A CytoSolve Research Division

Health Benefit Analysis Report

K9-701™ Health Benefits

Systematic Bioinformatics Analysis

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KEY TAKEAWAYS

- This is the first, to the best of CytoSolve Research Division's knowledge, a comprehensive systematic bioinformatics review of nutritional composition of ingredients in K9-701™
- This scientific review included analysis of 87 scientific studies, performed by 96 researchers, within 48 institution across the world, spanning 33 years of research
- The ten K9-701 ingredients comprised of 89 key molecules. Each of these molecules have documented nutritional benefits in the scientific literature.
- These 89 key molecules contained in D include:
    - 46 minerals
    - 28 phytochemicals
    - 11 vitamins
    - 4 other molecules
- This scientific review revealed that K9-701 ingredients have documented medicinal benefits for mammals including equine, across 10 different biological indications, references for which are listed in the report:
    - Immunomodulatory Effects
        - Anti-inflammatory and antioxidant effects
    - Blood glucose control/Diabetes
    - Bone Health
    - Pest Repellent/ Anti-parasitic Effect
    - Osteoarthritis
    - Neurodegeneration
    - Skin Health
    - Anti-Cancer
    - Women's Health
    - Atherosclerosis

Scientific Report

Systematic Bioinformatics Analysis of Heath Benefits of Ingredients in K9-701™

ABSTRACT

A systematic literature review is conducted to identify the nutritional components and their health benefits of K9-701™. The systematic bioinformatics process used for this study included: (1) Conducting search from disparate data sources including PubMed, Google Scholar, and multiple online databases; (2) Identifying the relevant studies; (3) Reviewing the relevant studies to identify nutritional profile; and (4) Identifying the health and nutritional benefits of K9-701 nutritional components. The systematic bioinformatics review indicates that K9-701 affects seventeen different biological functions in mammals, including equine. In this report, the active compounds of K9-701 are itemized, and where possible, the mechanisms of action of those active compounds affecting immunomodulation, osteoarthritis, heart health, women's health, brain health, bone health, skin health, cancer, diabetes, and pest repellent effects are described.

Keywords: K9-701, nutrition, systematic bioinformatics literature review, neurodegeneration, immune health, arthritis, heart health, CytoSolve®.

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1.0 INTRODUCTION

Understanding the complexity of ingredients in food, the effect of processing on the nutritional profile of food and their effect on diet and health is non-trivial. In this study, the research aim is to understand the nutritional profile and the health benefits of the ingredients in of K9-701™ on mammals including horses. We used the CytoSolve research methodology to perform exhaustive systematic bioinformatics literature review of here-to-fore known scientific literature to uncover the nutritional profile and health benefits of the following ingredients in K9-701:

1. Zeolite
2. Diatomaceous Earth (DE)
3. Methylsulfonylmethane (MSM)
4. Hyaluronic Acid
5. Red Raspberry Leaf
6. Fenugreek
7. Organic Kelp
8. Organic Turmeric
9. Organic Hawthorn Leaf and Flower
10. Spirulina

1.1 Research Aim

In this study, the research aim is to understand the nutritional profile of K9-701 and document the composition of the active molecules in K9-701 ingredients. We used the CytoSolve research methodology (Ayyadurai and Deonikar, 2022) to perform exhaustive systematic bioinformatics literature review of here-to-fore known scientific literature to uncover the nutritional profile of K9-701.

1.2 Organization of the Report

This manuscript is organized as follows: This Section 1.0 introduces and sets out the organization of this report. Section 2.0 provides the systematic literature review process and results to identify the health benefits of K9-701 for mammals including equine. Section 3.0 summarizes the health benefits of Zeolite. Section 4.0 summarizes the health benefits of Diatomaceous Earth. Section 5.0 summarizes the health benefits of Methylsulfonylmethane (MSM). Section 6.0 summarizes the health benefits of Hyaluronic acid (HA). Section 7.0 summarizes the health benefits of Red Raspberry Leaf. Section 8.0 summarizes the health benefits of Fenugreek. Section 9.0 summarizes the health benefits of Organic Kelp. Section 10.0 summarizes the health benefits of Organic Turmeric. Section 11.0 summarizes the health benefits of Organic Hawthorn Leaf and Flower. Section 12.0 summarizes the health benefits of Spirulina. Section 13 summarizes concluding remarks from this study. Section 14 contains the bibliography of references used in this study.

2.0 SYSTEMATIC LITERATURE REVIEW PROCESS

Based on the application of the search criteria through a parallel strategy, the literature collection of an initial set of 206 papers was derived from online databases such as PubMed and Google Scholar. The literature was restricted to studies conducted on mammals including equine. The precision search performed on the initial set was constrained to K9-701 composition and its health benefits within Titles or Abstracts of the papers from initial set and yielded the relevant set consisting of 87 papers. The relevant set was reviewed by the domain experts using CytoSolve user interface to identify the nutritional profile and health benefits of K9-701, which forms the basis of this systematic review. The final results of the systematic review are summarized in Figure 1.

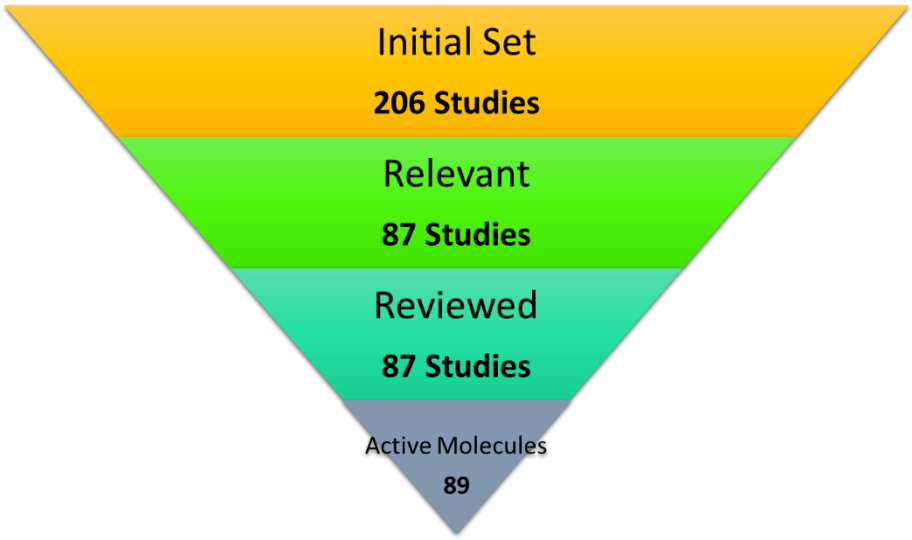

Figure 1: Systematic Review Results. There were 206 scientific papers (initial set), which met our search criteria. Of those, 87 (relevant set) appeared to be interesting based on the title and abstract, and were chosen as the quality studies upon which this systematic review is based. We identified 89 molecules from the study set.

3.0 HEALTH BENEFITS OF ZEOLITE

Zeolites have a wide range of applications in the medicinal field. Their general range of applications includes maintenance of homeostasis like the immune system, pH and balanced reactive oxygen species (ROS) levels. Their application with skin includes regeneration of wounds, skin reddening, acne and other common skin problems. In addition with the specific effects they are also involved in general health aspects and also reduce adverse effects of chemotherapy and radiotherapy (Laurino & Palmieri, 2015).

Natural zeolites – clinoptiloliteis, have been supplemented to the animals due to their antimicrobial property. As supplementation of hens with these zeolites have reported to have increased quality with egg production, egg weight and quality, they are advised for their effective action in improvement and against microorganisms. But the impact of these were mentioned to be affected by certain factors of their type, purity, size and proportion of zeolite composition (Prasai et al., 2017).

Due to their selectivity for calcium, retainment and release of such minerals, zeolites have been found to be beneficial in inducing calcium levels in bone enhancement which is due to their high affinity for calcium. Additional supplementation of zeolites even in calcium deficient diets have proved an improved growth properties. In addition to this, zeolites reduce toxic levels of ammonia and prevent mortality (Bintaş et al., 2014).

3.1 Immunomodulatory Effects of Zeolites

Clinoptilolite interacts with microfold cells (M-cells) in the intestine to modulate immune actions with the induction of immunoglobulin A (IgA). The intake of clinoptilolite by the M - cells within the Peyer’s

patches mediates mucosal mediated immune responses through a step by process initiated through the redox homeostasis process followed by impact on Peyer's patches and immune response. Additional points to be noted include the retention of clinoptilolite within the M-cells causes its prevention to enter into the blood cells and also that the other cells cannot participate in accordance with the intestinal cells (Pavelić et al., 2018).

While evaluating possible clinoptilolite immunomodulatory effects in the intestine, it should be emphasized that M-cells can uptake nano- and submicro - particles, which can probably induce changes in the redox homeostasis in a cell. It is important to note that M-cells apical and basolateral sides, which communicate with Peyer's patches, are polarized (Society for Mucosal Immunology, 2012) and one may hypothesize that, due to this particular phenotype, M-cells retain clinoptilolite particles or silica particles released from the clinoptilolite material (tuff), which does not enter the blood system (Nizet et al., 2018) and act locally on this tissue. The schematics of how zeolite (red diamond) affects immune system are shown in Figure 2.

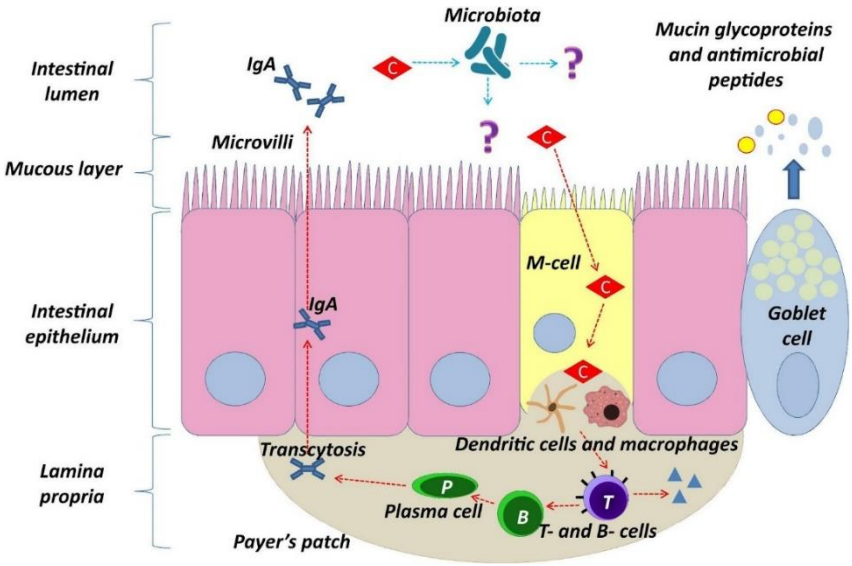

Figure 2: Zeolites impact on immunomodulatory effects in the intestine (Pavelić et al., 2018)

This clinoptilolite-induced M-cells' communication with Peyer's patches, as similarly shown by Pavelic et al. (2002), increases the immune response either through particle intake or microbiota effect (Sabbioni et al., 2016), and in particular, stimulates IgA producing B lymphocytes (plasma cells), a defensive mechanism of the intestinal tract against pathogenic bacteria. By means of this mechanism, the host can detect new bacterial types and ignore previously encountered bacteria in the intestine.

Lymphocytes stimulation by silicates, which also act as super-antigens, was already shown for different silicate materials in in vitro conditions and this mechanism may underlie immunomodulation activity of clinoptilolite in the intestine as well (Ueki et al., 1994; Aikoh et al., 1998). In this study, authors also proved that clinoptilolite materials were efficient in the removal of aluminum from aluminum chloride-intoxicated rats in vivo.). Interestingly, oral aluminum bioavailability is known to be increased by acidic pH, such as the pH in the human intestine, but in case of clinoptilolite tuff, it may be decreased, as this is a silicon-containing compound that releases certain amounts of dissolved silica (Jurkic et al., 2013).

3.2 Anti-inflammatory Effects of Zeolites

Natural zeolites are microporous crystalline aluminosilicates with channels and cavities of molecular dimensions, allowing for adsorption, ion exchange, water sorption/desorption, molecular sieving and excellent binding capacity for toxins and other harmful substances. Of particular interest herein is the use of zeolites in biomedical applications, namely as active ingredients in medical devices, carriers for drugs like antibiotics, wound healing accelerators, adjuvants in anticancer therapy and several other applications (Cervini-Silva et al., 2016).

In particular, a related study reported that zeolite from San Andrés, Cuba, composed primarily by clinoptilolite and mordenite, showed a high binding capacity of histamine, a neurotransmitter critical to the inflammatory response and pruritus. Edema forms due to increases of liquid level within the stroma, changes of vascular permeability, especially capillaries, and it is frequently associated to increased histamine levels as well as to the up-regulation of pro-inflammatory substances (e.g., cytokines IL-1, IL-6, and TNF- α). This zeolite sample can adsorb up to 16 mg of histamine per g under physiological conditions. The anti-inflammatory activity was attributed in part to the adsorption of histamine on zeolite by means of multiple π -charge-transfer and/or hydrogen bonding (Cervini-Silva et al., 2016).

