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Spirulina Demonstrated Protective Effects Against Thiamethoxam-Induced Renal Injury in Male Rats, Likely Through The Upregulation of Antioxidant Defenses

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ABSTRACT:

Background: Thiamethoxam (TMX), a common neonicotinoid pesticide, is widely used for insect control without causing genetic mutations. However, increasing concerns have been raised regarding its adverse effects on the environment and human health, including carcinogenicity, hepatotoxicity, and neurotoxicity. This study aimed to investigate the protective role of the natural antioxidant Spirulina platensis against TMX-induced renal damage.

Materials and Methods: Male rats were randomly divided into four groups: control, Spirulina-treated, TMX-treated, and TMX plus Spirulina-treated. Biochemical assays were conducted to assess oxidative stress markers, antioxidant enzyme activities, and renal function parameters. Histopathological examinations of kidney tissues were also performed.

Results: TMX exposure significantly increased oxidative stress markers, including hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), while depleting antioxidant defense systems such as catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and reduced glutathione (GSH). Furthermore, TMX administration resulted in renal dysfunction, evidenced by elevated creatinine and urea levels, and severe histopathological alterations. Pretreatment with Spirulina platensis effectively alleviated these effects by restoring antioxidant enzyme activity, reducing oxidative stress, and improving kidney function and tissue structure.

Conclusion: The findings suggest that Spirulina platensis exhibits strong antioxidant and renoprotective properties against TMX-induced renal toxicity. These results highlight its potential as a natural therapeutic agent to mitigate pesticide-induced kidney damage. Further studies are warranted to elucidate the underlying mechanisms, particularly its modulation of signaling pathways and inflammatory responses.

Keywords: Spirulina platensis; Thiamethoxam; Nephrotoxicity; Oxidative stress; Antioxidant enzymes; Renal function; Histopathology; Immunohistochemistry

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1. INTRODUCTION

The extensive and frequent application of pesticides has brought about numerous anomalies in target and non-target exposed species. Neonicotinoids are widely used in agro-production sectors among other pesticides [1]. With a global market share of over 25%, neonicotinoids have emerged as the most popular class of pesticides. They belong to a class of neuroactive pesticides that share chemical similarities with nicotine. The nitro group is present in thiamethoxam, a second-generation neonicotinoid neonicotinoid insecticide which is used worldwide and is a member of the thianicotinyl subclass [2].

Although thiamethoxam (TMX) is crucial for protecting the crop from pest attacks, it has also been shown to have negative effects on both people and animals. Neonicotinoids have been once thought to have little potential for toxicity in mammals, yet their widespread use has revealed that they have substantial toxic effects on both invertebrates and vertebrates. Significant eco-toxicological risks to aquatic and terrestrial creatures in the environment are also posed by extensive pesticide use [3].

The neonicotinoids class, which includes the synthetic organic pesticide TMX, is the most significant new class of insecticides that have been created in the last 30 years. Due to their potential mild toxicity to humans and capability for combating insects that are resistant to other classes of pesticides, neonicotinoids have been the insecticide class with the highest rate of development [4]. At the postsynaptic insect nAChRs, neonicotinoids serve as agonists with a much higher affinity [5]. Non-mutagenic, broad-spectrum neonicotinoid pesticide TMX [6] affects nicotinic acetylcholine receptors (nAChRs) in mammals and insects. It is most selective for insect nAChRs [7]. A total of three main TMX metabolites—CGA265307, CGA330050, and CGA322704—report [8] as suppressing iNOS in mice. Known to cause cellular damage, such inhibition may produce formaldehyde (HCHO) or reactive oxygen species (ROS). Among the several cellular components— carbohydrates, proteins, nucleic acids, and lipids—these reactive compounds might have negative effects on each one.

Historically, people have utilized spirulina, a blue-green algae that is a member of the Oscillatoriaceae family, as a source of vitamins and protein[9] . Much study was done on the two most often used and prevalent species, Spirulina maxima and Spirulina platensis in the medical and food area [10]. Apart from the 4% vitamin by weight and 60–70% protein content. Spirulina also abound in vital minerals, fatty acids, amino acids, and antioxidants[11].

