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UNDERSTANDING THE POTENTIAL HEALTH BENEFITS OF SPIRULINA: A SYSTEMATIC REVIEW

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Abstract

Spirulina, a type of cyanobacterium, is known for its high vitamin, protein, and mineral content and possesses antioxidative and anti-inflammatory properties, drawing attention because of its potential health improvements. Despite its common use, a thorough understanding of the underlying mechanisms causing these benefits is still elusive. This systematic review explores Spirulina's prospective health benefits, providing valuable insights into how it works and its potential impact on health. Studies consistently indicate that Spirulina has a component that reduces inflammation by reducing specific cytokines like TNF-α and increasing levels of IL-10 to help moderate reaction to inflammation. Additionally, it has been found to improve lipid profiles by lowering cholesterol and triglyceride levels, attributed to compounds like β-carotene and linolenic acid. Studies also highlight the immune-modulating activities of Spirulina, indicating its potential to promote health through various mechanisms. Histopathological examinations have demonstrated Spirulina's positive impacts on tissues and organs, indicating improvements in conditions such as arthritis and liver damage. Furthermore, Spirulina's potential as a safe and effective health supplement is further supported by its comparable effects to established medications such as Indomethacin, Atorvastatin, and Dexamethasone. These findings have implications for a variety of medical and health concerns, offering a basis for future investigations and therapeutic strategies. This systematic review provides a comprehensive understanding of Spirulina's underlying mechanisms and its potential health benefits, emphasizing the need for further research to enhance our understanding and pave the way for future discoveries.

Keywords

Spirulina, cyanobacterium, health, inflammation, PRISMA

1. Introduction

Arthrospira, commercially known as *Spirulina*, is a cyanobacterium that has been long used as a food and food supplement (Kulshreshtha, *et al.*, 2008); it continues to gain popularity and the interest of researchers, commercial firms, and the healthy food industry due to its nutritional value and potential health benefits (Wan, *et al*., 2016; Khan, Wu and Kuca, 2016; Jung, *et al*., 2005; Grosshagauer, Kraemer and Somoza, 2020). *Spirulina* is an ideal food and dietary supplement for the 21st century by the Food and Agriculture Organization (FAO) of the United Nations (Ali and Saleh, 2012) and was declared by the World Health Organization (WHO) as the best food for the future, winning "The Best Natural Food" award in Germany's International Food Expo, as well as it, has been a successful dietary supplement or compact food used by NASA for an astronaut on space missionary (Kulshreshtha, *et al*., 2008; Ali and Saleh, 2012; Karkos, *et al*., 2011). Various toxicological studies on several Spirulina species have been conducted, and their safety has been proven. It now belongs to the roster of Generally Recognized as Safe (GRAS) substances by the US Food and Drug Administration (Karkos, *et al*., 2011). *Spirulina* has been recognized for its biochemical compositions that are beneficial to the needed nutrients of the body for growth and maintenance. It is highly rich in protein and also contains a variety of vitamins such as vitamin C, vitamin D, vitamin E, vitamin B1, B2, B3, B6, B9, and incredibly high amounts of B12, and minerals, especially iron, and other antioxidative and anti-inflammatory properties (Kulshreshtha, *et al*., 2008; Wan, *et al*., 2016; Khan, Wu and Kuca, 2016; Jung, *et al*., 2005; Karkos, *et al*., 2011). This makes Spirulina a potent source of nutrients. While it is thriving in the agriculture, food, and pharmaceutics industries, it has also been through various and thorough investigations using in vivo and in vitro studies to evaluate its impacts relating to health maintenance and preventive care. Its potential health effects include anti-cancer, anti-diabetic, anti-inflammatory, antioxidant, and immune-modulatory (Jung, *et al*., 2005 and Karkos, *et al*., 2011). Studies establish the positive effects of Spirulina in health management, showing overwhelming evidence for its potential therapeutic applications in the aforementioned various areas. Despite the undoubted and highly acknowledged nutritional values, biochemical compositions, and possible health effects, the underlying mechanisms behind such benefits, mode of action, and implications are yet to be fully understood. This systematic review follows the PRISMA guidelines to provide an overview of Spirulina's mode of action and how it relates to its possible health benefits. Therefore, this presents a comprehensive understanding of Spirulina's pathophysiological, metabolic, and nutritional pathways to provide a deeper understanding of its potential health benefits. The research aims to uncover more about the activities of Spirulina that contribute to its health benefits. It will be a valuable guide for researchers as they work to understand the complexities of how Spirulina works and its overall impact on human health.

2. Methodology

This systematic review was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

3.1 Inclusion and Exclusion Criteria

The inclusion and exclusion criteria for this systematic review are guided by the PICO items. More specifically, the inclusion criteria for this systematic review are as follows: Studies that evaluate or investigate the pathophysiological, metabolic, and nutritional pathways and effects of Spirulina, such that it induces antioxidant, anti-inflammatory, antihyperlipidemic, and antinociceptive effects through human immunomodulatory activities, were included. Studies that conducted experiments on humans and animal models were considered eligible for inclusion. Non-English studies, review articles, systematic reviews, and case studies that evaluated the health benefits and effects of Spirulina were excluded. Studies that focused on the health benefits of spirulina for non-human subjects were also excluded. Furthermore, studies that assessed the effects of Spirulina along with other variables were also not considered.

3.2 Search Method

From database origin to August 2023, a systematic search was carried out on online databases Google Scholar, Science Direct, and PubMed for research articles investigating the underlying mechanisms behind the health benefits of Spirulina using the following search terms: "health benefits of spirulina," "immunomodulation of spirulina," "health effects and activity of spirulina," "pathophysiological of spirulina," metabolic of spirulina," and "nutritional pathways of spirulina." The articles included as references were carefully reviewed to identify pertinent and significant published research works.