3.3 Antioxidant Effects of Zeolites

Homeostasis in normal cells includes a balance between ROS production and antioxidant defense activity. When ROS production exceeds antioxidant capacity, we usually perceive the process as “oxidative stress” that leads to organic damage. Recently, a preliminary efficacy study performed on patients with dyslipidemia has also shown a positive effect of clinoptilolite supplementation on lowering the total lipid count and LDL (low density lipoproteins), which may also be indirectly correlated with its general antioxidative effect (Cutovic et al., 2017).

When hepatectomized rats were supplemented with a micronized clinoptilolite preparation, ‘Froximun,’ MDA levels were significantly lower, while liver tissue antioxidant mechanisms were strengthened, as witnessed by a significantly higher activity of Cu-Zn SOD and GSH (Saribeyoglu, 2011). Upon supplementation of pups with clinoptilolite, oxidative damage was restored and levels of

GSH-Prx were substantially ameliorated in the cerebral cortex and medulla oblongata. For instance, tribomechanically-micronized zeolite increased SOD activity in a transgenic mouse model of the Alzheimer disease in the hippocampus and cortex, while it concomitantly reduced Aβ (x-42) amyloid beta levels in the hippocampus (Montinaro et al., 2013).

Increasing evidence suggests that chronic hyperglycemia can cause excessive production of free radicals, particularly reactive oxygen species (ROS) (3–5). clinoptilolite was used in our study for the following reasons. clinoptilolite is the most abundant zeolite in Iran, and it has a total cation-exchange capacity of about 200 mEq. In addition, it has been proposed that zeolites tend to neutralize the solutions and exchange electrons and also that zeolites, such as clinoptilolite, may modify the disorders in a redox state and arrest the generation of peroxides and free radicals through their amphoteric character, as in the clinoptilolite –iron oxidase system (46, 50).

3.3 Blood Glucose Control Effects of Zeolites

Zeolites are among natural remedies proposed to reduce blood sugar. Blood glucose levels decreased in the clinoptilolite-treated healthy rats during the study ($p<0.05$). Blood glucose levels differed significantly between groups across time ($p<0.001$). Although significant differences were not found between the experimental groups, the data reveal that clinoptilolite reduced MDA levels more than NCLN in the normal group ($p<0.05$), but MDA levels mildly increased in both clinoptilolite - and NCLN- supplemented diabetic rats.

Clinoptilolite has been shown to mildly reduced blood glucose levels in nondiabetic rats. One study of type 1 diabetes in a non-obese diabetic animal model showed that Ca^{2+} -zeolite used in combination

with insulin could modify hyperglycemia through deterrence of the sodium-glucose transporter. It has been observed that dietary administration of clinoptilolite could alleviate deleterious effects of nitrates, including hyperglycemia and impairment of protein metabolism.

3.4 Bone Health Effects of Zeolites

Skeletal injuries are common in athletic horses. A recent study evaluating the role dietary silicon showed beneficial effects in racehorse injuries. Supplementation of dietary silicon from sources such as zeolite led to reduction in mineral loss from the cannon bone, and prevention of bone injury. Supplementation of silicon increased the horses' ability to complete the races with fewer injuries. It was proposed that the mechanism of action for reduced injury rates in horses supplemented with dietary silicon could be the change in bone turnover rate (Neilson, 2023).

4.0 HEALTH BENEFITS OF DIATOMACEOUS EARTH

Diatomaceous earth is white, cream or ash colored. The material is mined and ground, then graded for countless uses. Diatomaceous earth is a naturally occurring siliceous sedimentary mineral compound composed of the microscopic skeletal remains of the unicellular algae like plants whose cell walls are impregnated with silica. As Diatomaceous earth passes through the digestive system, it rubs against parasites and being very abrasive, causes serious damage causing the parasite to die and pass out of the animal with no negative side effects making the effects on the animal nothing but beneficial.

Benefits of using diatomaceous earth when used in feed additives include; natural, environmental friendly control of insects, internal parasites and worms, healthier appearance of the animals, including equine, and better feed conversion ratio, a marked reduction of vet bills and losses, reduces odor and moisture in the barns and stalls, stops dirt licking and corral gnawing, reduced flies (fly larva are killed

by the diatomite's left in the manure) and manure odor. Generally, higher weight gains were reported among the treatments as compared to the control diet. Diatomaceous earth was observed to improve the general appearance of equine as they had healthier coat appearance and their healthy status improved.

4.1 Pest Repellent Effects of Diatomaceous Earth

Pests such as bed bugs are obligatory hematophagous insects feeding commonly on humans. These ectoparasites have a long history of presence in mammals, including equine, with drastic consequences. For many years, they have been the main issue of public health and probably one of the most common ectoparasites on people with global spread due to human activities. Infestations occur in all ethnic groups and at all socioeconomic levels. Several publications have reported the effectiveness of diatomaceous earth in controlling bed bugs (Akhtar and Isman 2013, Singh et al. 2016). Diatomaceous earth adheres to the body of the bed bug and damages the protective waxy layer of the bed bug cuticle by sorption and abrasion. These particles penetrate the bed bug body and get stuck between its exoskeleton joints. When the bed bug moves, these sharp particles physically cut the bug organs. Consequently, it causes the loss of water from the bed bug's body and ultimately death.

4.2 Effects of Diatomaceous Earth on Bone Health

A new generation of bioactive coatings has emerged with the silicon substituted hydroxyapatite thin films (Si–HA). This hydroxyapatite, modified with the inclusion of small concentrations of silicon has been demonstrating to improve the osteoblast proliferation and the bone extracellular matrix production. A recent study that consisted in the comparison of dietary silicon intake with bone mineral

density in humans concluded that the bone mineral density was positively and significantly linked to dietary Si intake in men and premenopausal women (Alvarez et al 2009).

A recent study that consisted in the comparison of dietary silicon intake with bone mineral density in humans concluded that the bone mineral density was positively and significantly linked to dietary Si intake in mammals, including equine. Within the all available techniques to produce the Si–HA coatings, the pulsed laser deposition (PLD) technique is very promising to obtain high quality coatings. The proliferation was significantly higher ($p<0.05$) on the control than to the both Si–HA coatings and indicates the healthy stage of the cells.

Although initially there were no significant differences ($p>0.05$) in the osteoblastic activity between both Si–HA coatings, at the 7 day the ALP activity values for the cells adhered to the Si–HA from diatomaceous earth was significant higher ($P<0.01$) than to the Si–HA from silica. This higher osteoblastic activity at the diatomaceous coating can be probably explained by the presence of the minority elements at the coating composition.

5.0 HEALTH BENEFITS OF METHYLSULFONYLMETHANE

Health claims associated with MSM include relief of pain, inflammation, arthritis, allergies, certain parasitic infections and asthma (Martin, 1988; Lawrence, 1998; Jacob et al., 1999). It is also used to nourish skin, hair and fingernails, due to its sulfur concentration, which contributes to cysteine, a sulfur amino acid that is required for the production of keratin (Richmond, 1986). The data suggest that MSM

is nontoxic at relatively high dosages, for example, the no-observed-adverse-event level (NOAEL) is listed at 5 g/kg when given orally or intraperitoneally to small mammals. This study reported the acute oral LD50 of 17,020 mg MSM/kg of body weight. Currently, MSM is most commonly used as a treatment for arthritis and interstitial cystitis (Butawan et al., 2017).

5.1 Antioxidant and Anti-inflammatory Effects of MSM

Inflammation and oxidative stress have an interdependent relationship, whereby excess ROS can induce proinflammatory cytokine production and/or cytokine signaling can include ROS production. In short, a proinflammatory priming signal triggers NLRP3 monomer transcription and translation. Upon activation by one of several signals including ROS generation, NLRP3 monomers oligomerize to form the activated NLRP3 inflammasome, which can then promote the maturation and secretion of proinflammatory cytokines (Butawan et al., 2020). During the priming step, MSM may reduce the activity of the proinflammatory NFκB transcription factor. This reduces the transcription of the NLRP3 monomer, thus, resulting in impaired NLRP3 inflammasome assembly and reduced suppression on Nrf2. The schematics of anti-inflammatory and anti-oxidant effects of MSM are shown in Figure 3.

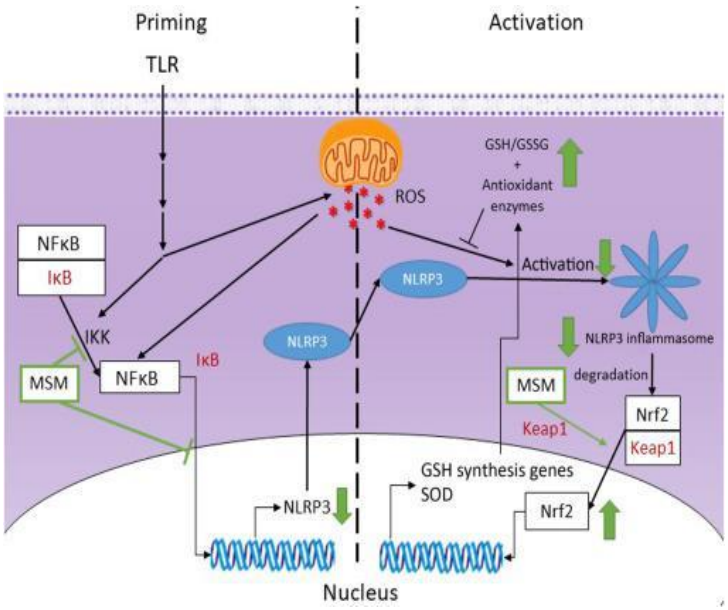

Figure 3: MSM exhibiting antioxidant activity with the inhibition of NFkB and NLRP3 activation (Butawan et al., 2020)

5.2 Effect of MSM on Osteoarthritis

The demand for arthritis pain control has resulted in the widespread use of palliative drugs, e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and cyclooxygenase-2 (COX-2) inhibitors, surgical interventions, and in recent years, the use of complementary and alternative medicine (CAM). A dietary supplement with increasing use is MSM often in combination with glucosamine and chondroitin sulfate has numerous efficacy trials supporting its use in osteoarthritis. MSM is popularly used for arthritic and rheumatic pain.

MSM may have anti-inflammatory activities, chemopreventive properties, prostacyclin (PGI2) synthesis inhibition, anti-atherosclerotic action, salutary effect on eicosanoid metabolism, and free radical scavenging activity. In murine models, MSM was shown to effect inflammatory conditions such as rheumatoid arthritis and lupus. The changes in the physical function in the MSM group were also

greater than in the placebo group at 12 weeks, p = 0.045. The pain and physical function mean decreases from baseline to 4, 8 and 12 weeks in the MSM group were greater compared to the placebo group. In the MSM group pain decreased by 14.6 mm (25.1%), and in placebo it decreased by 7.3 mm (13.2%) at 12 weeks. For physical function, stiffness and total symptoms, the decreases in MSM group were 15.7 mm (30.4%), 10.1 mm (19.7%), and 13.4 mm (25.1%) and the decreases in placebo group 8.8 mm (16.7%), 6.5 mm (11.7%), and 7.5 mm (13.8%), respectively.

Use of MSM significantly decreased the mean pain index from 1.53 ± 0.51 to 0.74 ± 0.65, and combination treatment resulted in a highly significant decrease in the mean pain index (1.7 ± 0.47 to 0.36 ± 0.33; $p < 0.001$). At the end of 12 weeks treatment, the mean swelling index significantly decreased from 1.43 ± 0.63 to 0.52 ± 0.51 with Glu, and from 1.30 ± 0.65 to 0.39 ± 0.50 with MSM, while there was a greater decrease in swelling index with combination therapy, from 1.43 ± 0.63 to 0.14 ± 0.35, after 12 weeks ($p < 0.05$ compared with Glu and MSM alone). A recent investigation used Lequesne index to compare the efficacy of Glu and MSM and their combination with placebo, to evaluate the effect on inflammatory, functional and day-to-day activity of the knee joint in osteoarthritis. At baseline all the groups had similar indices suggesting a comparable degree of osteoarthritis. Treatment with Glu and MSM, individually, decreased the Lequesne index significantly from 13 ± 0 to 8.85 ± 3.2, and 12.48 ± 2.25 to 8.48 ± 1.89 ($p < 0.001$) at the end of 12 weeks' treatment, respectively (Usha & Naidu, 2004).

5.2 Effect of MSM on Neurodegeneration

Chronic uncontrollable stressors are related with risk of developing cardiovascular diseases and neurodegenerative disorders that includes Alzheimer disease (AD). The exposure to environmental stressors such as pollutants, toxic chemicals and radiation induce oxidative stress accompanied with chronic inflammation. One of the exogenous environmental stressors is ionizing radiation (IR) that present in our lives originating from natural and manmade sources. Bisphenol A (BPA) was implicated in several chronic diseases such as diabetes, cardiovascular diseases, kidney diseases, cancer and adverse neurological effects, especially with brain development and promotion of neurodegenerative diseases (Abdel-Rafei & Thabet, 2020).

In addition, MSM acts as a source of sulfur in formation of sulfur-containing amino acids, which might intensifies its antioxidant efficacy via rebalance of glutathione cycle. MSM concurrent administration powerfully reversed this condition since it significantly elevated ($p < 0.001$) relative nuclear to cytoplasmic Nrf-2 ratio by 5.5 and 6 folds and by 5.9 and 8.5 folds in cortex and hippocampus, respectively of BPA + MSM and BPA + R + MSM groups, respectively as compared to BPA and BPA + R groups, respectively. MSM treatment efficiently recovered antioxidant machinery by about 2 and 3.2 folds for GSH, by about 1.7 and 2.5 folds for GPx, by about 1.6 and 2.2 folds for SOD and by about 1.8 and 3.1 folds for CAT in cortex of BPA + MSM (Abdel-Rafei & Thabet, 2020). The schematics of MSM effect on neurodegeneration is shown in Figure 4.