A cyanobacterium, Spirulina platensis (SP) can be defined as a microalga noted for its nutritional worth and other health advantages. Essential vitamins, minerals, beta-carotene, protein, phenolic acids, and tocopherols abound in such blue-green alga. SP stands especially for strong antioxidant and anti-inflammatory properties. Those qualities make it somewhat popular as a nutritional supplement for humans and a feed additive for poultry and aquaculture [12]. Moreover, studies by [13] show that SP and its bioactive component, C-phycocyanin, show a broad spectrum regarding pharmacological properties, like immunomodulatory, anti-inflammatory, nephroprotective, hepatoprotective, neuroprotective, anti-hypertensive effects. SP has also been proven to protect against different heavy metal caused organ toxicity [14].

Toxicological studies [14] have not showed any negative effects of many Spirulina species on the kidney given either chronic or acute dosages. Generally speaking, utilizing spirulina as a dietary supplement should be limited to no more than 15 grammes daily [15]. Spirulina has been investigated in many studies for its antioxidant properties. These investigations have shown that Spirulina may efficiently neutralize free radicals, greatly increase the activity regarding antioxidant enzymes, and prevent against DNA damage as well as lipid peroxidation [16].

Reducing oxidative stress also helps spirulina protect mice from colitis, neurotoxicity, and hepatonephrotoxicity [17]. Through controlling the synthesis regarding important cytokines, like interleukin (IL-1β), IL-4, IL-2, IL-10, IL-6, and TNF-α, Spirulina shows clear immunomodulatory as well as anti-inflammatory properties [18]. This work sought to examine how thiamethoxam affected oxidative stress, non-enzymatic and enzymatic antioxidants, immunohistochemical and histopathological changes in male rat kidneys. The research also looked at how natural antioxidant Spirulina might help to reduce the negative impacts of thiamethoxam.

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2- Methods and Materials:

2.1. Experimental compounds and doses used

Spirulina extract has been obtained along with thiamethoxam (TMX). TMX dosage used was 1/10 LD50, or 156 mg/kg BW (the oral rat LD50 is 1563 mg), per the research by [19]**.** The advised dose of Spirulina extract is 800 mg/kg BW, per [20].

2.2. Animals and experimental design

A total of twenty male Albino rats that weighed 175 ± 10 g and 12 weeks of age have been utilized in the research. The animals were imported from Iraq's University of Karbala. The Institutional Animal Care & Use Committee's (IACUC) local ethical requirements as well as the Guide for Care and Use of Lab Animals guided the research's conduct, and all procedures have been completed in accordance with the Committee's guidelines. A simple diet and unlimited access to tap water have been provided to the rats. The animals were kept under conventional atmospheric conditions for the experiments, which included a temperature of 25º ± 5ºC, a 12hr light/day cycle, and a of 50–70% humidity level. Using a ball-tipped curved intubation needle, the animals have been randomly assigned to 4 equal groups of five rats after two weeks of acclimation. Group 1 received distilled water and TMX, Group 2 received 156 mg/kg BW of TMX, Group 3 received 800 mg/kg BW of Spirulina extract, and Group 3 received TMX plus Spirulina extract. The animals were given their daily doses orally for three weeks.

2.3. Blood sample collection and tissue preparations

Following the trial, 100% isoflurane (2 mg/kg body weight or 2% breathed in a desiccator) was used to put the animals to sleep. Blood samples were taken via cardiac punctures for extracting the serum. Those samples have been centrifuged for a period of 20 minutes at 860 xg and after that kept at a -20ºC temperature until the measured parameters were analyzed. In order to remove any adhering connective tissue or adipose deposits, the kidneys were carefully washed with cooled 0.9% saline solution after excision. After that, a Potter-Elvehjem homogenizer was used for homogenizing the kidneys. In this process, kidney tissue was suspended 10% (w/v) in 1.15% KCl and ice-cold sodium phosphate buffer (0.01M, pH 7.4). After that, the homogenate has been centrifuged for 20min at 10000 xg and 4 Celsius. The resulting supernatant, free of cellular debris, was carefully collected and stored at -20ºC for a further analysis of specified parameters.

2.4. Biochemical parameters

[21] technique of urea level in serum helped to estimate it.. [22] developed an approach for assaying the serum creatinine levels. [23] approach helped one ascertain the lactate dehydrogenase activity [9] developed an approach for assaying alkaline phosphatase activity in serum [24] devised a technique that helped one ascertain the protein concentration.