3.3 *Study Selection*

One of the authors assessed the studies for duplication and significance in the current investigation. Moreover, another author independently reviewed the keywords and double-checked the titles and abstracts for accuracy to ensure all pertinent study was included. The review author retrieved the full-text versions of the articles upon screening. The retrieved articles are then assessed for eligibility to determine the composition of scholarly articles that are to be included in the review. The articles that matched the inclusion criteria were considered eligible for inclusion.

3.4 Data Extraction

Data extraction was conducted by two authors and verified by another author. Data extraction from each study included bibliometric indices and the PICO items. Participant Population: Research/Studies were deemed eligible for inclusion if the health effects and activity of *Spirulina* species were evaluated on human participants and animal models, specifically rats and mice. Intervention: Research/Studies were deemed eligible for inclusion if they evaluated the health effects and activity of *Spirulina* through bioassays and induced health conditions. Comparison: Research/Studies were deemed eligible for inclusion if the study assessed the

health effects of *Spirulina* in comparison with a placebo or with other established nutraceuticals or pharmaceuticals. Outcome: The research findings did not form part of the criteria for eligibility to be included but were categorized into primary and secondary outcomes. The primary outcomes include the impact of spirulina treatment, which provides for anti-/pro-inflammatory, antioxidant, and antihyperlipidemic effects on induced health conditions, and its various mechanisms of action attributed to its health benefits. Secondary outcomes include the safety of *Spirulina* treatment, its comparison to established medications, and histopathological outcomes.

3.5 Assessment of risk of bias in included studies

This systematic review comprises randomized and non-randomized studies on the health benefits of Spirulina. For non-randomized studies, the review authors utilized the Cochrane Risk of Bias in Non-Randomized Studies of Intervention (ROBINS-I Tool), which was used to assess the risk of bias in the results of non-randomized studies comparing the health effects of two or more interventions, while Cochrane Risk of Bias Tool (RoB 2), a second version of the Cochrane-risk-of-bias tool was used for the randomized studies to assess its risk of bias in a series of questions to help gather information about features of the trial that are relevant to the risk of bias.

4. Results

4.1 Search Results

During the initial database search, 3,312 studies were found, but only 221 were selected for further review. After assessing their eligibility, the researchers identified ten (10) studies that fulfilled the inclusion criteria and were deemed eligible for the review. This information is summarized in Figure 1.

4.2 Characteristics of Included Studies

As summarized in Table I, the ten (10) publications eligible for inclusion in this systematic review investigated the underlying mechanisms of action behind the health benefits of *Spirulina* through immunomodulatory activity such as anti/pro-inflammatory (Parages, *et al*., 2012; Ali, Barakat and Hassan, 2015; Santos, *et al*., 2020; Perez-Juarez, *et al*., 2022; Kuhad, *et al*., 2006; Jadaun, Yadav and Bisen, 2017), antioxidant (Ali, Barakat and Hassan, 2015; Arteaga-Salvador, *et al*., 2020; Grover, *et al*., 2021; Perez-Juarez, *et al*., 2022; Ebrahim and Khouly, 2020; and Moradi-Kor, *et al*., 2020), antihyperlipidemic (Arteaga-Salvador, *et al*., 2020), and antinociceptive (Grover, *et al*., 2021) properties. These studies were conducted in Spain (Parages, *et al*., 2012), Egypt (Ali, Barakat and Hassan, 2015; Ebrahim and Khouly, 2020), Peru (Arteaga-Salvador, *et al*., 2020), Brazil (Santos, *et al*., 2020), India (Grover, *et al*., 2021; Kuhad, *et al*., 2006; Jadaun, Yadav and Bisen, 2017), Iran (Moradi-Kor, *et al*., 2020), and Mexico (Perez-Juarez, *et al*., 2022). Parages, *et al*. (2012) tested the capacity of an acidic polysaccharide fraction from *Spirulina* (A. *platensis*) to induce

proinflammatory cytokine TNF-alpha in macrophages. Ali, Barakat and Hassan, (2015) tested the antioxidant properties of *Spirulina* (*A. platensis*) against Complete Freund's Adjuvant-Induced Arthritis (AIA) in a rat model through an in vivo study for arthritis swelling evaluation and in vitro study for antioxidant effect evaluation. Arteaga-Salvador, *et al*. (2020), determines the antihyperlipidemic properties of *Spirulina* (*A. jenneri*) in mice models through in vivo and in vitro studies. Santos, *et al*. (2020) characterize the antinociceptive and anti-inflammatory properties of *Spirulina (A. platensis*) SP-LEB18 biomass and their mechanism of action via the opioid system in a Complete Freund's AIA inflammation in mice model. Grovel, *et al*. (2021) evaluated the antioxidant effects of C-phycocyanin from *Spirulina (A. platensis*) in mice models through an in vivo toxicity study. Pérez-Juárez, *et al*. (2022) explore the anti-inflammatory and antioxidant effects of *Spirulina (A. maxima)* against ethanol-induced liver damage in rat models. Ebrahim & Khouly, (2020) evaluated the effects of *Spirulina (A. platensis*) against thyroid dysfunction associated with alteration in liver functions and lipid profile in rat models. Kuhad, *et al*. (2006) investigated the renoprotective potential of *Spirulina* (*A. fusiformis*) on gentamicin-induced oxidative stress and renal dysfunction in rat models. Jadaun, Yadav and Bisen (2017) studied the inhibitory effect of *Spirulina* (*A. platensis*) on high glucose oxidative stress mitochondrial damage-mediated apoptosis in cardiomyoblasts. Moradi-Kor, *et al*. (2020) investigated the neuroprotective effects of *Spirulina* (*A. platensis*) against adolescent stress-induced oxidative stress.