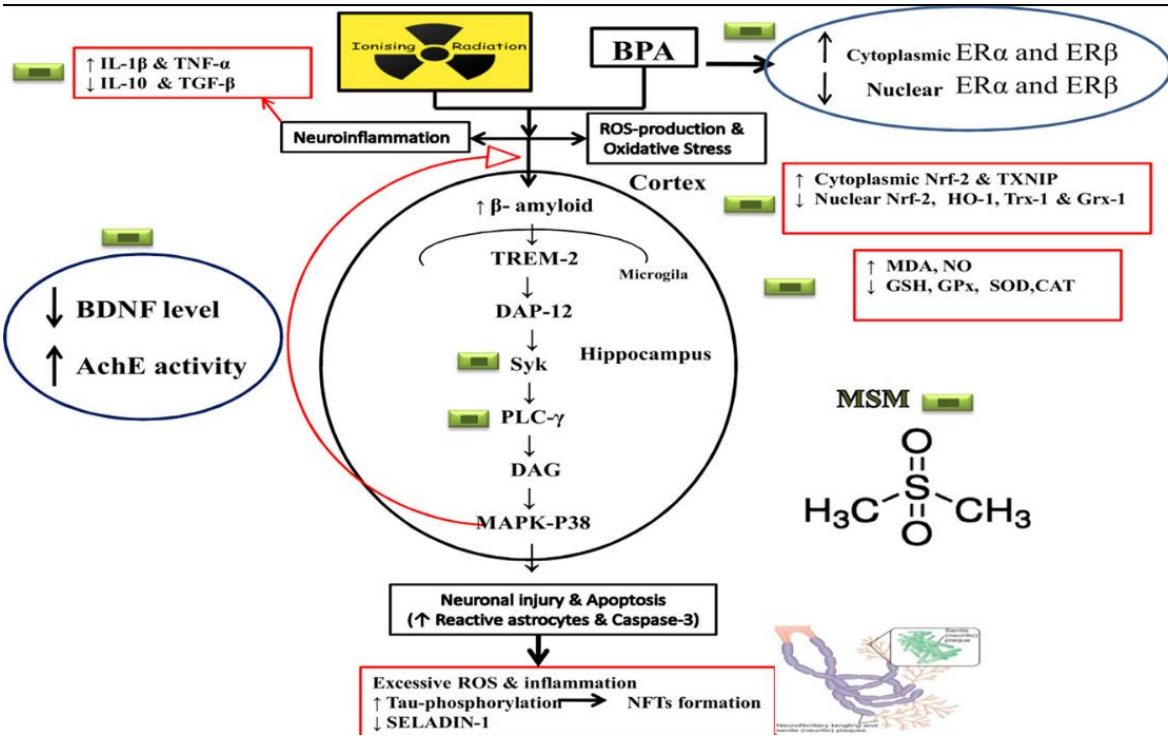

Figure 4: BPA induced neurodegeneration ameliorated by methylsulfonylmethane (MSM) (Abdel-Rafei & Thabet, 2020)

6.0 HEALTH BENEFITS OF HYALURONIC ACID

The biological functions of HA include maintenance of the elastoviscosity of liquid connective tissues such as joint synovial and eye vitreous fluid, control of tissue hydration and water transport, supramolecular assembly of proteoglycans in the extracellular matrix, and numerous receptor-mediated roles in cell detachment, mitosis, migration, tumor development and metastasis, and inflammation in mammals, including equine (Balazs et al., 1986; Toole et al., 2002; Turley et al., 2002; Hascall et al., 2004).

In addition to its lubricating and cushioning properties, demonstration of some in vitro anti-inflammatory activity and a possible disease-modifying effect for hyaluronic acid in animals has

prompted its investigation as a treatment in osteoarthritis and, to a much lesser extent, in rheumatoid arthritis. Hyaluronic acid 20mg, as weekly intra-articular injections for 3 to 7 weeks, improved knee pain and joint motion in patients with osteoarthritis (Goa & Benfield, 1994).

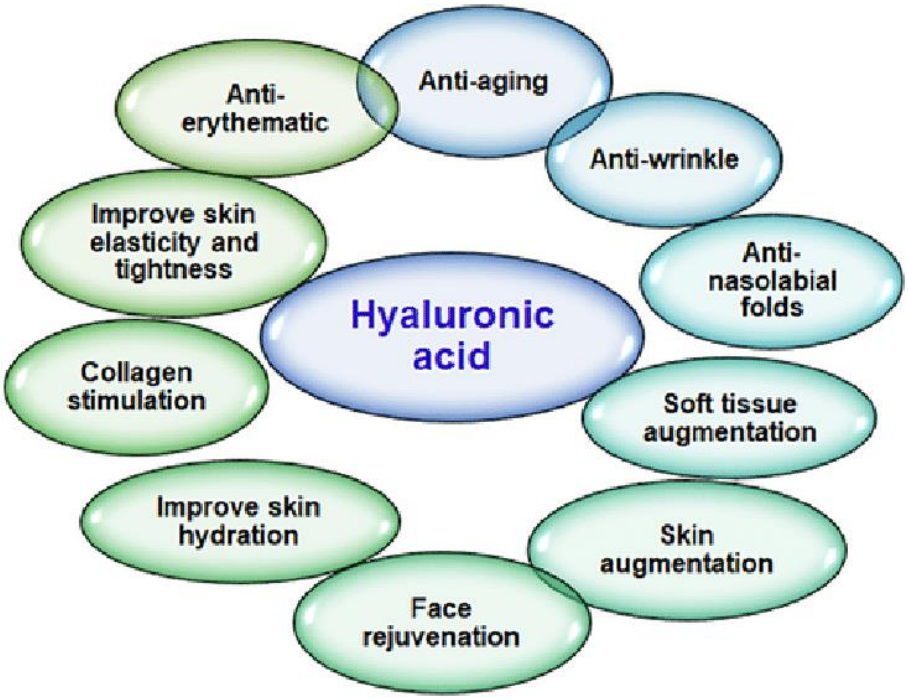

Figure 5: Overall health benefits of Hyaluronic acid supplementation (Bukhari et al., 2018)

Being an important component of the ECM and due to its available derivatization scenarios, HA is widely used in drug delivery through several routes: cutaneous, ocular (intravitreal, periocular, subretinal), topical, nasal, oral, etc. HA can be conjugated with drug molecules (in the form of prodrugs) or can be incorporated in several molecular architectures (nanoparticles, microparticles, microspheres, gels, polyplexes, polymersomes, liposomes, micelles, implants, etc.).

6.1 Effect of Hyaluronic Acid on Skin Health

The skin naturally achieves hydration through dermal glycosaminoglycans (GAGs) such as HA, indicating to the importance of selecting HA of the proper molecular weight in effective skin formulations. Supplementation of HA over week 6 with continued improvement ($p < 0.001$) in all attributes: smoothness (64%), plumping (60%), hydration (63%), fine lines (31%), wrinkles (14%), and overall global assessment (43%). The improvement was probably due to the enhanced water holding capacity of the topically applied HA, which was effective for all Fitzpatrick skin types regardless of the degree of photo damage.

The assessment showed that the HA group had significantly higher stratum corneum water content in the facial measurement sites ($p = 0.02$) as compared with the placebo group at 12 weeks after ingestion. The assessment showed that the HA group had significantly lower transdermal water transpiration in the face versus the placebo group after 12 weeks of treatment ($p = 0.009$). Higher values of percutaneous water transpiration were associated with rougher and unhealthy skin (Hsu et al., 2021). A daily HA dose of 120 mg ($n = 17$) significantly increased skin moisture in the lower left part of the eye compared with a daily placebo dose ($n = 18$) 2 weeks after HA was consumed in a randomized, double-blind, placebo-controlled study for subjects with dry skin (average age ± S.E; 31.5 ± 13.3) (Kawada et al., 2014)

Because aging is associated with a decrease of HA in the skin, a randomized, double-blind, placebo-controlled study of middle-aged and elderly female subjects with dry skin (average age ± S.E.; 43.6 ± 4.6) was conducted [17]. The HA group ($n = 19$), which ingested 120 mg/day of HA, was found to have

a significant increase of skin moisture, and a tendency for the skin moisture to increase in the face, compared with the placebo group (n = 20) after 3 and 6 weeks of ingestion (Kawada et al., 2014).

6.2 Effect of Hyaluronic Acid on Osteoarthritis

Osteoarthritis is characterized by decreases in the amount and viscosity of endogenous hyaluronic acid. Injecting exogenous hyaluronic acid corrects these abnormalities. French legislation requires that intra articular hyaluronic acid injections be prescribed and administered by a rheumatologist, orthopedic surgeon, or physical rehabilitation physician. All the clinical efficacy criteria (pain, function, and quality of life) were significantly improved three months after hyaluronic acid therapy compared to the three previous months, and further improvements in pain and function were achieved during the fourth through sixth month after hyaluronic acid (Mazières et al., 2007). Intra-articular use of hyaluronic acid (HA) was shown to improve symptoms of synovitis and osteoarthritis in horses by reducing the release of inflammatory cytokines such as IL-1, IL-6, IL-10, TNF-α, (Neuenschwander et al., 2019)

Intra-articular (IA) injection of HA has been recommended to alleviate pain and improve joint function in patients with knee osteoarthritis. Chondroprotective and analgesic properties inherent to HA suggest that HA can delay total knee replacement (TKR) surgery, a treatment popular enough that it has become a key driver of health care costs. Kurihara et al. reported that HA is decomposed into 2–6-membered polysaccharides by enteric bacteria, and these polysaccharides are partially absorbed into the body by the small intestine. Following the decomposition of HA by enteric bacteria to a low MW form, free polysaccharides are known to migrate into the joints and other tissue.

Oral HA binds to an intestinal receptor (Toll-like receptor-4; TLR-4). Cytokine array analysis showed that HA enhanced the production of interleukin-10 (IL-10), an anti-inflammatory cytokine. DNA array analysis of tissue from the large intestine showed that HA up-regulates suppressor of cytokine signaling 3 (SOCS3) expression and down-regulates pleiotrophin expression. These results suggest that the binding of HA to TLR-4 promotes IL-10 and SOCS3 expression and suppresses pleiotrophin expression leading to anti-inflammation of arthritis (Fig. 6) (Oe et al., 2016).

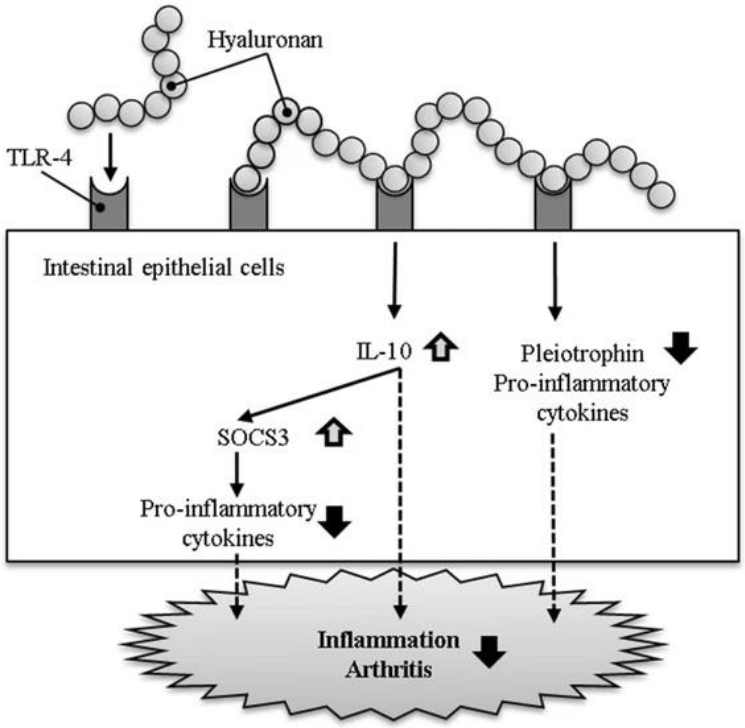

Figure: 6: Hyaluronic acid mediating reduced effect on osteoarthritis (Oe et al., 2016)

7.0 HEALTH BENEFITS OF RED RASPBERRY LEAF

Tea made from the leaves of *Rubus idaeus* L. (raspberry) has been used for centuries as a folk medicine to treat wounds, diarrhea, and colic pain. The bioactive potential of raspberry leaves in exhibiting

cytoprotective activity on human laryngeal carcinoma and colon adenocarcinoma has been documented. Antithrombotic activity of raspberry leaves was reported by Han et al.

The antioxidant actions of berries are well known, and they top the list with highest antioxidant capacity. The anthocyanin's in raspberry help in improvement of endothelial function by its protective action on endothelial cells with less oxidative stress. The polyphenols as anthocyanin's in red raspberry and ellagic acid stimulates insulin secretion in animals. Red raspberry's anthocyanins acts as an anti-obesity agent by changing lipid metabolism as by enhancing lipolysis in adipocytes. We know that raspberry is richly blessed with polyphenols which helps in reducing oxidative stress, inflammation and improvement in insulin signaling there is promising chance for treatment for Alzheimer disease (Singh et al., 2020).