2.5. Assessment of oxidative stress biomarkers and antioxidant status

The levels of MDA have been assessed for lipid peroxidation in kidney homogenates using [25] approach and the thiobarbituric acid reactive substances (TBARS) assay. A modified method from [26] was used to quantify reduced glutathione (GSH) levels. Protein precipitation with the use of the metaphosphoric acid has been followed by colorimetric detection with the use of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) at 412nm. Catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase, glutathione S-transferase (GST**)** [27]**,** and antioxidant enzyme activities—glutathione reductase (GR) and glutathione peroxidase (GPx)—have been determined by using the approaches that have been described by [28]**.**

2.6. Kidney histopathological examination

Every rat had its kidney taken, then promptly fixed in 10% formalin. The tissue was next sectioned after being fixed in paraffin and run through a sequence of graded alcohols as well as xylene. Following [29]**,** the sections have been stained with Hematoxylin and Eosin (H&E).

2.7. Detection of KI67 marker in kidney using Immunohistochemistry technique

describe immunohistochemical analysis regarding KI67 expression in deparaffinized kidney sections (5 µm) with the use of Avidin-Biotin-Peroxidase (ABC) approach (Elite–ABC, Vector Laboratories, CA, US). KI67 receptor subunits were detected using a monoclonal anti-KI67 antibody (dilution 1:100; DAKO Japan Co, Tokyo, Japan)[30].

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2.8. Statistical analysis

The data have been expressed as mean ± standard error (SE). SPSS 17 was utilized to perform the statistical analyses, which employed one-way analysis of variance (ANOVA). In the case when differences have been found, post hoc multiple comparisons using the Duncan multiple range test (DMRT) were utilized in order to determine differences between specific treatments. The threshold for statistical significance has been set to the value of P̂ 0.05.

RESULTS AND DISCUSSION

Urea, creatinine, enzyme activities, and protein content

Serum creatinine and urea levels considerably increased ($P < 0.05$) in patients treated with TMX, although kidney protein content dramatically decreased in comparison to the control. Serum urea, creatinine, and protein concentration did not significantly change when Spirulina (SP) was administered alone. In the case when SP was present with TMX, the levels regarding the tested parameters stayed closer to the typical values.

Xenobiotics can induce a spectrum of renal toxicities, impacting both tubular cells and glomerular structures [31]. Because of the high expression regarding transporters involved in xenobiotic secretion as well as reabsorption, renal proximal tubules are especially vulnerable to xenobiotic-induced damage. The kidney becomes more susceptible to harm when toxic substances accumulate inside it due to changes in the expression or functions of such transporters. This can occur through decreased efflux of toxicants and their metabolites or enhanced uptake of xenobiotics via carrier proteins. Furthermore, interference with protein synthesis by xenobiotics can result in elevated levels of urea, the primary nitrogenous waste product of protein metabolism [32].

Table1: Effects of thiamethoxam (TMX), spirulina (SP), and their combination on urea, creatinine, enzyme activities, and protein of male rats' kidneys

Parameter	Experimental Groups			
	Cont.	SP	TMX	SP+TMX
Urea (mg/dl)	36.1±0.89^{c}	34.2±1.02^{c}	50.6±1.68^{a}	44.6±0.95^{b}
Creatinine (mg/dl)	0.647±0.020^{c}	0.607±0.020^{c}	0.893±0.030^{a}	0.765±0.021^{b}
LDH (U/mg protein)	815±30.15^{c}	767±15.62^{c}	1098±33.68^{a}	952±23.45^{b}
ALP (U/mg protein)	194±6.01^{a}	206±7.04^{a}	126±3.26^{c}	160±5.48^{b}
Protein (mg/g tissue)	75.1±2.57^{a}	79.4±1.57^{a}	51.2±1.17^{c}	61.4±2.15^{b}

There are five rats in each group, and the data are shown as mean ± standard error (S.E.). Statistically significant differences ($p < 0.05$) between groups are shown by superscript letters (a-g), where 'a' denotes the greatest mean value and the other letters follow in descending order, with 'g' denoting the lowest mean value.

LDH = Lactate dehydrogenase, ALP = alkaline phosphotase

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Renal failure is indicated by elevated urea as well as plasma creatinine levels in the present study. A high blood urea level is common in people with moderate-to-advanced chronic kidney disease (CKD). Urea is an indirect and direct uraemic toxin, as shown by numerous studies, especially those pertaining to cardiovascular disease [33].