4.3 Characteristics of Excluded Studies

Four reviews, two for human welfare and agricultural studies, thirty-eight with no reports regarding *Spirulina's* mechanism of action, three studies simultaneously investigating the effect of Spirulina with other variables, two direct effect evaluation only, three morphology and histopathology observations only, four bioactive compounds only, and two cross-over trials were excluded.

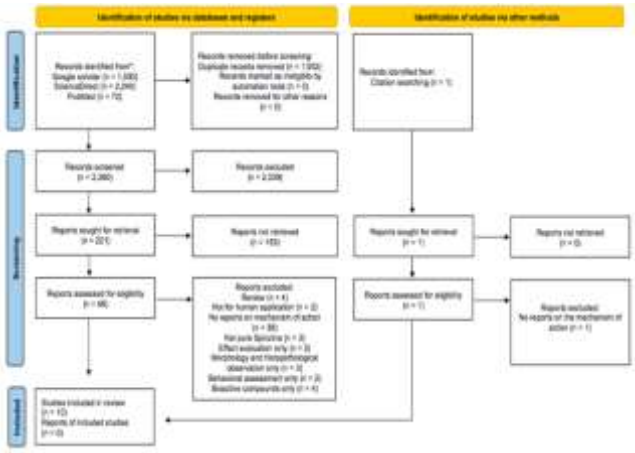

Figure1. Prisma Flowchart

Table 1. Characteristics of included studies

First author, year (Country of origin)	Study design	Participants (Disease)	Intervention Used	Outcomes	Results	
Ali, 2015 (Egypt)	Experimental study	Healthy male Wistar rats (Complete Freund's Adjuvant-Induced Arthritis)	Spirulina *(A. platensis)* at 200 mg/kg and 400 mg/kg vs Indomethacin at 5 mg/kg/day	Cytokines TBARS level Histopathological examination	*Spirulina* (200 mg/kg) - Decreased TBARS level - Reduce IL-6 and VEGF, and COX 2 level - grade 2 synovial proliferation and cartilage erosion (400 mg/kg) - Significantly decreased TBARS level - Reduce IL-6 and VEGF, and COX 2 level - fairly normal joint architecture and grade 1 synovial proliferation	Comparator (5 mg/kg/day) Lessened serum TNF-α, IL-6 and VEGF levels without effect on serum COX 2 Decreased synovial proliferation and cell infiltration.
Arteaga-Salvador, 2020 (Peru)	Randomized trials study	Mus musculus var. swiss (Balb/c) (Triton X-305-induced hyperlipidemia)	*Spirulina* (*A. jenneri)* at 0.05g / 100g and 0.3g / 100g vs. Atorvastatin 10mg / 1mL	Cholesterol level Triglyceride Level Catalase Activity	*Spirulina* Cholesterol values 97.80 ± 19.51 (*Spirulina* 0.03) 92.92 ± 5.20 (*Spirulina* 0.05) Triglyceride values 116.25 ± 29.14 (*Spirulina* 0.03) 98.78 ± 0.071 (*Spirulina* 0.05) CAT activity 1188.25 ± 704.15 (*Spirulina* 0.03) 807.81 ± 144.11 (*Spirulina* 0.05)	Comparator Cholesterol values 73.42 ± 3.82 (atorvastatin) Triglyceride values 82.12 ± 2.63 (atorvastatin). CAT activity 898.647 ± 326.204 (atorvastatin)

Santos, 2020 (Brazil)	Experimental study	Male Swiss mice (Complete Freund's Adjuvant (CFA) induced inflammation and pain	Spirulina (*A. platensis)* LEB-18 biomass at 200 mg/kg vs. Dexamethasone at 2 mg/kg	Mechanical nociceptive threshold, Paw edema, and Latency time in the tail flick test.	*Spirulina* Prevented overexpression of TNF-α and IL-1β. Increased the production of IL-10 Comparator Similar to *Spirulina*
Grover, 2021 (India)	In vivo study	Six weeks old Balb/c mice	C-Phycocyanin *(A. platensis)* at up to 2000 mg/kg	Toxicity cytokines	Does not cause acute and subchronic toxicity Suppresses the synthesis of pro-inflammatory cytokines
Perez-Juarez, 2022 (Mexico)	In vivo study	Male wistar rats (Ethanol-Induced Damage)	Spirulina *(A. maxima)* at 200 mg/kg	Histological Indicators Glucose, Albumin levels	Decrease in apoptosis, necrosis, and congestion. Restored glucose and triglyceride levels; normalized albumin values
Ebrahim, 2020 (Egypt)	Experimental study	Male albino rats Sprague-Dawley (Gamma radiation)	Spirulina *(A. maxima)* at 300mg/kg	- Superoxide dismutase (SOD) - Catalase (CAT) -Malondialdehyde (MDA)	Increase SOD Elevated CAT Decrease in MDA
Kuhad, 2006 (India)	Experimental study	Wistar rats (gentamicin-induced oxidative stress)	Spirulina *(A. fusiformis)* at (500, 1000, 1500 mg/kg	- Thiobarbituric acid reacting substances (TBARS) - Superoxide dismutase (SOD) -Histopathological changes	Produced a significant and dose-dependent reduction in TBARS Significantly and dose-dependently improved SOD levels Regressed the changes in the cortex and outer medulla of renal morphology
Jadaun, 2018 (India)	Experimental study	Rat cardiomyoblast (High-glucose induces oxidative stress)	Spirulina *(A. platensis)*	Cytotoxicity	Showed no cytotoxicity Significant decrease in intracellular reactive oxygen species induced by high-glucose treatment in cells.
Moradi-Kor, 2017 (Iran)	Randomized study	Wistar female adolescent rats (Adolescent Stress-Induced Oxidative Stress)	Spirulina *(A. platensis)* at 200 mg/kg	- Superoxide dismutase (SOD) -Malondialdehyde (MDA)	Increase SOD Decrease MDA