7.1 Red Raspberry Leaves Mediated Effects as Antioxidant and Anticancer Agent

According to several studies, raspberry leaves are a rich source of flavonoid derivatives, represented by quercetin derivatives, as well as phenolic acids, triterpenes, mineral salts, and vitamin C. The mostly appreciated and well-established bioactive compounds of raspberry leaves are ellagic acid and ellagitannins, whose contents are highly affected by the cultivar and geographical location of the plant's origin. Survival curves and the highest nontoxic concentrations of raspberry leaf extract were determined after 1 and 2 hours of cell treatment because in this period of time, biologically active compounds are stable enough to accomplish their biological effect without the formation of secondary metabolites or spontaneous degradation products, which could influence the results overall.

In just one hour of incubation of HEp2 cells, raspberry leaf extract increased the total level of GSH in comparison with the control. In cells treated with H2O2 and raspberry leaf extract the level of GSH significantly decreased in comparison with the control treated with H2O2. Leaf and bark extracts containing the highest quantity of polyphenolic compounds showed the highest radical-scavenging activity. The activity of extracts prepared from winter stems was significantly lower compared to that of spring stem and leaf extracts (Garjonyte et al., 2022).

7.2 Red Raspberry Leaves Extract Mediated Effects as Women's Tonic

As a uterine tonic, uterine relaxant, and uterine stimulant as well as for its nutritive properties, red raspberry leaf has a variety of benefits for women's health. Drank as a tea or taken as a tincture over the course of the menstrual cycle (or when taken in higher doses during menses), it can help alleviate dysmenorrhea. Its astringent action can help curb excessive menstrual flow and when taken over an extended period of time (3 cups daily for 1-3+ months), can prevent excessive bleeding. The astringent properties are useful when treating vaginal infections, such as Chlamydia. This tonic helps to prepare the uterus for birth, relaxing and relieving cramps, it is so effective that many women report pain-free or virtually pain-free labors thanks to red raspberry leaf tea (Zheng et al., 2010).

The findings suggested that raspberry leaf can be taken safely during pregnancy to shorten labor with no expected side effects for women or their babies. The study also reported a decreased likelihood of pre- and post-term gestation, and fewer obstetric interventions, including decreased amniotomy, caesarean section, forceps delivery, and vacuum extraction in the group that had taken raspberry leaf when compared with the control group (Romm, 2010).

8.0 HEALTH BENEFITS OF FENUGREEK

Fenugreek is one of the oldest medicinal plants and the medicinal properties are well documented in the ancient medical literature. In Ayurveda, the traditional Indian medical system, fenugreek was used as a digestive aid and ancient Egyptians used it to incense and embalm mummies and also as lactation aid. In traditional Chinese medicine, fenugreek was used to treat edema in the legs. There are number of folkloric uses of fenugreek, including the treatment of lung congestion and sinus, indigestion, baldness in men, hair tonic and conditioner and as galactogogue. There have been several preliminary animal and human trials that demonstrated fenugreek exhibiting hypoglycemic, hypolipidemic, and hypocholesterolemic effects. Fenugreek has also been reported to possess anti-fertility, anticancer, anti-parasitic, and antimicrobial effects (Zameer et al., 2018).

8.1 Fenugreek mediated effects on diabetes

Numerous herbs/ plants are used to treat T2DM that but fenugreek seeds (FGS) are considered to be more effective. Fenugreek seeds help in lowering glucose levels in blood. FGS contains chemicals which have anti-diabetic activity like fiber, saponins, amino acids etc. As the result of this study it is concluded that fasting blood glucose level effectively decrease and showed beneficial effects in the treatment of type 2 diabetes mellitus. Hypoglycemic effect of fenugreek seeds is reported to be due to the rich components called dietary fiber that is 45.4% (13.3 soluble and 32% soluble) and galactose. Hence it is implied that BMI also has a contributory role in determining glucose tolerance in patients which might possibly exert an effect on FBG as well as macro and microvascular complications of diabetes in the patients (Anderwald et al., 2011; Janghorbani & Amini, 2008). In a clinical trial where two groups were checked at baseline, after 1 month and then after two months, it was reported that

fenugreek seed reduces blood glucose level as well as decrease in HbA1c levels of the group of participants that undertook fenugreek seeds treatment.

8.2 Hypolipidemic Effect of Fenugreek

A number of adipogenic and lipogenic factors such as peroxisome proliferators-activated receptor-γ (PPAR-γ), the CCAAT enhancer element-binding proteins-α, β, δ (C/EBP-α, β, δ) and the sterol regulatory element-binding proteins (SREBPs) as well as fatty-acid binding protein aP2 etc., are upregulated in differentiated cells (9,10). These stand as possi- ble therapeutic targets for lipid abnormality-related metabolic diseases.TEFS treatment (50 μg/ml) to cells differentiated for 6 days resulted in significant decrease in fat accumulation on day 12 (Figure 2a C vs. D–F and 2b C vs. D–F) (Vijayakumar et al., 2010).

In adipocytes, inactivation of insulin signaling molecules reduces the translocation of intracellular glucose transporter 4 (GLUT4) to the plasma membrane and consequently impairs glucose import into the cells (Luan et al., 2018). Treatment with four fenugreek flavonoid glycosides significantly reduced the expression of PPARγ and C/EBPα compared to fully differentiated control adipocytes. All of the four fenugreek flavonoid glycosides dramatically decreased the expression level of FAS. Our results revealed that isoorientin enhanced glucose uptake in dexamethasone-induced insulin resistance 3T3-L1 adipocytes, similar to rosiglitazone. In addition, the phosphorylation level of Akt2 and AMPK was increased in the presence of isoorientin (Luan et al., 2018).

8.3 Fenugreek mediated effects as anticancer agent

Diosgenin is the most abundant steroidal sapogenin in fenugreek seeds. Diosgenin is a phytosteroidal saponin and a major bioactive compound found in the seeds of T. foenum-graecum, commonly known

as fenugreek, and in the roots of wild yam (Dioscorea villosa). Several studies have demonstrated the diverse biological activities of diosgenin, such as hypolipidemic, anti-inflammatory, anti-proliferative, hypoglycemic activity, and as a potent anti-oxidant. In addition, diosgenin inhibited cancer cell proliferation and induced apoptosis in a variety of cancer cell lines including colorectal, hepatocellular, breast, osteosarcoma, and leukemia (Sethi et al., 2018).

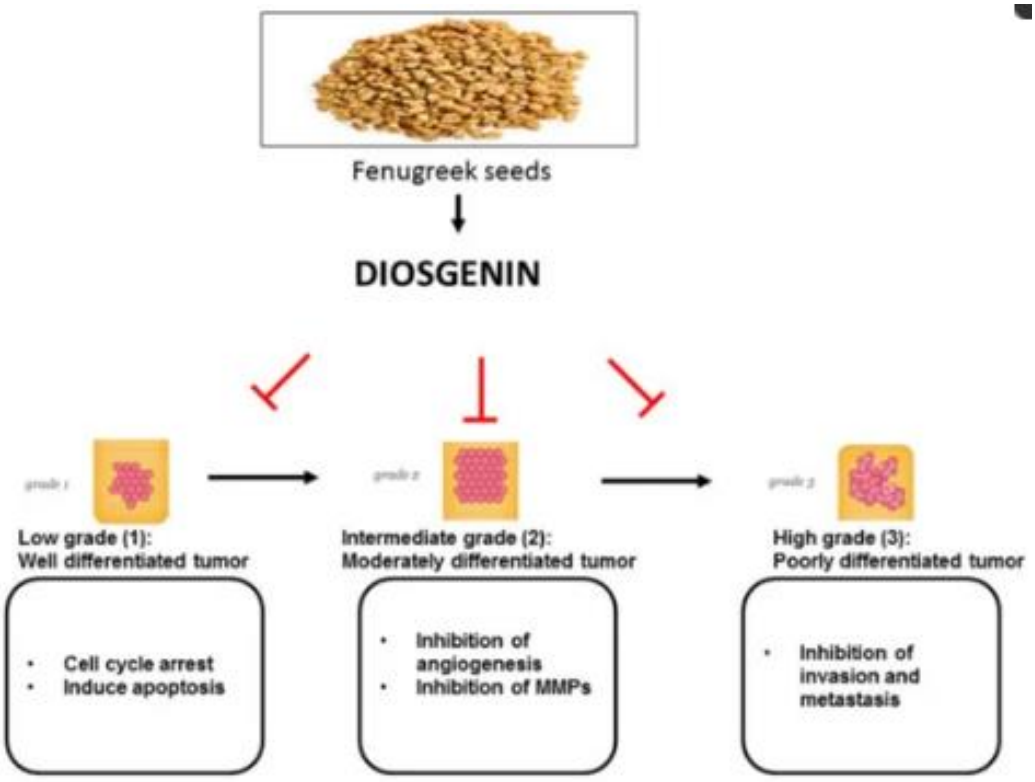

Figure: 7: Fenugreek seeds mediated health benefits especially as inhibiting cancer progression (Sethi et al., 2018)

Diosgenin has been shown to potently suppress constitutively-activated pro-inflammatory and pro-survival signaling pathways in a variety of cancer cells, and induced apoptosis. Diosgenin also suppressed STAT3 transcriptional activity and the expression of its downstream gene products involved

in proliferation, invasion and metastasis. ome of the earlier studies by Shishodia and Aggarwal reported that diosgenin abrogated TNF-α-induced NF-κB activation and suppressed osteoclastogenesis in RAW 264.7 macrophage cells. Diosgenin is also a potent inhibitor of cancer cell invasion, migration, and tumor-associated angiogenesis.

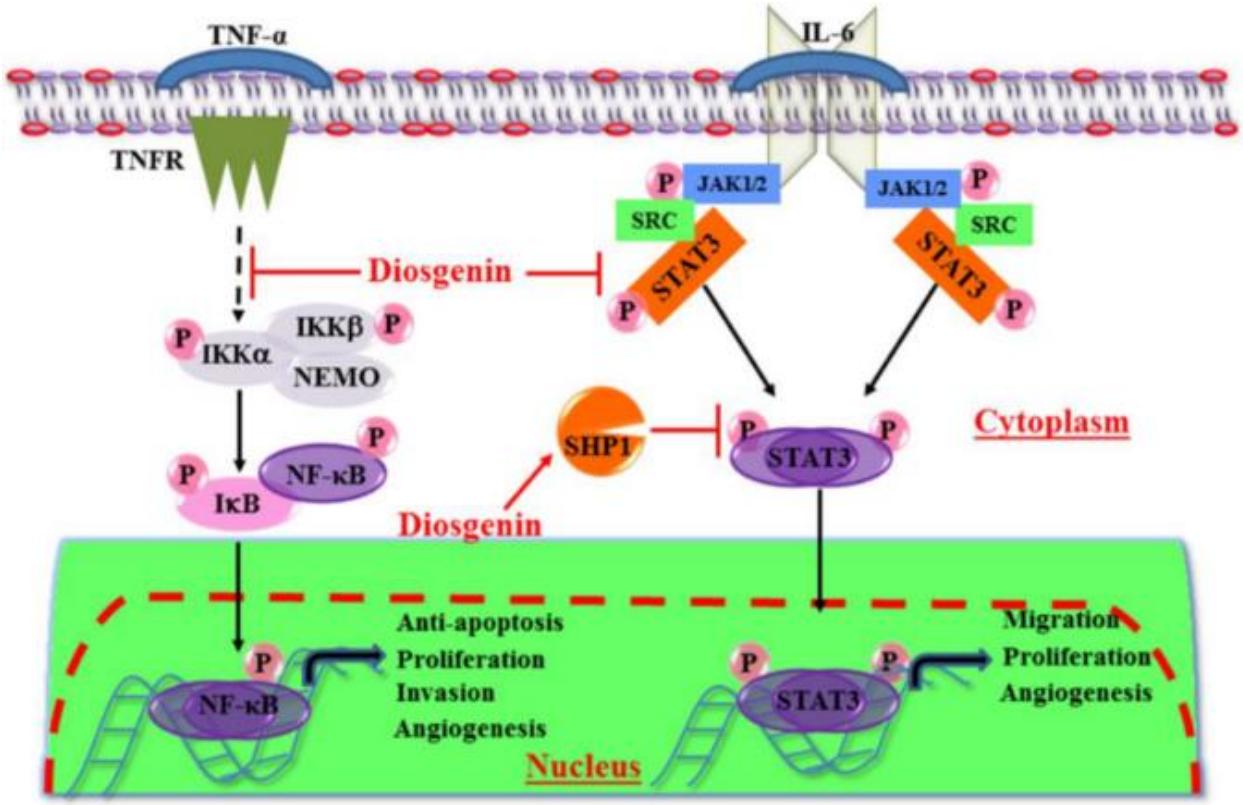

Figure 8: Diosgenin - major component of fenugreek mediated effects as anticancerous agent (Sethi et al., 2018)

9.0 HEALTH BENEFITS OF ORGANIC KELP

Kelp possesses ample of nutrients and minerals which make them highly bioactive. Anticoagulants, antibiotics, antiparasites, antihypertensives, reducers of blood cholesterol, dilatory drugs and

insecticides are made with the help of such properties. Due to its numerous medicinal benefits, it has been used in traditional Chinese medicine since centuries and is listed in the Chinese pharmacopeia. It is often used to control edema, as an expectorant and antitussive, and as a remedy for testicular pain and swelling. Despite its salty character, the herb is slightly hypotensive (S. K. Kim & Bhatnagar, 2011).