Because of increased activity regarding the urea-synthesizing enzyme arginase, elevated blood urea levels are frequently linked to accelerated protein degradation in animals and/or enhanced conversion of ammonia to urea. Animal immune system dysfunction, central nervous system, cancer, kidney, and cardiovascular problems have all been linked to increases in arginase activity [10].

Given urea is a consequence of protein catabolism, increased creatinine and blood urea levels in TMX-treated rats are regarded as major markers of renal failure. Those spikes might also be reflections of underlying metabolic problems, maybe including hepatic dysfunction. Xenobiotics also change salt transport and boost kidney acid-secretory activity [32]. Mostly resulting from muscle breakdown, creatinine is generated at a rather consistent rate. Higher serum creatinine readings point to reduced kidney function. Consistent with the development of renal impairment, this investigation found a modest rise in serum creatinine in TMX-treated group over the control animals. Although glomerular filtration rate (GFR) is frequently measured using serum urea and creatinine, in cases of renal failure their accuracy could be limited. Still, such criteria—especially the combination regarding serum creatinine and blood urea nitrogen (BUN)—could offer insightful analysis of GFR. Comparable results have come out of earlier rat investigations [20]. Especially, Spirulina treatment showed a protective effect against kidney damage caused by TMX-induced by means of notable changes in serum creatinine and urea levels.

Lactate dehydrogenase (LDH) is one suggested marker of xenobiotics' toxicity [34]. Changes in the dehydrogenase activity in TMX-treated rats could have resulted from major cellular damage, which increased hydrogenase release and impeded protein as well as carbohydrate metabolism. Furthermore mentioned were metabolic problems and a clear response to energy deprivation based on lactate's increase [35].

An indicator that is sensitive to alterations brought on by pesticide toxicity is alkaline phosphatase (ALP), a biochemical measure. The enzyme phosphatase is essential to biological processes. Its activities include metabolism, detoxification, and the production of energy macromolecules needed for many vital processes. Tissue necrosis may cause the membrane-bound biomarker enzyme ALP to escape into the bloodstream, which might explain its decline in kidney and liver tissues [13]. According to [36] , vepacide may also interfere with ALP, resulting in tissue damages, biochemical impairment of cellular processes, and increased plasma membrane permeability. Through waste product excretion and essential material reabsorption, the kidney aids in maintaining the body's equilibrium. Compared to other bodily tissues, the kidneys are exposed to xenobiotics at a very high level. According to [11], the nephrotoxic effects of xenobiotics can vary from modest to severe cell necrosis, and their functional changes can range from slight modifications in tubular function to severe renal failure. One of the primary cellular constituents that is vulnerable to damage by free radicals is protein. According to. [37], excessive loss through nephrosis was the primary cause of protein depression. Furthermore, increased proteolytic activity, decreased protein synthesis, or degradation might be the cause of the protein decline.

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Kidney oxidative stress, enzymatic and non-enzymatic antioxidants

Table 2 shows that kidney H2O2 and TBARS concentrations (a marker of lipid peroxidation, or LPO) were considerably (P<0.05) greater in rats treated with TMX alone than in the control group. Rats administered SP alone, on the other hand, exhibited significantly lower levels of H2O2 and TBARS (P<0.05) compared with the controls. The combination group (SP + TMX) had considerably (P < 0.05) lower levels of H2O2 and TBARS compared with TMX-treated group. On the other hand, rats given TMX had a considerably (P<0.05) reduced glutathione content (GSH) in comparison with the controls. GSH content of rats treated with SP alone has been substantially higher than that of the control group (P < 0.05). Additionally, when SP and TMX were administered together, the GSH content was much higher than in the group that received TMX.

Table2: Effects of thiamethoxam, spirulina, and their combination on TBARS, H_2O_2, and GSH content of male rat kidneys

Parameter	Experimental Groups			
	Cont.	SP	TMX	SP+TMX
TBARS (nmol/g tissue)	22.7±0.48^{c}	19.2±0.45^{d}	31.7±0.55^{a}	26.9±0.40^{b}
H_2O_2 (µmol/g tissue)	42.4±1.42^{c}	35.3±1.12^{d}	57.7±1.18^{a}	49.5±1.84^{b}
GSH (mmol/mg protein)	2.41±0.070^{b}	2.86±0.065^{a}	1.40±0.048^{d}	1.93±0.049^{c}

There are five rats in each group, and the data are shown as mean ± standard error (S.E.). Statistically significant differences (p < 0.05) between groups are shown by superscript letters (a-d), with 'a' denoting the greatest mean value and the other letters following in descending order.
TBARS: Thiobarbituric acid reactive substances; H2O2: Hydrogen peroxide; GSH: Reduced glutathione

The dual roles of ROS and RNS are well known due to the fact that they can either be detrimental or beneficial to the biological systems. Oxidative stress, which is specified as an imbalance between the elimination and production of ROS, can be defined as a critical factor and a common denominator of various chronic diseases, including neurological disorders (for example, Alzheimer's and Parkinson's diseases), metabolic, cardiovascular, and cancer [38].