4.4 Risk of Bias of Included Studies

Figures 2 summarize and illustrate the author's assessment of the risk and biases using the Cochrane Risk of Bias in Non-Randomized Studies of Intervention (ROBINS-I Tool). The study by Ali, Barakat and Hassan, (2015) recorded the highest risk across domains in bias due to deviations from intended interventions and missing data, while

studies from (Parages, *et al*., 2012; Santos, *et al*., 2020; Grover, *et al*., 2021; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; Jadaun, Yadav and Bisen, 2017) were labeled with moderate risk of bias across domains.

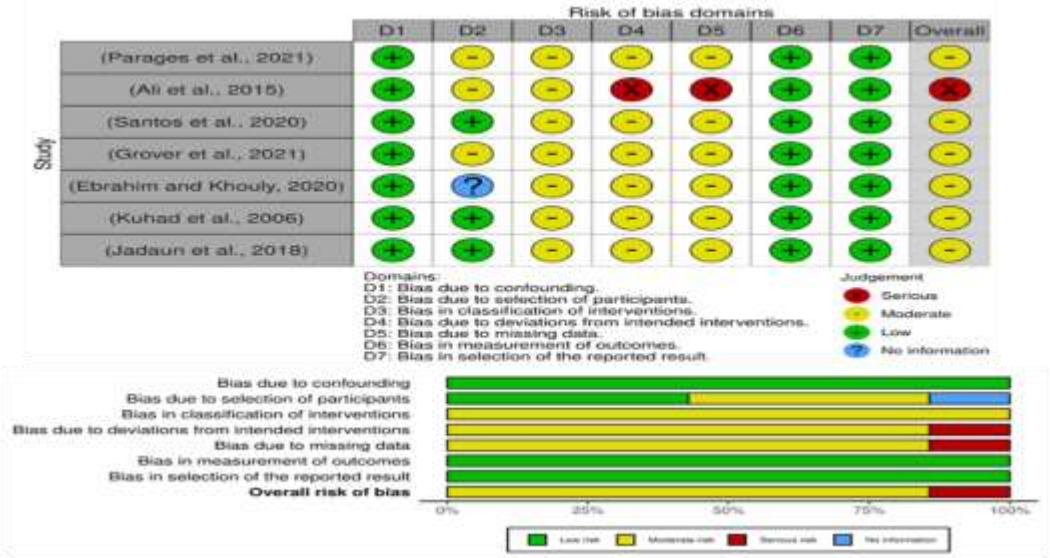

Figure 2. Risk of bias assessment of studies that did not randomize participants into groups.

Figure 3 summarizes the author's judgment on the risk of bias assessment of randomized studies using the Cochrane Risk of Bias Tool (RoB 2), where all three (Arteaga-Salvador, *et al*., 2020; Perez-Juarez, *et al*., 2022; Moradir-Kor, *et al*., 2017) studies recorded a high risk across domains. Although such studies are least likely to be biased in the measurement of outcome data, they all lack discussion on the randomization process. They are biased due to deviation from intended interventions and missing outcome data. In non-randomized studies, there is a moderate risk of bias due to issues such as the classification of interventions, deviations from intended interventions, and missing data. On the other hand, randomized studies carry a high risk of bias due to deviations from intended interventions and missing outcome data.

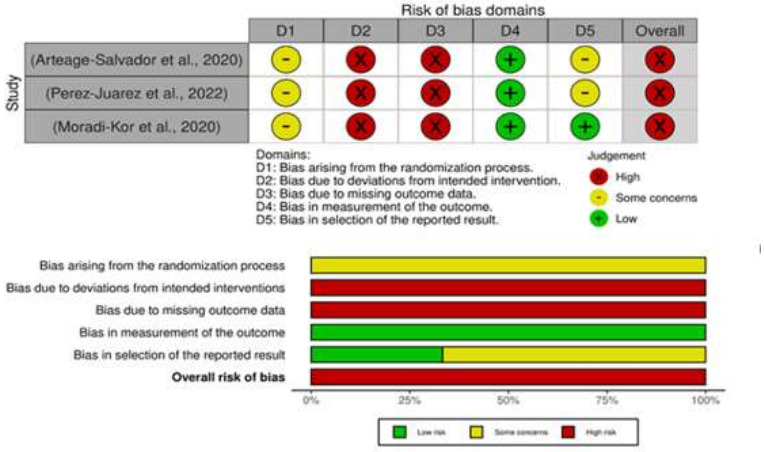

Figure 3: Risk of bias assessment of studies that randomized participants into groups.