9.1 Kelp as an antioxidant agent

Among these active compounds, polyphenolic phlorotannins, a group of complex molecular assemblages from the polymerization of phloroglucinol [2], represent a large class of well-characterized brown algae secondary metabolites [3]. Phlorotannins are a group of major polyphenol secondary metabolites found only in brown algae and are known for their bioactivities and multiple health benefits (Bai et al., 2020).

Phlorotannins encapsulated by PVP nanoparticles (PPNPS) with different loading ratios were prepared for characterization. As expected, H2O2 treatment significantly increased cellular ROS production in HaCaT keratinocytes, while PPNPS treatment significantly reduced ROS production. When the concentrations of PPNPS treatment were 6.25 and 12.5 μg/mL, the proportion of ROS production decreased by 12 and 18%, respectively. The results are consistent with previous reports that phlorotannin could be developed as a potential ROS inhibiton. The cell viabilities among 6.25, 12.5 and 25 μg/mL PPNPS (1:8, w/w) were not significant ($p > 0.05$), but the cell viability significantly increased at the concentration of 50 μg/mL PPNPS (1:8, w/w) ($p < 0.05$). These results indicated that PPNPS were not cytotoxic to HaCaT keratinocytes. It can safely be used on skin or as functional chemicals in cosmetics.

10.0 HEALTH BENEFITS OF TURMERIC

Turmeric (Curcuma longa) has been used for 4,000 years to treat a variety of ailments. Turmeric has long been used in both Ayurvedic and Chinese medicine as an anti-inflammatory, to treat digestive and liver problems, skin diseases, and wounds. The curcumin in turmeric has been shown to stimulate the production of bile by the gallbladder. Curcumin is also a powerful antioxidant; antioxidants scavenge damaging particles in the body known as free radicals, which damage cell membranes, tamper with DNA, and even cause cell death (Bhowmik et al 2015).

Turmeric is a valuable intestinal antiseptic. The rhizome, its juice or dry powder, mixed in buttermilk or plain water is highly beneficial in intestinal problems, especially chronic diarrhea. It also helps prevent flatulence. About 20 drops of the juice of raw turmeric, mixed with a pinch of salt, taken first thing in the morning daily is considered an effective remedy for expelling worms. Turmeric, being rich in iron is useful in anemia. Turmeric is useful in the treatment of measles. Turmeric with its antiseptic properties is a useful remedy for chronic cough and throat irritations.

Turmeric exerts cardio-protective effects mainly by antioxidant activity, lowering lipid peroxidation, anti-diabetic activity and inhibiting platelet aggregation. Turmeric also inhibits ulcer formation caused by stress, alcohol, Indomethacin, reserpine, pyloric ligation, increasing gastric wall mucus in rats subjected to these gastrointestinal insults. Curcumin, in small doses of turmeric has been shown to protect against chromosomal damage caused by gamma radiation (Rafieian-Kopaei et al., 2014).

10.1 Curcumin mediated effects on Cancer

Curcumin (1,7-bis(4-hydroxy-3- methoxyphenyl)-1,6-heptadiene-3,5-dione), a natural polyphenolic compound derived from the rhizomes of Curcuma longa (turmeric), is one such agent that is high accessible, cost-effective and safe. Curcumin has been demonstrated to possess anti-inflammatory, antioxidant, anti-microbial, cardioprotective and anticancer properties. The anticancer effects of curcumin have been extensively studied (Wang 2019).

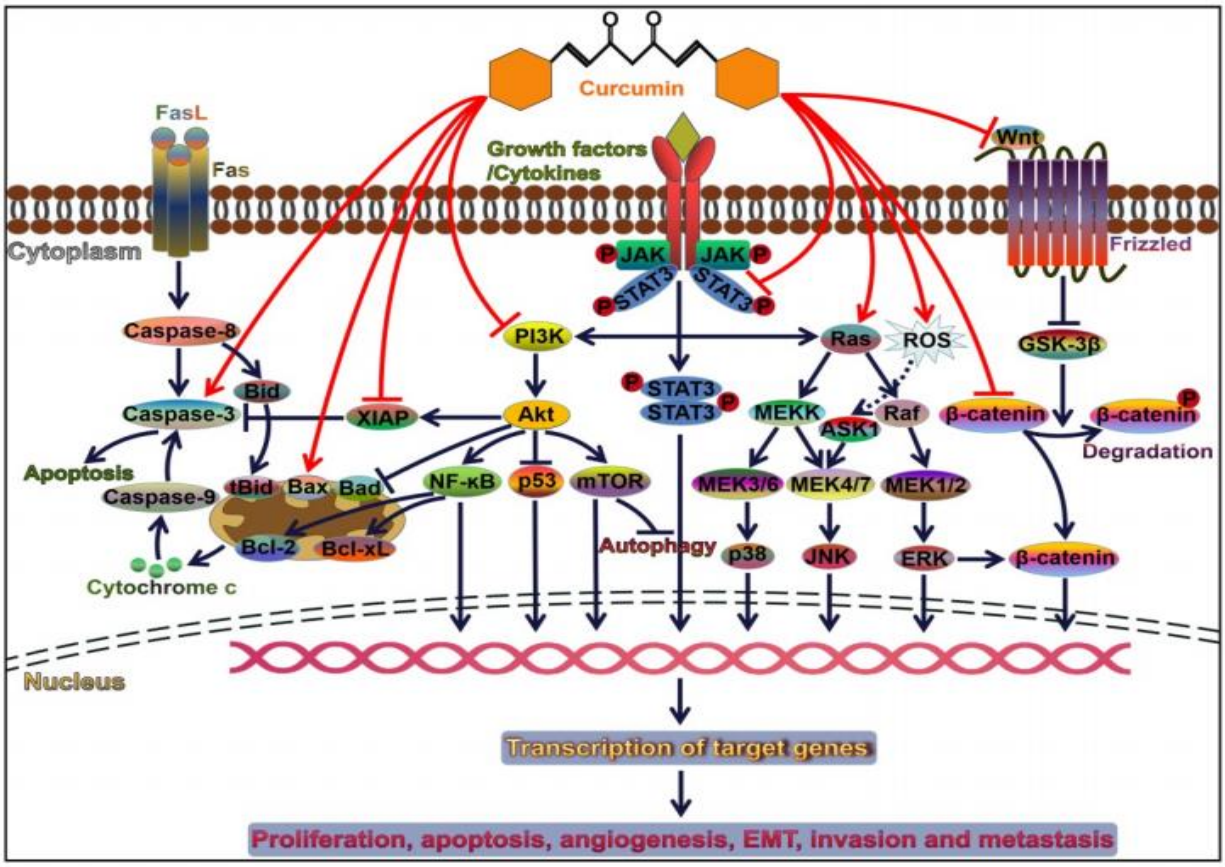

Figure 9: Curcumin exhibiting as an anticancer agent by inhibiting several signaling pathways (Wang et al., 2019)

Curcumin serves as a vital player in cancer progression through interference with multiple cellular signaling cascades including Wnt/β-catenin signaling, phosphoinositide 3-kinase (PI3K)/protein kinase

B (Akt) pathway, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, mitogen-activated protein kinase (MAPK) pathway, p53 signaling and nuclear factor-κB (NF-κB) pathway. Recently, curcumin has been found to target oncogenic and tumor-suppressive miRNAs.

Dysregulation of this pathway (Wnt/β-catenin pathway) causes the accumulation of β-catenin in the nucleus and enhances the expression of multiple oncogenes including c-myc and cyclin D1. Mechanistic analysis indicated that curcumin blocked the Wnt/β-catenin signaling and reduced glypican-3 (GPC3) expression. Curcumin depressed β-catenin accumulation and also downregulated its target genes c-myc, vascular endothelial growth factor (VEGF) and cyclin D1. Curcumin repressed growth and induced apoptosis of gastric cancer (GC) cells by downregulating target genes (Wnt3a, LRP6, β-catenin, c-myc and survivin) of the Wnt/β-catenin pathway. Curcumin obviously suppressed the expression of β-catenin and p-glycogen synthase kinase-3β (GSK-3β), as well as the expression of downstream cyclin D1 and c-myc.

The misregulation of PI3K/Akt signaling pathway is commonly correlated with carcinogenesis and cancer progression. Curcumin induced cell cycle arrest by downregulating cell division cycle 25 (CDC25) and CDC2 while upregulating p21. It also inactivated the Akt/mammalian target of rapamycin (mTOR) signaling and the downstream targets, which were upstream of cell cycle proteins, indicating that curcumin might induce cell cycle arrest through suppression of the Akt/mTOR pathway. In addition, curcumin reduced B-cell lymphoma-2 (Bcl-2) and upregulated Bcl-2-associated X protein (Bax) and cleaved caspase-3, thus promoting breast cancer cell apoptosis.

The JAK/STAT pathway may represent a potential target for cancer management and drug development. In terms of mechanism, curcumin reduced the phosphorylation of JAK1, STAT1 and STAT3. Mechanistic analysis revealed that curcumin suppressed the phosphorylation of JAK and STAT3, which led to the downregulation of VEGF, B-cell lymphoma-extra-large (Bcl-xL) and cyclin D1. MAPK signaling cascades govern a variety of biological processes including cell proliferation, immune responses and carcinogenesis [66]. Curcumin was able to activate caspase-3/9 and thus promoted retinoblastoma cell apoptosis. Moreover, curcumin also triggered the phosphorylation of JNK and p38 MAPK. Inhibition of JNK/p38 MAPK offset curcumin-induced cancer cell apoptosis. More importantly, curcumin induced the activation of c-JNK, p38 and ERK, suggesting that MAPK signaling pathways were involved in curcumin-induced apoptosis of lung adenocarcinoma cells. In addition, curcumin inhibited the invasion of human monocytic leukemia cells by descending the expression of MMP-2/9.

Activation of the p53 signaling could prevent cancer cell proliferation and induce cell apoptosis [72, 73]. Mechanistic investigation demonstrated that curcumin could increase the expression of p53 and p21, thus activating the p53 signaling pathway. Curcumin is an inhibitor of NF-κB. Studies have shown that curcumin significantly suppressed the phosphorylation of inhibitor of kappa Bα (IκBα), which in turn resulted in the degradation of NF-κB. Thus, curcumin synergistically boosted the pro-apoptotic effect of irradiation through blockade of the NF-κB pathway.

10.2 Anti-inflammatory Effects of Turmeric

Inflammation is typically an adaptive response caused by harmful stimuli and conditions (such as infection and tissue damage) to keep the body homeostasis. The acute inflammation lasts only a short

time and is usually beneficial to the host. When inflammation persists for a long time, it becomes chronic and can contribute to a variety of chronic diseases, such as obesity, diabetes, arthritis, pancreatitis, cardiovascular, neurodegenerative, metabolic diseases, and some types of cancer.

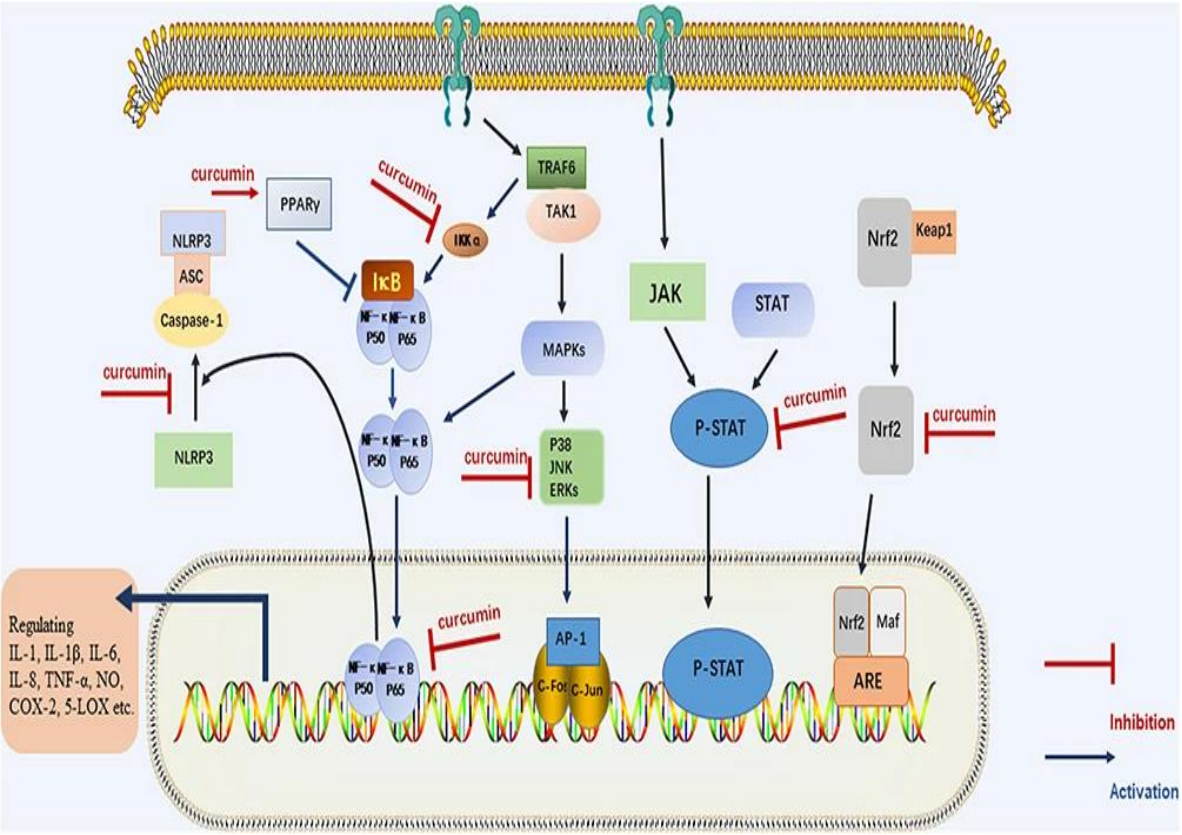

Figure 10: Curcumin mediating effects as anti-inflammatory agent (Peng et al., 2021)

In general, anti-inflammatory effects of drugs mainly include: acting on receptors and signaling pathways, regulating the response of target tissues to inflammatory mediators; reversing the effect of the medium on the target tissue; produce anti-inflammatory mediators and so on. Curcumin can down-regulate NF-κB through acting on Peroxisome proliferator-activated receptor gamma (PPARγ).