A harmful state known as oxidative stress causes lipids, proteins, and DNA to oxidize, which damages cells and ultimately leads to their death [39]. Hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), and superoxide radical (O^2) are among ROS that pose a continuous threat to organisms with aerobic metabolism. By causing oxidative damage to biological components, the very toxic superoxide anion significantly impairs regular metabolism.

Among the worst effects on cells is peroxidation regarding membrane lipids. Compromised biological membrane integrity could have quite serious effects. Increased permeability resulting from

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disturbances to the membrane structure as well as function lets other solutes, including potassium, flow through it. This imbalance finally helps to cause cellular demise [9]**.** Oxidative stress results from an imbalance between the capacity of a biological system to detoxify ROS and their too high production in tissues and cells. Although ROS are essential for several physiological functions including cell signalling, their generation is mostly resulting from cellular metabolism. But environmental stresses—including ionizing radiation, UV radiation, heavy metals, and pollutants—along with xenobiotics like some medications could greatly increase ROS generation. This increased ROS generation throws off the delicate balance and causes oxidative stress and consequent damage of tissues and cells [40]**.** The results of our investigation of increased H_2O_2 and TBARS and decreased GSH after TMX exposure point to the function of oxidative stress in TMX-induced nephrotoxicity. Various antioxidants, like the flavonoids, vitamin E, spirulina, and polyphenols, have lately been employed for their real or allegedly therapeutic benefits against oxidative stress[4, 15] **.**

Co-administration of SP can reduce the level of hydroxyl radicals and scavenge ROS. [12] have discovered that spirulina restored the abnormal blood levels of TNF-a, nitric oxide, oxidative stress, biochemical markers, and tissue lipid peroxidation. Our results are consistent with their findings. The significant decrease in GSH levels in rats administered TMX might increase the susceptibility of renal tissue to damage from free radicals. Spirulina could help reduce the oxidative hazard posed through TMX because of its scavenging properties and ability to stop the generation of radicals. Spirulina could prevent free radicals as well as activated oxygen species from reacting with biological structures or from becoming out of hand. Most free radicals and activated oxygen species must be eliminated by oxidation of the endogenous antioxidants, which mainly scavenge and reduce molecules. In this sense, the present work shows that, on the contrary with the negative effects of TMX, administering SP significantly enhances kidney function.

The activity of kidney antioxidant enzymes CAT, SOD, GR, GPx, and GST is shown in Table 3. Antioxidant enzyme activity was considerably ($P < 0.05$) lower in rats given TMX compared with the controls. Yet, in the case when SP was administered alone, antioxidant enzyme activities in the rat kidneys rose dramatically ($P < 0.05$). However, in comparison to the TMX-treated group, antioxidant enzyme activity significantly recovered in animals given SP supplementation prior to TMX treatment.

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Vol, 33; S.2 | 2025

Table3: Effects of Spirulina, thiamethoxam, and their combination on activities of the antioxidant enzymes of male rat kidneys

Parameters	Experimental Groups			
	Cont.	SP	TMX	SP+TMX
SOD (U/mg protein)	66.9±2.20^{b}	77.5±2.11^{a}	39.9±1.33^{d}	53.2±1.93^{c}
CAT (µmol/hr/mg protein)	51.2±1.14^{b}	61.2±2.04^{a}	29.8±1.01^{d}	39.8±1.15^{c}
GPx (U/mg protein)	25.7±0.93^{b}	30.0±0.97^{a}	15.6±0.43^{d}	20.4±0.71^{c}
GR (nmol/min/mg protein)	10.3±0.36^{b}	11.8±0.38^{a}	6.2±0.14^{d}	8.2±0.27^{c}
GST (µmol/hr/mg protein)	0.53±0.018^{b}	0.62±0.017^{a}	0.33±0.011^{d}	0.42±0.012^{c}

There are five rats in each group, and the data are shown as mean ± standard error (S.E.). Statistically significant differences ($p < 0.05$) between groups are shown by superscript letters (a-d), where 'a' denotes the greatest mean value and 'd' the lowest.

