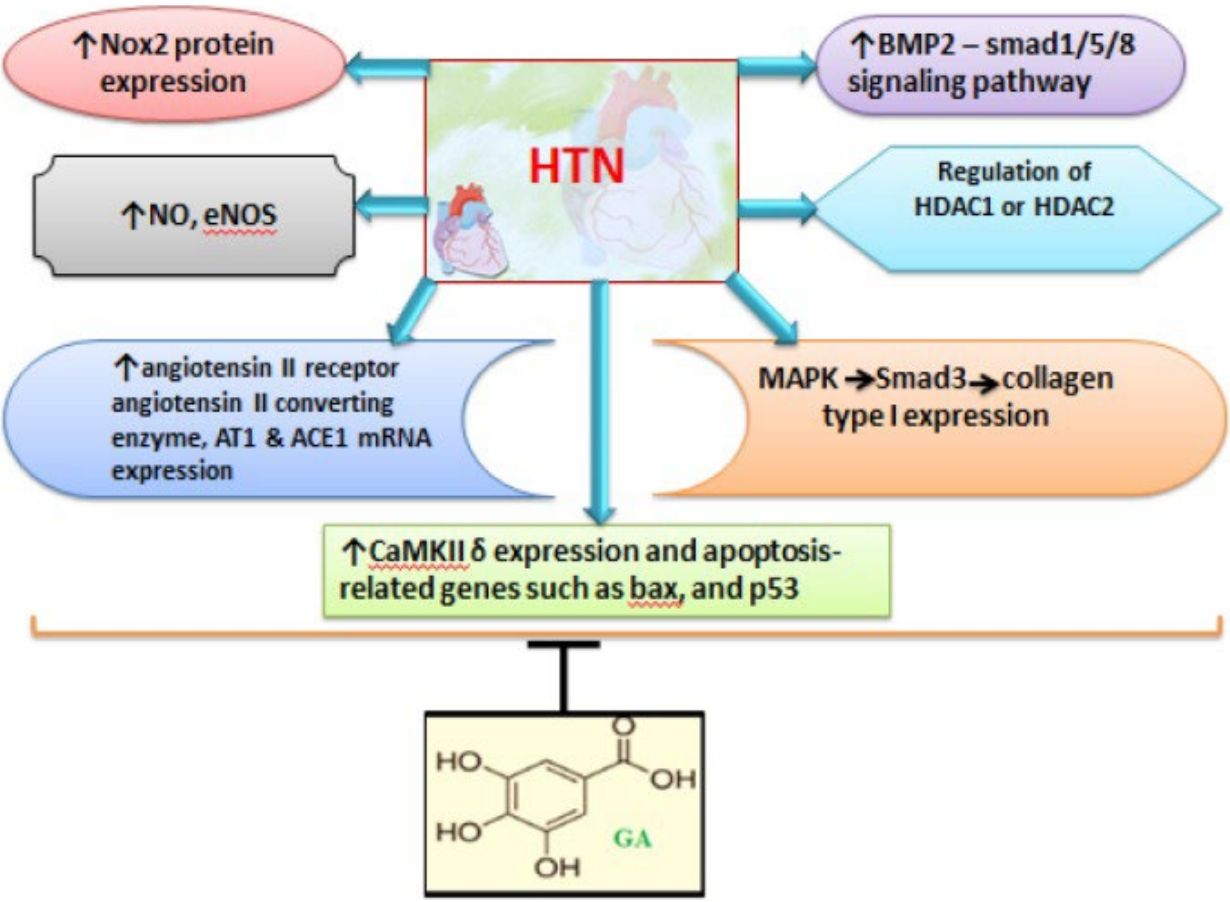
Abstract / Objective:
The prevalence of cardiovascular diseases (CVDs) is growing. CVDs are the major cause of mortality and have become one of the most important health challenges in developing countries. Gallic acid (GA) is a natural phytochemical which has been widely used against multiple conditions. The present review was designed to evaluate molecular mechanisms underlying the protective effects of this agent against CVDs.

Materials and Methods: Data discussed in this review were collected from the articles published in databases such as Science Direct, Scopus, PubMed, and Scientific Information Database between 1993 and 2018.

Results: According to the experimental studies, GA has protective actions against CVDs through increasing antioxidant enzymes capacity, inhibition of lipid peroxidation and decreasing serum levels of cardiac marker enzymes, modulation of hemodynamic parameters, recovery of electrocardiogram aberrations, and preservation of histopathological changes.