Curcumin could directly restrain the assembly of NLRP3 inflammasome, or inhibits the activation of NLRP3 inflammasome by inhibition of NF-κB pathway, which may be one of the mechanisms of

curcumin for the treatment of inflammatory diseases. In the studies of inflammatory cells and animals, curcumin decreased levels of pro-inflammatory mediators such as Interleukin-1 (IL-1), IL-1β, IL-6, IL-8, IL-17, IL27, Tumor necrosis factor-α (TNF-α), Inducible nitric oxide synthase (iNOS), NO, Regulated upon activation normal T cell expressed and secreted factor(RANTES), Granulocyte colony-stimulating factor (G-CSF), and Monocyte chemotactic protein-1 (MCP-1). Therefore, maintaining Th17/Treg balance is conducive to the maintenance of immune homeostasis and the treatment of inflammatory diseases.28 Curcumin inhibits Th17 differentiation, and regulate Treg/Th17 rebalance is by inhibit the IL-23/Th17 pathway. Recent evidence suggests that curcumin can reduce the pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-1 and IL-8 via interaction with several signaling and transcription molecules such as NF-κB, JAKs/STASs, MAPKs and β-catenin (Kahkhaie et al., 2019).

The inhibitory action of curcumin on JAK/STAT signaling pathway has been confirmed in a study conducted by Kim et al. where it was shown that curcumin suppresses phosphorylation of JAK1, JAK2, and their downstream molecules such as STAT1 and STAT3 in IFN-γ, gangliosides, or LPS-activated microglial cells. As a result, the expression of several pro-inflammatory mediators including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2), MCP-1 and ICAM-1 were impaired in activated microglial cells (Kim et al. 2003). In this regard, the activation of Src homology 2 domain-containing protein tyrosine phosphatases (SHP)-2, a key negative regulator of JAK activity is one of the several molecular mechanisms by which curcumin mediates the suppression of JAK activation (Kim et al. 2003). In addition, another in vitro study indicated that pretreatment of murine microglia cell line N9 with curcumin and demethoxycurcumin (DMC) could reduce LPS-induced phosphorylation of p38, JNK, and ERK1/2 MAPKs pathways, resulting in inhibition of the production of ROS by microglial cells (Zhang et al. 2010b).

10.3 Turmeric Mediated Neuro-Protective Effect

NDDs are characterized by degenerative processes and are thought to share several pathobiological pathways. Inflammation, impaired protein processing, mitochondrial dysfunction, oxidative stress, and disruptions in the autophagy-lysosomal system (Vidoni et al., 2016; Wolfe, 2018) are proposed as pathobiological pathways where initial perturbations in one pathway may cascade into a vicious cycle of cellular impairment and neurodegeneration. One anti-oxidative compound that has received considerable attention is the natural polyphenol curcumin, one of the major components of turmeric (Abrahams et al., 2019).

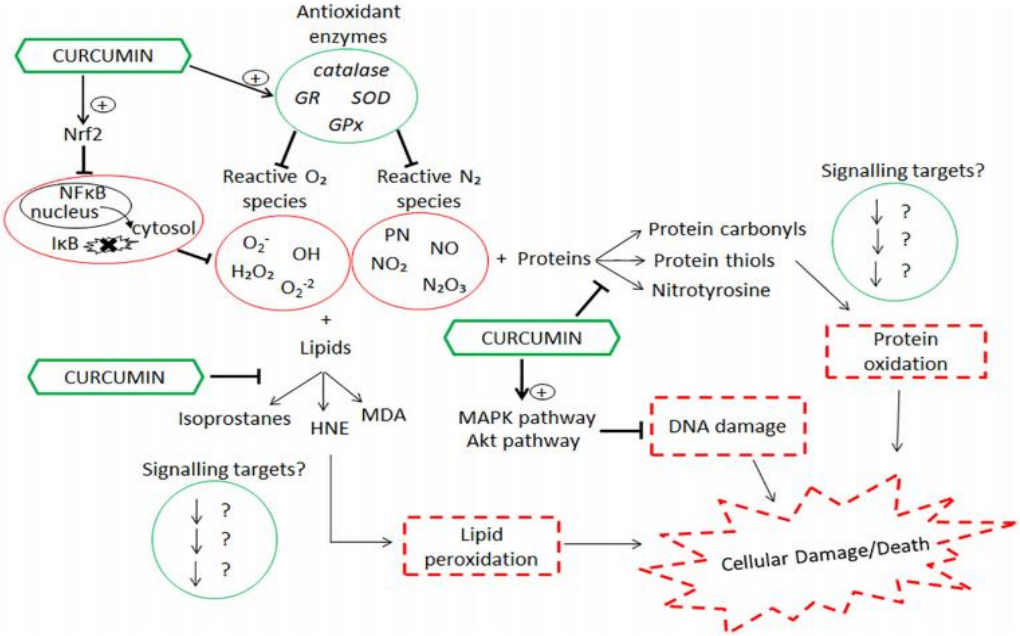

Figure 11: Curcumin inhibiting several signaling molecules related to oxidative stress and neural damage (Abrahams et al., 2019)

Curcumin pre-treatment reduced amyloid-β induced ROS production by 10% and 18%, respectively. From immunofluorescence imaging, the authors proposed that the antioxidant effect of curcumin was

mediated via the nuclear factor erythroid 2–related factor 2 (Nrf2) and apurinic/apyrimidinic endonuclease 1 (APE1) pathways.

In another study in which both mouse Neuro-2a neuroblastoma and human HEK293 kidney cells were used, curcumin was given as a pre-treatment followed by H2O2 exposure (Morales et al., 2017). Curcumin protected against cell damage mediated by oxidative stress in these cells. Curcumin reduced ROS levels in paraquat-exposed cells and increased expression of the antioxidant genes, SOD and GPx. Curcumin and its bioconjugates were shown to reduce levels of ROS, MDA and H2O2 while only curcumin, additionally, increased GSH and glutathione S-transferase (GST) protein levels.

Curcumin was also shown to reduce iNOS levels and thereby prevent the toxic overexpression of RNS. Ortiz-Ortiz et al. (2009) also showed that curcumin protected against the degradation of Iκβ, prevented the translocation of NF-κB, and thereby suppressed nitrosative stress in paraquat-treated N27 cells. Furthermore, the authors showed that the protective action of curcumin acted via the cell growth-death MAPK (mitogen-activated protein kinases) and Akt (serine/threonine protein kinase) pathways. Curcumin also inhibited unregulated DNA damage by stimulating Akt and MAPK pathways.

11.0 HEALTH BENEFITS OF HAWTHORN LEAF AND FLOWER

Various parts of hawthorn plant—in particular, the berries, flowers, and leaves—are rich in nutrients, and have been traditionally associated with many health, medicinal or nutraceutical beneficial health effects. This wild plant has been used as a traditional medicine, herbal drug, and food supplement for centuries. According to the holistic and traditional approach, hawthorn leaves and flowers are used to prepare infusions that can be used to control palpitations, tachycardia, and nervousness. Away from

meals, hawthorn has been used against hypertension and, before sleeping, for its relaxing and sedative actions.

The most common traditional use of hawthorn is as a food, whether eaten as a fruit or in hawthorn products, which are very popular. Hawthorn has ethnomedicinal value for its digestive and anti-cardiovascular properties. Hawthorn also contains bioactive components with great potential for the pharmaceutical industry, including polyphenols and flavonoids. The active ingredients in hawthorn are the basis for the wide range of health benefits it exerts. For example, polyphenolic compounds are good antioxidants and immunomodulators, flavonoids have anti-inflammatory and anti-atherogenic activity, lignans have antibacterial and antioxidant activity, while triterpenoids have anticancer, anti-inflammatory, and anti-proliferative activities. Hawthorn isolated compounds have shown anti-tumor activity both in vivo and in vitro and are considered a promising drug for the treatment of melanoma. Components of hawthorn such as bioflavonoids and proanthocyanidins have beneficial effects on the blood coagulation system (Zhang 2022).

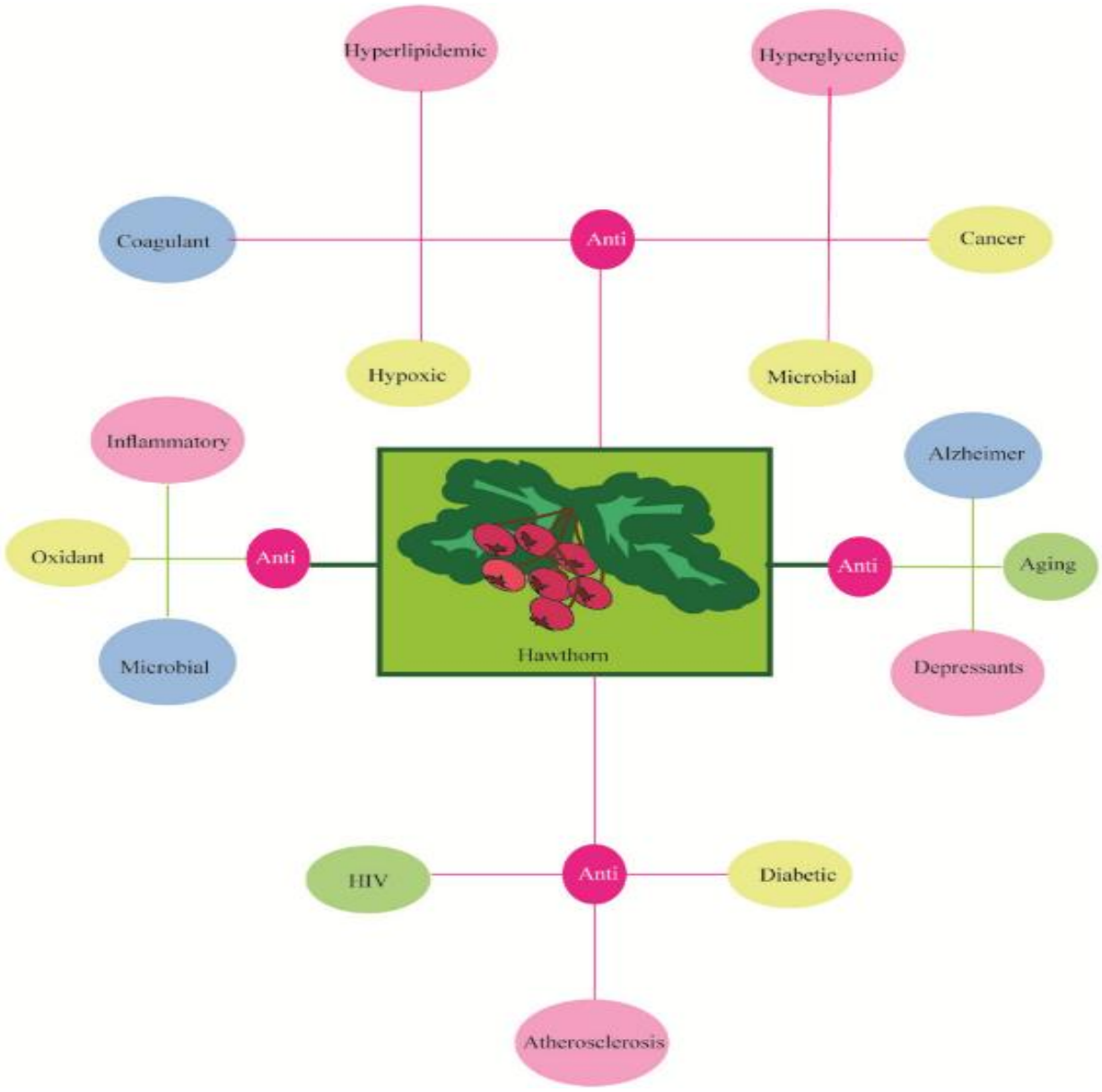

Figure 12: Various health benefits associated with hawthorn plant extract (Nazhand et al 2020)

11.1 Hawthorn induced effects on Atherosclerosis

Hawthorn is one of the recognized medicinal plants in European medicine, as Dioscorides described its cardiovascular actions in the first century (Petrovska, 2012). The primary pathogenesis of CVDs is

atherosclerosis, which could lead to dramatic clinical events, such as unstable angina or myocardial infarction (Reiner et al., 2011). The underlying pathophysiological mechanisms of atherosclerosis are oxidative stress damage, lipid deposition, inflammatory responses, and vascular endothelial dysfunction (Wu et al 2020).