SOD = Superoxide dismutase, GPX = Glutathione peroxidase, GR= Glutathione Reductase, CAT = Catalase, GST = Glutathione s-transferase.

For the purpose of counteracting harmful ROS effects, organisms have evolved a complex, 3-line antioxidant defense mechanism. The best first-line defense mechanism includes antioxidant enzymes like GPx, CAT, and SOD. This line of defense is essential for dismutation superoxide radicals (O2•−) as well as hydrogen peroxide (H2O2). Through elimination of superoxide radicals, SOD maintains physiologically significant level of nitric oxide (NO•) and prevents the synthesis regarding the far more dangerous peroxynitrite ONOO− (O2•− + NO•− ONOO−), an essential component in neurotransmission, vasodilation, and inflammation. The second-line anti-oxidant defense mechanism includes exogenous small-molecule anti-oxidants from the diet. The third-line antioxidant defense is ensured by a variety of enzyme systems that repair or remove oxidized proteins as well as other macromolecules [38]**.** In aerobic organisms, SOD is an essential part of the antioxidant defense system. Superoxide anions (O2-) are dismutated by this enzyme into hydrogen peroxide (H2O2) and molecular oxygen (O2-).

Subsequently, hydrogen peroxide is further reduced to water by the action of catalase [27]**.** Consequently, it is believed that SOD and CAT prevent ROS from building up. Both under stress conditions and as byproducts of regular cellular metabolism, ROS can be generated in cells. Numerous pesticides have the potential to cause oxidative stress, which changes antioxidants and produces free radicals [41].

The antioxidant enzymes' counterbalancing effect is lost because pesticides either indirectly or directly generate excessive ROS [42]**.** Hydrogen peroxide (H2O2) accumulates when the enzymes that eliminate free radicals are inhibited. Detrimental effects of such accumulation may include DNA damage, lipid peroxidation, altered gene expression, and eventually cell death [43, 44]**.**

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Through reducing production of free radicals, spirulina might improve the activity of antioxidant enzymes in rats given TMX. The improvement of tissue thiol pools as well as the ability of spirulina to chelate reactive lipid peroxidation products and free radicals are two possible explanations for such protective effect. These processes are consistent with the known functions of glutathione metabolism-related enzymes and antioxidants. Spirulina's abundance regarding antioxidant-active components might be the cause of its protective qualities**. [16]** list these as C-phycocyanin, minerals, β-carotene, vitamins, vital amino acids, lipids, fatty acids, proteins, and carbs, all of which have strong anti-inflammatory and antioxidant qualities.

Kidney histopathological examination

In the control group (Figure 1A), the kidney exhibited the characteristic bean-shaped morphology. Histologically, it displayed a typical tubular structure composed of numerous uriniferous tubules, which constitute the primary component of renal tissue. The kidney was encapsulated by a firm connective tissue layer containing collagenous as well as some elastic fibers. In the spirulina group (Figure 1B), the histological examination revealed normal renal architecture, characterized by intact Bowman's capsules and renal tubules, comparable to the control group. Kidney sections regarding the thiamethoxam group (Figure 1C) showed marked degeneration and dilatation of kidney tubules, congestion of blood vessels with RBCs, multiple hemorrhagic foci, coagulative necrosis, intertubular hemorrhage, and severe leucocytic infiltrations. Kidney sections of cotreatment of thiamethoxam with spirulina group (Figure 1D) showed mild intertubular hemorrhage, moderate leucocytic infiltrations, and mild atrophied glomeruli.

The results acquired are consistent with the findings regarding [19], who discovered that exposure to acetamiprid caused histopathological distortions. Likewise, glomerular congestion, hypercellularity, and inflammatory cell infiltration were observed upon exposure to 1/10 LD50 of Lufenuron insecticide [45]**.** found that rats subjected to higher dosages of imidacloprid showed tubular abnormalities in their kidneys as well as increased weight in their study of imidacloprid nephrotoxicity[4]. Additionally found that mice receiving greater doses of imidacloprid experienced tubular alterations and an increase in kidney weight, which is indicative of nephrotoxicity**,** [10].