4.5 Primary Outcomes: Anti-inflammatory

Activity Seven studies (Parages, *et al*., 2012; Ali, Barakat and Hassan, 2015; Santos, *et al*., 2020; Grover, *et al*., 2021; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; Moradir-Kor, *et al*., 2017) have examined the anti-inflammatory and pro-inflammatory effects of Spirulina species. Specifically, three studies (Ali, Barakat and Hassan, 2015; Santos, *et al*., 2020; Grover, *et al*., 2021) consistently demonstrated that the treatment of *Spirulina* (*A. platensis*), *A. platensis* LEB-18 biomass, and a novel protein C-phycocyanin extracted from *A. platensis* resulted in decreased serum levels of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α). This decrease was observed in response to various inflammatory stimuli, such as induction of Complete Freund's Adjuvant-Induced arthritis in rats and inflammation in mice in a dose-dependent manner (400mg/kg and 200mg/kg, respectively). Similarly, (Grover, *et al*., 2021) exhibited a suppression in the synthesis of TNF-α, also in a dose-dependent manner where the decrease in such serum levels was significant in doses 500 and 1000 mg/kg. On the other hand (Santos, *et al*., 2020) *A. platensis* and (Grover, *et al*., 2021) its novel protein C-phytocyanin treated mice had exhibited an increase in the production of anti-inflammatory cytokine, Interleukin-10 (IL-10). Furthermore, (Ali, Barakat and Hassan, 2015) and (Kuhad, *et al*., 2006) showed a significant decrease in the serum Thiobarbituric Acid Reactive Substances (TBARS level), a byproduct of lipid peroxidation associated with oxidative stress, upon treatment with (Ali, Barakat and Hassan, 2015) *A. platensis* 400mg/kg and (Kuhad, *et al*., 2006) *A. fusiformis* 1500mg/kg, both a dose-dependent reduction. Also, a decrease in Malondialdehyde (MDA) level, another lipid peroxidation byproduct associated with oxidative stress, was observed in (Ebrahim and Khouly, 2020) gamma-irradiated rats and (Moradir-Kor, *et al*., 2017) restraint stress-subjected rats treated with *A. platensis*. Meanwhile, in the study of Parages, *et al*., (2012), the acidic polysaccharide of *A. platensis* improved the TNF-α production in macrophages, exhibiting pro-inflammatory activity and stimulating the immune response of the cells.

4.6 Primary Outcomes

Antioxidant Six studies (Grover, *et al*., 2021; Perez-Juarez, *et al*., 2022; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; Jadaun, Yadav, and Bisen, 2017; Moradir-Kor, *et al*., 2017) whose experiments have shown the antioxidant activities exhibited by *Spirulina* species, *A. platensis* (Grover, *et al*., 2021; Jadaun, Yadav, and Bisen, 2017); *A. maxima* (Perez-Juarez, *et al*., 2022; Moradir-Kor, *et al*., 2017) and [15], [17*] A. fusiformis* (Kuhad, *et al*., 2006; Moradir-Kor, *et al*., 2017) have provided evidence that treatment with *Spirulina* enhanced antioxidant enzymes, which in turn protect cells and tissues from oxidative stress caused by free radicals and reactive oxygen species. The serum level of Superoxide dismutase (SOD) has been observed to significantly increase in studies where orally administered C-phycocyanin at doses of 500 mg/kg and 1000 mg/kg (Grover, *et al*., 2021). Additionally, *Spirulina* treatment has been found to increase SOD levels, significantly reversing reductions induced by gamma radiation in rats and chronic stress in the basolateral amygdala (Ebrahim and Khouly, 2020).

Spirulina intervention showed increased activity following chronic stress in the basolateral amygdala (Moradir-Kor, *et al*., 2020), and the *Spirulina*-treated groups (Perez-Juarez, *et al*., 2022) exhibited restoration to normal activity levels to ethanol-induced damage. Furthermore, an increase in catalase (CAT) enzymes was also observed in studies (Perez-Juarez, *et al*., 2022; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006) [13]. Just like in SOD serum level, (Perez-Juarez, *et al*., 2022) CAT enzyme activities were restored to normal levels in response to ethanol-induced liver damage, while irradiated rats treated with *Spirulina* showed significantly higher CAT enzyme levels compared to both the control and radiation groups (Ebrahim and Khouly, 2020). Lastly, the reduction of CAT activity via Gentamicin administration has been dose-dependently improved by *Spirulina* treatment (Kuhad *et al*., 2006). A study by Ali, Barakat, and Hassan (2015), on the other hand, showed an increase in the level of glutathione (GSH), another antioxidant that helps protect the cells and tissues against oxidative stress, has been successfully increased by spirulina treatment in comparison with complete Freund's adjuvant-induced arthritis in rats. In contrast, a study by Jadaun *et al*. (2017) showed that pretreatment with *Spirulina* extract resulted in a significant decrease in intracellular reactive oxygen species induced by high-glucose treatment in cells.

4.6 Primary Outcomes: Antihyperlipidemic Activity

In three studies (Arteaga-Salvador, *et al*., 2020 Perez-Juarez, *et al*., 2022; and Ebrahim and Khouly, 2020), the lipid-lowering effects of two types of *Spirulina*, *A. jenneri* and *A. maxima*, were investigated in mice and rat models. The studies found that *Spirulina* treatment reduced cholesterol and triglyceride values in the treated groups, with about 27% and 23% reductions, respectively, at a dose of 0.5g/100g. Lower doses of 0.3g/100g also showed decreased cholesterol and triglyceride values at 20% and 15%, respectively. Furthermore, *Spirulina* treatment significantly reduced total cholesterol and triglyceride values compared to the irradiated group, indicating an improved lipid profile. Additionally, one of the studies (Perez-Juarez, *et al*., 20022) demonstrated that animals treated with *Spirulina* showed improvements in glucose levels and a tendency to normalize albumin values in the liver, which had been damaged by ethanol-induced effects.