Conclusion: GA has potential cardioprotective action. Therefore, it has been suggested that this agent can be administered in underlying of CVDS.

Figure 2. Schematic presentation of the molecular mechanisms underlying GA effects on HTN



Schematic presentation of the molecular mechanisms underlying GA effects on HTN

IFUS Point 22: Does Gallic Acid contain Neuroprotective efficacy?

IFUS Point 22a:

Does pistacia lentiscus contain carotenoids? Pistacia lentiscus contains carotenoids, including lutein, zeaxanthin, and β-carotene (1,2,3,4,5).

Ref. (1 & 4) Identification and quantitation of tocopherols, carotenoids and triglycerides in edible Pistacia lentiscus oil from Tunisia, F. Mezni, et.al, . Mater. Environ. Sci., 2020, Volume 11, Issue 1, Page 79-84,

Ref. (2) 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. (3) Bouakline H, Bouknana S, Merzouki M, Ziani I, Challioui A, Bnouham M, Tahani A, El Bachiri A. The Phenolic Content of Pistacia lentiscus Leaf Extract and Its Antioxidant and Antidiabetic Properties. ScientificWorldJournal. 2024 Feb 7;2024:1998870. doi: 10.1155/2024/1998870. PMID: 38356989; PMCID: PMC10866636.

Ref. (5) Ghzaiel I, Zarrouk A, Nury T, Libergoli M, Florio F, Hammouda S, Ménétrier F, Avoscan L, Yammine A, Samadi M, Latruffe N, Biressi S, Levy D, Bydlowski SP, Hammami S, Vejux A, Hammami M, Lizard G. Antioxidant Properties and Cytoprotective Effect of Pistacia lentiscus L. Seed Oil against 7β-Hydroxycholesterol-Induced Toxicity in C2C12 Myoblasts: Reduction in Oxidative Stress, Mitochondrial and Peroxisomal Dysfunctions and Attenuation of Cell Death. Antioxidants (Basel). 2021 Nov 5;10(11):1772. doi: 10.3390/antiox10111772. PMID: 34829643; PMCID: PMC8615043.

Addendum 3: Most common plant-based Phenolic Acids per Robbins, Rebecca. (2003). Phenolic Acids in Foods: An Overview of Analytical Methodology. Journal of agricultural and food chemistry. 51. 2866-87. 10.1021/jf026182t. Phenolic acids are aromatic secondary plant metabolites, widely spread throughout the plant kingdom. Existing analytical methods for phenolic acids originated from interest in their biological roles as secondary metabolites and from their roles in food quality and their organoleptic properties. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease,

stroke, and cancers). High performance liquid chromatography (HPLC) as well as gas chromatography (GC) are the two separation techniques reviewed. Extraction from plant matrixes and cleavage reactions through hydrolysis (acidic, basic, and enzymatic) are discussed as are the derivatization reagents used in sample preparation for GC. Detection systems discussed include UV-Vis spectroscopy, mass spectrometry, electrochemical, and fluorometric detection. The most common tandem techniques are HPLC/UV and GC/MS, yet LC/MS is becoming more common. The masses and MS fragmentation patterns of phenolic acids are discussed and tabulated as are the UV absorption maxima.

R_2	R_3	R_4	R_5	X	code	common name
H	H	H	H	a	**1**	cinnamic acid
–OH	H	H	H	a	**2**	*o*–coumaric acid
H	H	–OH	H	a	**3**	*p*–coumaric acid
H	–OH	H	H	a	**4**	*m*–coumaric acid
H	$–OCH_3$	–OH	H	a	**5**	ferulic acid
H	$–OCH_3$	–OH	$–OCH_3$	a	**6**	sinapic acid
H	–OH	–OH	H	a	**7**	caffeic acid
H	H	H	H	b	**8**	benzoic acid
–OH	H	H	H	b	**9**	salicylic acid
H	H	–OH	H	b	**10**	*p*–hydroxybenzoic acid
H	$–OCH_3$	–OH	H	b	**11**	vanillic acid
H	$–OCH_3$	–OH	$–OCH_3$	b	**12**	syringic acid
H	–OH	–OH	H	b	**13**	protocatechuic acid
–OH	H	H	–OH	b	**14**	gentisic acid
–OH	–OH	–OH	–OH	b	**15**	gallic acid
H	$–OCH_3$	$–OCH_3$	H	b	**16**	veratric acid
H	$–OCH_3$	–OH	$–OCH_3$	c	**17**	syringealdehyde
H	$–OCH_3$	–OH	H	c	**18**	vanillin