Also, in agreement with the present results, treatment of rats jointly with acetamiprid and clove preserved the histoarchitectural pattern of the kidney, and the nephropathic alterations induced by acetamiprid were improved [45]. Hemorrhages and localized coagulative necrosis, as well as vacuolar degeneration of tubular epithelial cells, were seen in the kidneys. However, many xenobiotics have nephrotoxic effects that might affect the kidneys, which are the main organs involved in detoxification. Imidacloprid-induced lesions were found in kidney tissues by histopathological analysis.

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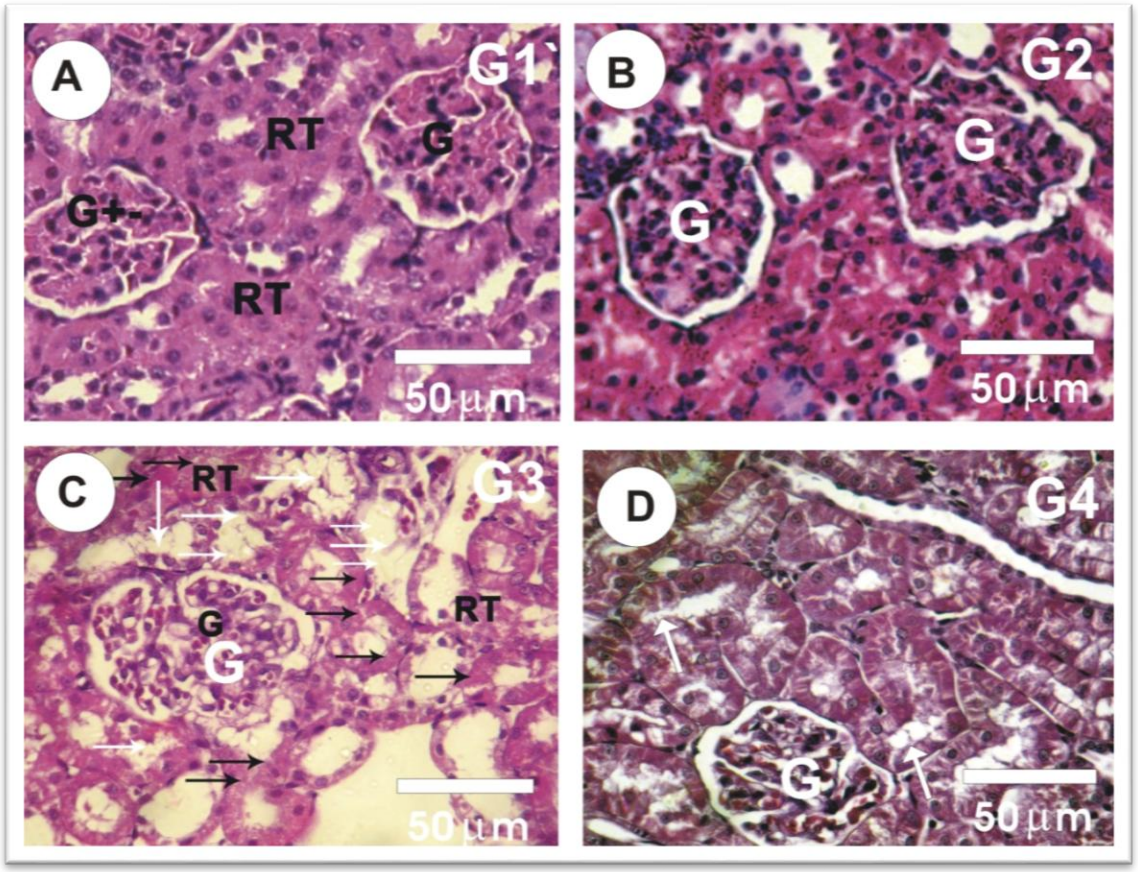

Figures 1A-D: H&E staining was used to examine rat kidney sections from various experimental groups histologically. A&B: Rat kidney sections from the spirulina and control groups showed that the renal tubules and Bowman's capsules were structurally normal. C: Kidney sections from the thiamethoxam group demonstrated severe coagulative necrosis (black arrows), dilatation of the kidney tubules (white arrows), and RBC-filled blood vessels. D: Kidney sections from rats given thiamethoxam and spirulina revealed considerable leucocytic infiltrations, minor intertubular hemorrhage, and kidney tubules that had deteriorated (white arrows).