4.7 Secondary Outcomes: Mortality and Toxicity

Three studies have examined the mortality (Ali, Barakat and Hassan, (2015) and toxicity (Grover, *et al*., 2021 and Jadaun, Yadav and Bisen, 2017) effects of *Spirulina*. The results of Ali, Barakat and Hassan, (2015) indicated that *A. platensis* at a dose of 400 mg/kg showed no mortalities during the experiment, while a 12.5% mortality rate was observed at a dose of 200 mg/kg in rats induced with complete Freund's adjuvant arthritis. For toxicity, on the other hand, 2000 mg/kg C-phycocyanin-treated mice underwent acute and sub-acute toxicity studies (Grover, *et al*., 2021). The study on acute toxicity revealed no instances of mortality behavioral changes, or no obvious toxic symptoms over the course of the research. In addition, there were no substantial variations in terms of body weight, organ weight, and relative organ weights between the C-phycocyanin-treated group and the no-treatment

group. In the sub-acute toxicity study, there were no signs of abnormalities such as behavioral changes, skin and fur color changes, or changes in the eyes and mucus membrane. Furthermore, no tremors, salivation, or diarrhea were observed, and there were no reports of signs and symptoms of toxicity. Moreover, the study by Jadaun, Yadav and Bisen ,(2017) found that *A. platensis* showed no cytotoxicity from 0 to 8 µg/mL with more than 98% cell viability. When high glucose-challenged cells demonstrated a significant decrease in cell viability, *Spirulina* extract treatment led to a significant decrease in this effect.

4.8 Secondary Outcomes: Histopathological Examination

Three studies have investigated the histopathological effects of various types of *Spirulina*: *A. platensis* (Ali, Barakat and Hassan, 2015), *A. maxima* (Perez-Juarez, *et al*., (2022), and *A. fusiformis* (Moradir-Kor, *et al*., 2020). In the study of Ali, Barakat and Hassan (2015), rats treated with *Spirulina* at a dose of 200 mg/kg showed moderate proliferation of synovial cells with four layers of reactive synoviocytes, increased mitotic activity, and mild or absent invasion of synovial cells into adjacent bone and connective tissue. There was also localized deep degradation of cartilage based on the scoring system used. In contrast, rats administered with *Spirulina* at a specified dosage of 400 mg/kg showed normal joint structure and mild synovial cell growth that is characterized by two to four layers of active synovial cells. These results differed significantly from those observed in the Adjuvant-Induced Arthritis group, which demonstrated synovial membranes multiplying and wearing away at the cartilage, heavy mononuclear cell infiltration, pannus formation, and extensive deep cartilage degradation. Additionally, histopathological examination of ankle portions that were dyed using the Masson's trichrome staining method revealed that treatment with *Spirulina* at both 200 mg/kg and 400 mg/kg resulted in suppression of the area percentage of synovial membrane compared to the Adjuvant-Induced Arthritis group. Furthermore, Spirulina at 200 mg/kg led to collagen fibers that have been slightly increased in thickness, while Spirulina at 400 mg/kg ameliorated the area percentage for the articular cartilage. On the other hand, Perez-Juarez, *et al*. (2022) observed an increase in apoptosis, necrosis, and congestion, indicating the presence of inflammation and steatosis in the histological sections of ethanol-induced damage in rats. However, rats treated with *Spirulina* exhibited a reduction in the severity of these histological changes. Furthermore, a study by (Moradir-Kor, *et al*. (2020) examined the histopathological changes in renal morphology of rats with gentamicin-induced oxidative stress and found that treatment with spirulina reversed the changes observed in the gentamicin-treated groups. These changes included alterations in the cortex and outer medulla, loss of tubular brush border, swelling in the interstitial spaces, and death of cells in the epithelial tissue.

4.9 Secondary Outcomes: Comparison with Established Medication Three

studies (Ali, Barakat and Hassan, 2015; Arteaga-Salvador, *et al*., 2020; and Santos, *et al*., 2020) have compared *Spirulina* with established medications. Study by Ali, Barakat and Hassan (2015) compared

Spirulina (*A. platensis*) with Indomethacin in terms of (1) Mortality: The group treated with Indomethacin (5mg/kg) showed a 37.5% mortality rate, while the group treated with *Spirulina* (200 mg/kg) showed a 12.5% mortality rate in rats induced with Complete Freund's Adjuvant-Induced Arthritis. However, the group treated with *Spirulina* (400 mg/kg) did not show any fatalities that occurred during the conduct of the trial. (2) TBARS and GSH levels: *Spirulina* markedly reduced serum levels when compared to rats treated with indomethacin. These findings aligned with the indicated increase in serum levels in the group of Adjuvant-Induced Arthritis rats. (3) TNF-α, IL-6, COX 2, and VEGF: Indomethacin demonstrated a reduction in these markers, except for COX 2, while Spirulina lowered all indicators in comparison to the group of Adjuvant-Induced Arthritis rats. However, the difference in results with indomethacin is not significant. (4) Histopathological examination: Rats treated with Indomethacin exhibited reduced growth of tissue in the joint and infiltration of cells, while ankles from *Spirulina* (200 mg/kg) treated rats indicated grade 2 increase in the synovial lining. However, a higher dose of *Spirulina* (400 mg/kg) demonstrated typical joint structure and mild synovial tissue growth at level 1. Both indomethacin and *Spirulina* (200 or 400 mg/kg) significantly reduced the total histological scores compared to those of Adjuvant-Induced Arthritis rats. Additionally, indomethacin and Spirulina (400 mg/kg) ameliorated the area % for articular cartilage. Study by Arteaga-Salvador, *et al.* (2020) compared the antioxidant and antihyperlipidemic effects of two different doses of *Spirulina* (*A. jenneri*) (0.03g/100g and 0.05/100g) with Atorvastatin (10 mg/kg). The results showed that the groups treated with atorvastatin demonstrated the highest reduction in cholesterol and triglycerides compared to both doses of Spirulina in a hyperlipidemic mice model. On the other hand, Santos, *et al.* (2020) compared the effects of *Spirulina* (*A. platensis*)-LEB-18 biomass with Dexamethasone. They found that SPLEB18 was more effective than Dexamethasone for antinociceptive effect. However, for anti- and pro-inflammatory activity, the impact of SPLEB18 is similar to that of dexamethasone. As for the anti-edematogenic activity of SPLEB18, its efficacy was similar to that of dexamethasone, but its effect was shorter-lasting. inflammatory.