To better elucidate the mechanism of hawthorn leaves in the treatment of CHD, the 44 targets were screened out in the compound-CHD targets. Coincidentally, 14 targets, namely IL6, VEGFA, IL1B, MMP9, CXCL8, CCL2, PTGS2, IL10, ESR1, EGFR, MMP2, CRP, SERPINE1, and ICAM1, have been identified as key pathogenic genes in the PPI network of CHD target proteins. The data showed that some of the compound- target pairs had good docking affinity, such as, PTGS2, MMP2, EGFR, MMP9, and ESR1, which means that the protective effects of hawthorn leaves in CHD may be achieved to some extent by regulating these genes above. Our results demonstrated that the increased PTGS2, MMP2, and MMP9 protein levels induced by LPS could be reversed by quercetin, kaempferol and isorhamnetin, respectively, in RAW264.7 cells. Our research showed that quercetin, kaempferol and isorhamnetin reduced protein expression of IL-1β, IL6, TNFα induced by LPS in RAW264.7 cells. These data further manifest that the effective compounds of hawthorn leaves could inhibit the production of pro-inflammatory cytokines stimulated by LPS in macrophage, confirming that hawthorn leaves may play a therapeutic role in CHD by inhibiting inflammatory pathways (Ding et al 2022).

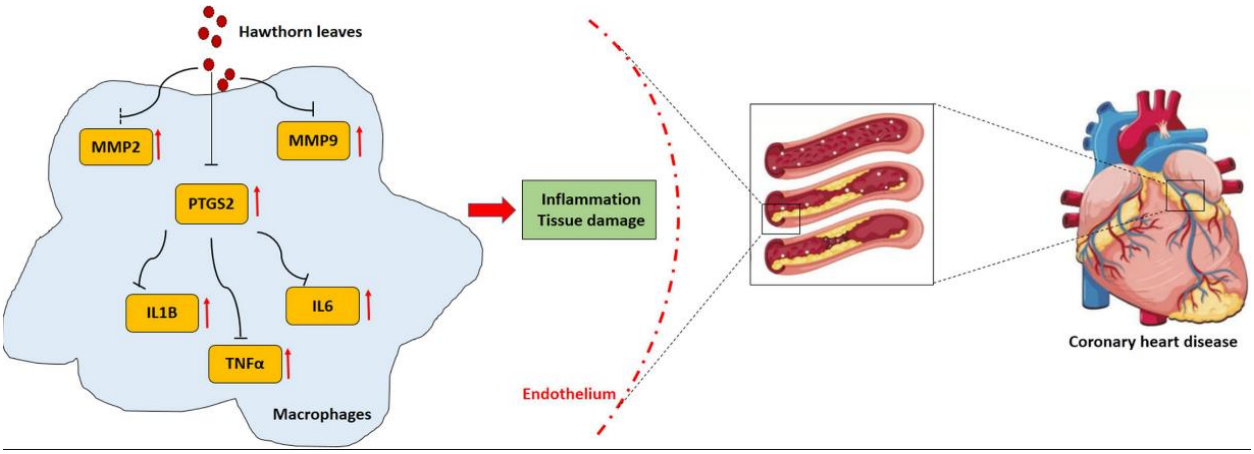

Figure 13: Hawthorn leaves exhibiting inhibiting effects on coronary heart disease (Ding et al 2022).

Lipid retention is an essential and critical initial step in the atherosclerotic cascade, and without this event, atherosclerosis could not be induced in animal models (Olofsson and Boren, 2005). Multiple steps: First, it inhibits cholesterol absorption by downregulating the expression and activity of intestinal acyl-CoA cholesterol acyltransferase (ACAT).). Second, the total flavonoids attenuate the expression of two essential liver enzymes involved in lipid biosynthesis, hydroxy methylglutaryl coenzyme A reductase (HMG-CoA) and cholesterol-7-alpha-hydroxylase (CYP7a). Moreover, the total flavonoids of C. pinnatifida inhibit the mature adipocyte secretion of the leptin and plasminogen activator inhibitor (PAI)-1 (Liu et al., 2009c) and decrease adipogenesis-related gene expression, including sterol regulatory element-binding proteins-1c (SREBP), fatty acid synthase (FAS), triacylglycerol hydrolase (TGH), and hormone-sensitive TG lipase (HSL). In vitro data showed HLF inhibited the formation of foam cells by promoting cellular cholesterol efflux via the upregulation of the ATP-binding cassette transporter A1 (ABCA1), PPARg, and downregulation of CD36, thus preventing the progression of atherosclerotic lesions (Liu et al., 2009a).

LDLs in their native state cannot trigger the lipid retention cascade before oxidation by free radicals. Therefore, oxidative stress is crucial to illustrate atherogenic mechanisms. In contrast to native LDLs,

only the modified or oxidized LDLs can drive the development of atherosclerosis. It has been demonstrated that extracts from C. pinnatifida exert potent scavenging properties against DPPH, hydroxyl radicals, and copper-II and peroxyl radical-induced LDL cholesterol oxidation (Zhang et al., 2001; Liu et al., 2009b), as well as hydrogen peroxide and superoxide species (Bahorun et al., 1996), which is partially due to interactions with antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) (Wang W. et al., 2011; Zhang et al., 2014).

Endothelium dysfunction occurs in the early stages of atherogenesis, characterized by a reduction of NO-mediated vasodilator responses and increased vasoconstriction due to excess endothelin (ET)-1 synthesis, resulting in enhanced vascular permeability. Hawthorn extracts resulted in decreased ET-1 and elevated NO levels in both human and animal experiments (Asher et al., 2012; Zhang et al., 2013). . Hawthorn extracts could induce vasorelaxation by increasing the phosphorylation of serine residue 1177 (Brixius et al., 2006). Hawthorn extract WS® 1442 increases cytosolic [Ca2+] i by suppressing sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA) and activating the inositol 1,4,5-trisphosphate (IP3) pathway, but without affecting the barrier function or endothelial cell contraction.

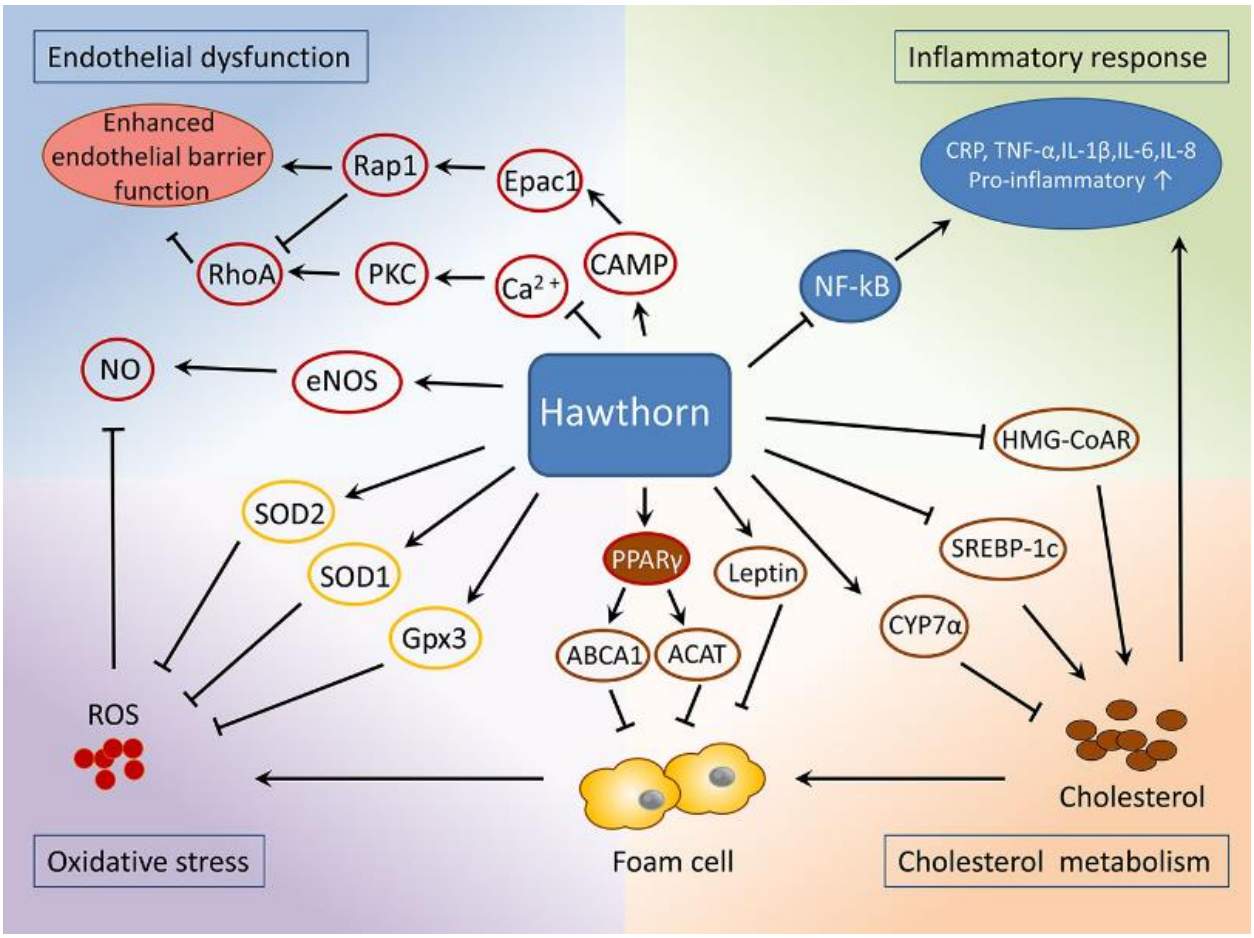

Figure 14: Hawthorn mediating inhibitory effects on various causative agents of atherosclerosis (Wu et al 2020).

12.0 HEALTH BENEFITS OF SPIRULINA

Compared to other foods or by weight, Spirulina is recognized as one of the most nutritious foods on the planet: high in proteins, containing all essential amino acids, also high in B vitamins, iron, magnesium, potassium and many other vitamins and minerals, as well as antioxidants. Spirulina has long been used as a dietary supplement by people living close to alkaline lakes where it is naturally found.

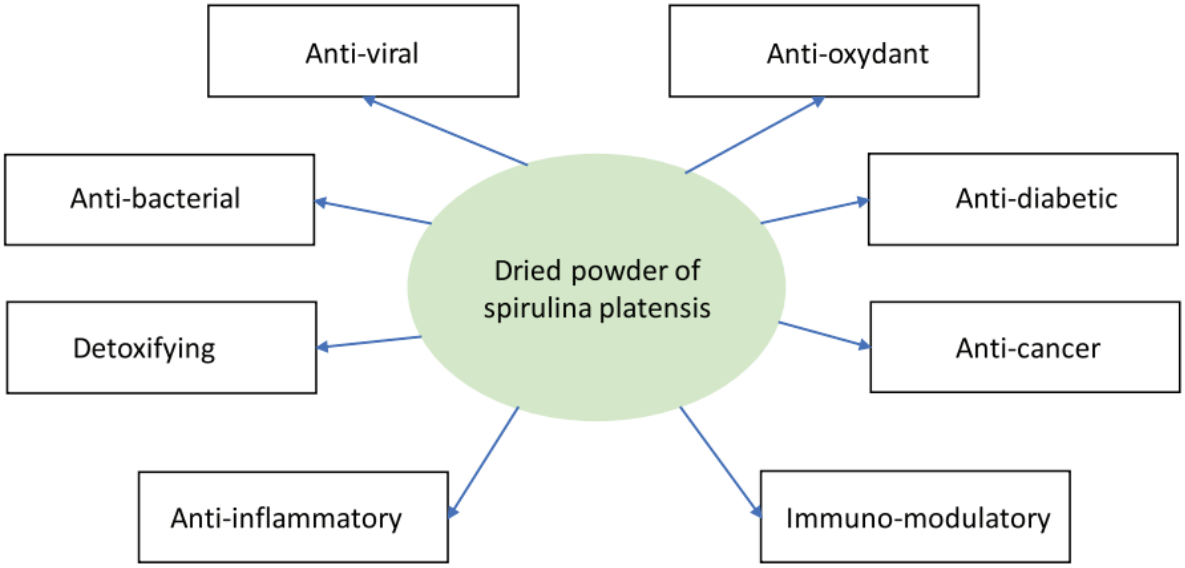

Figure 15: Spirulina exhibiting various health benefits (Jung et al., 2019)

Spirulina's fatty acids, such as α and γ linolenic acid, inhibited isoenzyme type 1 metabolism and improved the oily skin appearance. The polysaccharides contained in the alga extract, instead, stimulate the cell division process and contribute to the keratinization processes or to renew the stratum corneum. Vitamins, minerals and proteins contained into the alga may further contribute in improving skin microrelief and hydration (Ragusa et al., 2021).