5. Discussion

The results from the studies included in the review have provided an investigation into the mechanisms of action of *Spirulina* and its potential health benefits. Seven studies evaluated the anti-inflammatory activity of *Spirulina* species (Parages, *et al*., 2012; Ali, Barakat and Hassan, 2015; Santos, *et al*., 2020; Grover, *et al*., 2021; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; and Moradi-Kor, *et al*., 2020), six of which demonstrated anti-inflammatory activity (Ali, Barakat and Hassan, 2015; Santos, *et al*., 2020; Grover, *et al*., 2021; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; and Moradi-Kor, *et al*., 2020). Three studies reported a decrease in the serum level of the pro-inflammatory cytokine TNF-α in a dose-dependent manner, along with a decrease in Thiobarbituric Acid Reactive Substances (TBARS) and Malondialdehyde (MDA) serum levels, which are associated with oxidative stress (Ali, Barakat and Hassan, 2015; Santos, *et al*.,

2020; Grover, *et al*., 2021). Additionally, studies also observed an increase in the production of the anti-inflammatory cytokine Interleukin-10 (IL-10). Tumor necrosis factor alpha (TNF-α) is a cytokine that plays a key role in regulating inflammatory responses and is crucially involved in the pathogenesis of some inflammatory and autoimmune diseases like Rheumatoid Arthritis that is usually accompanied by lipid peroxidation and oxidative damage in both humans and animal models (Ali, Barakat and Hassan, 2015) that occurs due to the upregulation or imbalance in free radicals and reactive oxygen species (ROS) which is continuously produced inside the body. Thiobarbituric Acid Reactive Substances (TBARS) are produced as a byproduct of lipid peroxidation and used as oxidative stress markers. They help to detect lipid peroxidation by measuring Malondialdehyde (MDA), a stable lipid hydro peroxide, and provide an indicator of lipid peroxidation in biological tissue (Kuhad, *et al*., 2006). IL-10, on the other hand, is a major anti-inflammatory cytokine that counteracts the effects of TNF-α. It is involved in systemic inflammation and is part of a group of cytokines that stimulate the acute phase reaction of the immune response (Parages, *et al*., 2012; Santos, *et al*., 2020). In the context of inflammatory pain, *Spirulina* has been shown to decrease the serum levels of TNF-α, TBARS, and MDA while increasing the IL-10 (Ali, Barakat and Hassan, 2015; Santos, *et al*., 2020; Grover, *et al*., 2021; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; and Moradi-Kor, *et al*., 2020). These results strongly suggest that the probable mechanism of action of *Spirulina* under inflammatory conditions is the upregulation of IL-10, which in turn reduces the local levels of the pronociceptive cytokines TNF-α and IL-1β. The increase in MDA levels is attributed to the interaction of hydroxyl radicals with polyunsaturated fatty acids in cellular membranes (Ebrahim and Khouly, 2020). This effect was reduced after treatment with Spirulina. Additionally, *Spirulina* has been found to have a significant protective effect by lowering TBARS content, attributed to its antioxidant components such as C-phycocyanins, β-carotene, vitamins, and minerals (Ali, Barakat and Hassan, 2015). However, one study (Parages, *et al*., 2012) showed an improved production of TNF-α in RAW macrophages in response to an acidic polysaccharide extracted from *Spirulina*, stimulating the immune response of the cells. Six studies (Grover, *et al*., 2021; Perez-Juarez, *et al*., 2022; Ebrahim and Khouly, 2020; Kuhad, *et al*., 2006; Jadaun, Yadav and Bisen, 2017; and Moradi-Kor, *et al*., 2020) have found that Spirulina exhibits antioxidant properties and can increase the levels of antioxidant enzymes such as Superoxide dismutase (SOD) (Perez-Juarez, *et al*., 2022; Ebrahim and Khouly, 2020; and Kuhad, *et al*., 2006), catalase (CAT), and glutathione (GSH) (Jadaun, Yadav and Bisen, 2017). These enzymes protect cells from oxidative damage by scavenging hydrogen peroxide and superoxide ions. SOD is used as a marker for oxidative stress-induced diseases, and a decrease in its activity is associated with harmful oxidative changes (Grover, *et al*., 2021; Perez-Juarez, *et al*., 2022). Spirulina treatment has shown potential in restoring SOD and CAT activity, which may help in maintaining the structural