Several previous toxicity studies showed that there were no adverse effects in experimental animals after short-term and long-term ingestion of dietary Spirulina (S. platensis and Spirulina maxima), even after administration of high doses (Salazar et al., 1998; Hutadilok-Towatana et al., 2008). In addition, S. platensis has been reported to have various beneficial effects, such as anticancer (Dasgupta et al., 2001; Ismail et al., 2009; Grawish et al., 2010), hepatoprotective (Ismail et al., 2009; Lu et al., 2010), antiviral (Hayashi et al., 1996a, b), anti-allergic (Kim et al., 1998), cardioprotective (Khan et al., 2005), and neuroprotective effects (Bermejo-Bescos et al., 2008).

12.1 Spirulina mediated effects on skin health

Aged skin lacks of bounded water and has weak hydration networks, which make skin look less and less glowing and firm. Typically, UV radiation, pollution, a poor diet and an unhealthy lifestyle are the main causes of skin aging, and therefore of the loss of moisture together with the decrease of skin barrier (Ragusa et al., 2021). Delsin et al., in 2015 showed how spirulina improves the epidermis structure and acts as a hydration booster with positive results on skin barrier function, particularly to skin protection, antiaging, and oil control.

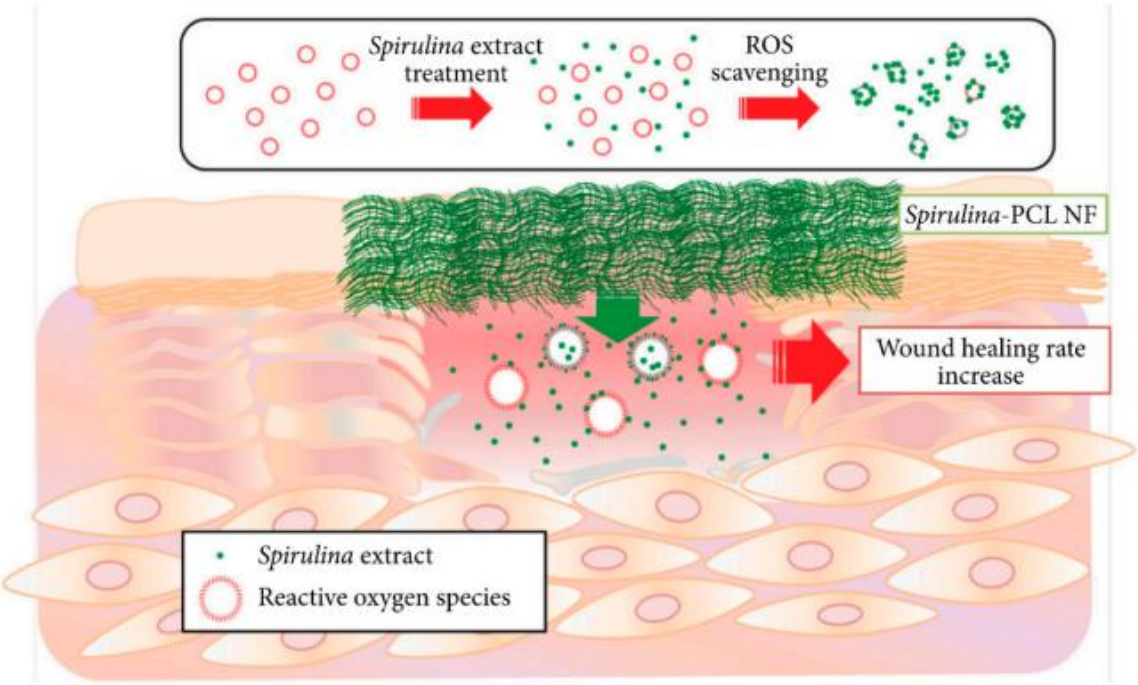

Figure 16: Supplementation of spirulina exhibits greater cell proliferation and wound healing effects (Ragusa et al., 2021)

Another important aspect related to skin aging is the peptides reduction in the dermis extracellular matrix. With this purpose, in 2006 a French research team patented a dermatological peptide extract of spirulina, claiming positive effects on the stimulation of fibroblast proliferation and on the

glycosaminoglycans and collagen's synthesis. As an antioxidant, spirulina may reduce skin hyperpigmentation and protect skin against sun-induced damages (e.g., photoaging) by inhibiting ROS-induced damage to the dermis.

It has been observed that, while in vitro experiment measures the formulation transmittance, the in vivo procedure determines the effective ability to prevent inflammatory reaction (erythema) triggered by solar radiation. This means that spirulina increases the light scattering properties without furnishing enough anti-inflammatory activity. Moreover, as previously stated by Delsin et al., topically applied spirulina regenerates the skin barrier and reduces the loss of water.

Both the abnormal loss and the overproduction of melanin may generate serious esthetical and dermatological skin disorders in humans, such as acanthosis nigricans, melasma, cervical poikiloderma, Lentigines, Periorbital hyperpigmentation, neurodegeneration associated with skin cancer risk and Parkinson's disease. As a result, the use of spirulina as a safer and greener tyrosinase inhibitor might have a huge potential application in the field. In particular, the IC50 values of tyrosinase inhibition with spirulina water and ethanol extracts were found as 1.4×10^{-3} and 7.2×10^{-3} g/mL, respectively. Another important component that could be responsible for this antimelanogenic effect is the c-phycocyanin, which has also antioxidant properties, as mentioned above.

Wound Healing Properties: The proliferation and growth stimulation activities of Spirulina extract seem to be directly connected to the presence of both phycocyanin and carotenoids, which synergistically contribute to the wound healing and tissue regeneration. The in vitro cell culture tests demonstrated that Spirulina extracts showed significant effects on fibroblast cell proliferation and

migration. Additionally, Spirulina platensis also revealed a strong antioxidant property, due to its superoxide dismutase (SOD) activity with values up to 8.0 U/mL of SOD in Spirulina extract.

In general, spirulina helped to regenerate wounds and enhanced skin regeneration, by improving the antioxidant mechanism against the reactive oxygen species (ROS) of fibroblast under oxidative stress. Nevertheless, the developed nanofibers had a restricted capability to moisturize wounded skin because of the hydrophobicity of polycaprolactone (PCL). In comparison to the earlier developed Spirulina PCL nanofibers, alginate improved the moisture preservation and adhesiveness of the Spirulina-Alg/PCL nanofibers, in addition it accelerated the regeneration of full thickness wounded skin in the rat model.

Anti-acne skin properties: The anaerobic cutibacterium acnes (also known as propionibacterium acnes) plays a role in the inflammation process because it hyperproliferates in the sebaceous lipid environment and produces ROS and pro-inflammatory compounds. The results shown that the water-based formulation was more effective in inhibiting bacteria proliferation than the oily-based one and confirmed the Spirulina anti-acne property.

12.2 Effects of Spirulina on brain health

Seaweed–microbiota interaction can lead to the production of small bioactive molecules, which can affect intestinal ecology and subsequently host brain health by growth-promoting (prebiotic) effects of specific bacterial genera involved in the production of neurotransmitters, such as GABA and serotonin [4,6]. In particular, spirulina administration was found to reduce the acute systemic inflammatory insult of lipopolysaccharide (LPS) in young rats, which led to a decline in neural stem cell proliferation. In this case, LPS-induced inflammatory IL-1β was reduced, while the LPS-induced inhibition of the

expression of antioxidant γglutamylcysteine ligase catalytic subunit (γGCLC), Nrf2, brain-derived neurotrophic factor (BDNF) was reversed, possibly via normalizing effects on phosphorylated AKT (pAKT) (Sorrenti et al., 2021).

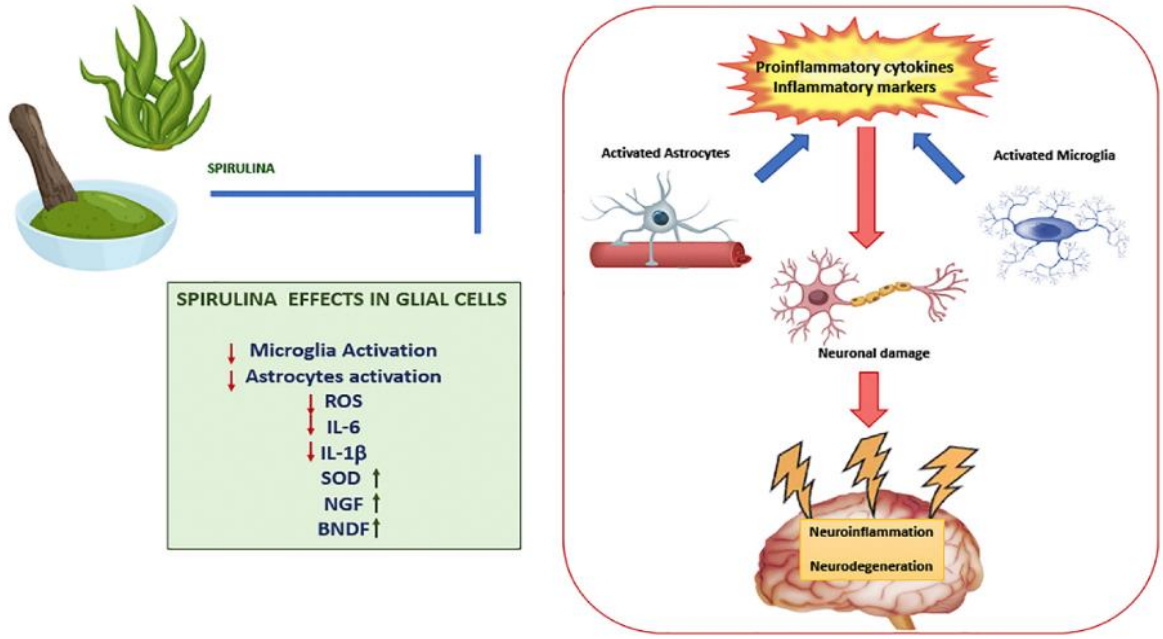

Figure 17: Spirulina mediating protective effects against neural damage (Trotta et al., 2022)

In a different model where a cognitive disorder was induced in mice by amyloid β 1–42 icv injection, treatment with ethanol extract of spirulina maxima ameliorated cognitive impairments by inhibiting the increased phosphorylation of glycogen synthase kinase-3 and increased glutathione and activating the BDNF/phosphatidylinositol-3 kinase/serine/threonineprotein kinase signaling pathway.

Spirulina platensis was also shown to be neuroprotective in a 6OHDA PD rat model, both in the behavioral test and in neuronal survival, an effect possibly associated with a decrease in the inflammatory enzymes iNOS and COX-2 [52]. Neuroprotective activity by spirulina in neurodegeneration has also been shown in other in vivo models and has been suggested to be mediated

by an antioxidant activity associated with the increase of superoxide dismutase, catalase, and glutathione peroxidase.

It was demonstrated that treatment with spirulina-enriched diets increases cerebellar glutathione (GSH) levels, reduces malondialdehyde (MDA) levels, reduces pro-inflammatory cytokines, and ameliorates both spatial and motor learning in aged rats. In cerebral ischemia, a condition marked by cerebral hypoxia with the generation of free radicals, ROS or reactive nitrogen species (RNS) and energy crisis, Spirulina treatment evidenced neuroprotective effects with progressive decline in TUNEL positive cells and caspase-3 activity in the ischemic hemisphere (Trotta et al., 2022).

Several studies have demonstrated the protective effects of spirulina or its components, in particular C-PC, against glial activation. In male Wistar rats treated with tributyltin chloride (TBTC), an environmental pollutant and potent biocide, C-PC effectively reduced ROS generation, decreased astroglia activation, counteracting the morphological alteration, and upregulation of GFAP, indices of astroglia activation.

The authors suggested that PCB, when released from C-PC in vivo, can be absorbed, cross the BBB, and by this way, exert antioxidant effects in the hippocampus, suppressing ROS production from activated microglia. There is considerably strong evidence to clarify that inflammation and oxidative stress play a fundamental role in the onset and progression of neurodegenerative diseases, and adequate scientific evidence has shown the beneficial effects of spirulina in PD, AD and multiple sclerosis (MS).

13.0 CONCLUDING REMARKS

A study was conducted to analyze the nutritional composition of K9-701 using a systematic

bioinformatics literature review. The conclusions of this study are as follows:

- This scientific review included analysis of 87 scientific studies, performed by 96 researchers, within 48 institution across the world, spanning 33 years of research
- The 10 K9-701 ingredients comprised of 89 key molecules. Each of these molecules have documented nutritional benefits in the scientific literature.
- These 89 key molecules contained in D include:
    - 46 minerals
    - 28 phytochemicals
    - 11 vitamins
    - 4 other molecules
- This scientific review revealed that K9-701 ingredients have documented medicinal benefits for mammals, including equine, across 10 different biological indications, references for which are listed in the report:
        - Immunomodulatory Effects
            - MSM
            - Zeolite
            - Diatomaceous Earth
            - Kelp
            - Turmeric
        - Blood glucose control/Diabetes
            - Diatomaceous Earth
            - Fenugreek
        - Bone Health
            - Diatomaceous Earth
        - Pest Repellent
            - Diatomaceous Earth
        - Osteoarthritis
            - MSM,
            - Zeolite
            - Hyaluronic Acid

        - Diatomaceous Earth
    - Neurodegeneration
        - Diatomaceous Earth
        - Turmeric
        - Spirulina
    - Skin Health
        - Hyaluronic Acid
        - Spirulina
    - Anti-Cancer
        - Raspberry leaves
        - Fenugreek
        - Turmeric
    - Women's Health
        - Raspberry leaves
    - Atherosclerosis
        - Hawthorn

APPENDIX I

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