integrity of liver cells and eliminating free radicals generated during intoxication (Perez-Juarez, *et al*., 2022). Additionally, the increase in SOD enzyme activities in the basolateral amygdala following chronic stress may be attributed to elevated glucocorticoid levels and a compensatory response to chronic stress (Moradir-Kor, *et al*., 2020). The increase in GSH is related to the abundance of phenolic compounds and ω-3 and ω-6 fatty acids in *Spirulina*, contributing to its antioxidant effects (Perez-Juarez, *et al*., 2022). In addition, three studies (Arteaga-Salvador, *et al*., 2020; Perez-Juarez, *et al*., 2022; Ebrahim and Khouly, 2020) have looked into the lipid-lowering effects of spirulina. They assessed cholesterol and triglyceride (TG) levels (Arteaga-Salvador, *et al*., 2020 and Ebrahim and Khouly, 2020) as well as glucose and albumin levels (Perez-Juarez, *et al*., 2022). All three studies consistently demonstrated a decrease in cholesterol and triglyceride levels, along with restoring glucose and albumin levels with *Spirulina* treatment. Using radiation-induced rat models showed a significant increase in serum TG, LDL, and TC, indicating hyperlipidemia (Perez-Juarez, *et al*., 2022). However, treatment with *Spirulina* significantly improved the levels of thyroid hormones that regulate cholesterol and increased the expression of hydroxylase, which converts cholesterol into bile acids. This led to an improvement in the lipid profile (Ebrahim and Khouly, 2020). Overall, these studies demonstrated a significant decrease in cholesterol and triglyceride levels, confirming the lipid-lowering effects of *Spirulina* (Perez-Juarez, *et al*., 2022). The hypolipidemic effect of *Spirulina* may be due to the presence of β-carotene and linolenic acid, both of which affect cholesterol synthesis (Ebrahim and Khouly, 2020). High levels of cholesterol and triglycerides can cause oxidative stress. To counteract these harmful effects, the body uses antioxidant enzymes such as SOD, CAT, and Glutathione peroxidase (GPx) (Arteaga-Salvador, *et al*., 2020). *Spirulina* is also rich in phycocyanin, which binds to bile acids in the jejunum and inhibits cholesterol absorption (Ebrahim and Khouly, 2020). Albumin, the most abundant protein in the blood, has antioxidant properties. Decreased albumin levels can result in inefficient defense against oxidative stress-induced damage. Treatment with *Spirulina* has been shown to restore albumin and glucose levels. This hepatoprotective effect is attributed to *Spirulina's* main antioxidant protein, C-phycocyanin, which can eliminate alkoxyl, hydroxyl, and peroxyl free radicals, reduce nitrite production, and inhibit hepatic microsomal lipid peroxidation. Phycocyanin also improves human serum albumin stability and antioxidant activity. *Spirulina* has been reported to exhibit insulin-like properties by stimulating pancreatic β cells, increasing insulin production, and reducing glucose levels (Perez-Juarez, *et al*., 2022). The results show that *Spirulina*-treated groups restored glucose and albumin to normal levels, indicating its hypoglycemic effect. Three studies (Ali, Barakat and Hassan, 2015; Perez-Juarez, *et al*., 2022; Moradir-Kor, *et al*., 2020) examined the histopathological effects of *Spirulina* in animal models. The study by Ali, Barakat and Hassan (2015) noted that *Spirulina* treatment resulted in dose-dependent moderate cell proliferation, increased mitotic activity, mild or absent invasion of adjacent bone and connective tissue by synovial

cells, and localized deep cartilage degradation. Higher doses showed normal joint architecture and mild cell proliferation with reactive synoviocytes. This contrasts with untreated subjects, where extensive synovial membrane proliferation eroded the articular cartilage, heavy infiltration of mononuclear cells, formation of pannus in multiple sites, and extensive pannus formation in two sites or more. Additionally, *Spirulina* treatment was found to suppress the expansion of the synovial membrane. The study by Perez-Juarez, *et al*. (2022) found that *Spirulina* reduced the degree of apoptosis, necrosis, congestion, inflammation, and steatosis and reversed changes in the cortex and outer medulla. While the study by Moradir-Kor, *et al*. (2020) reported that *Spirulina* also reversed tubular brush border loss, interstitial edema, and epithelium necrosis in gentamicin-treated groups. These results suggest *Spirulina* can inhibit or reduce histopathological lesions caused by potential infection or inflammation. In addition, several studies (Ali, Barakat and Hassan, 2015; Grover, *et al*., 2021; and Jadaun, Yadav, and Bisen, 2017) have evaluated the mortality and toxicity effects of *Spirulina*. They consistently reported that spirulina showed no toxicity or obvious toxic symptoms throughout the study. Grover, *et al*. (2021) found no indications of abnormal behavior, skin, fur, eye color, or mucus membrane, implying a wide margin of safety in its usage. These findings also align with the outcomes of spirulina's comparison with established medications. *Spirulina* was compared to Indomethacin (Ali, Barakat and Hassan, 2015), Atorvastatin (Arteaga-Salvador, *et al*., 2020), and Dexamethasone (Santos, *et al*., 2020) and demonstrated an effect close to these medications. This indicates that spirulina exhibits potential, efficient, and safe health benefits, making it a great potential contributor to various human health endeavors.

6. Conclusion

The systematic review of *Spirulina* reveals its significant potential health benefits, primarily attributed to its rich nutritional profile and bioactive compounds. *Spirulina* has been shown to exert lipid-lowering effects, as evidenced by multiple studies demonstrating reductions in cholesterol and triglyceride levels, alongside improvements in glucose and albumin levels in various animal models. These findings suggest that Spirulina may be crucial in managing hyperlipidemia and related metabolic disorders. Moreover, *Spirulina's* antioxidant properties are noteworthy, with studies indicating its ability to decrease serum levels of Thiobarbituric Acid Reactive Substances (TBARS), a marker of oxidative stress, and to enhance the activity of key antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). This antioxidant activity is particularly relevant in chronic stress and radiation exposure, where Spirulina has demonstrated protective effects against oxidative damage. Additionally, the modulation of inflammatory markers, including a decrease in TNF-α and an increase in IL-10, further underscores Spirulina's potential as an anti-inflammatory agent. The histopathological studies also support the safety and efficacy of *Spirulina*, showing dose-dependent improvements in cell proliferation and overall tissue health. However, while the evidence is promising, the review highlights a significant risk of bias in

many studies, particularly concerning methodological limitations and data reporting. Therefore, further rigorous research is warranted to elucidate the precise mechanisms of action and establish standardized dosages for therapeutic applications. *Spirulina* presents a multifaceted approach to health enhancement. Its lipid-lowering, antioxidant, and anti-inflammatory properties make it a valuable candidate for further investigation in clinical settings. Future studies should aim to address the existing biases and explore the long-term effects of Spirulina supplementation in diverse populations.

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