KI67 immunoreactivity in Kidney

The discovery regarding an antibody against Hodgkin's lymphoma cells led to the first identification of Ki-67 protein. Anti-Ki-67 antibodies have emerged as one of the useful tools in clinical investigations for the detection and evaluation of cell proliferation due to their robust expression in proliferating cells and low expression in quiescent cells. According to [36]**,** Ki-67 protein is frequently utilized as a marker of tumor proliferation. Ki67 expression is commonly used as a proliferation marker in routine pathological investigations and is closely associated with tumor cell proliferation and development. The nuclear protein Ki67 (pKi67) is a known predictive and prognostic indicator for assessing of cancer patient biopsies. pKi67 was shown to connect with metastases as well as the clinical stage of malignancies. Additionally, it was shown that malignant tissues with poorly differentiated

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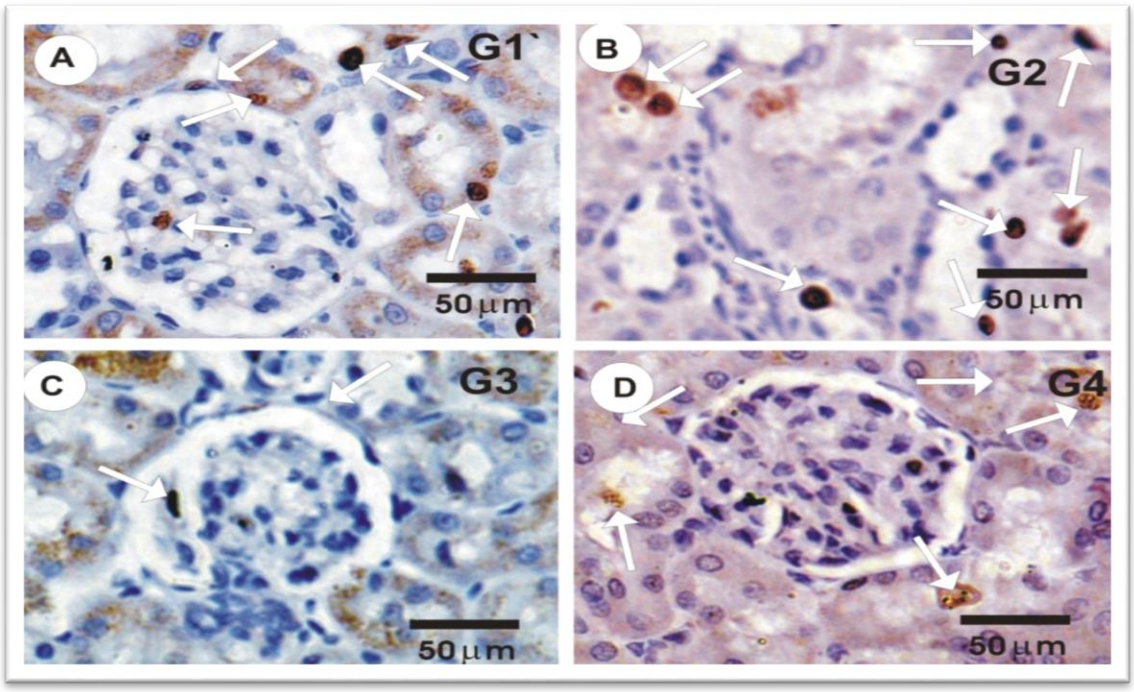
tumor cells produce Ki67 at significantly higher levels than normal tissue [46]. Figures 2 A to D displayed Ki67 immunoreactivity (Ki67-ir) detection and distribution in kidney slices from each group. The kidney sections from the spirulina and control groups showed a fairly good reactivity for Ki67-ir (grade 4) in the urinary tubules and glomeruli (Figs 2 A&B). However, the thiamethoxam-treated rats' kidney sections displayed somewhat positive Ki67-ir (grade 1) responses (Fig 2 C). However, thiamethoxam and spirulina-treated rats' kidney sections displayed somewhat positive Ki67-ir (grade 2) responses (Fig 2D).



Figures 2 (A-D): Histological sections of rat kidneys from each experimental group were stained with an anti-Ki-67 antibody for immunohistochemical analysis. **A&B**: Moderate Ki67-ir positive reaction (arrows) in the urinary tubules and glomeruli in control and spirulina groups. **B:** Faint positive reactions for KI67 in the thiamethoxam group (arrows). **D:** Mild positive reactions for KI67 in the cotreatment group of thiamethoxam with spirulina (arrows).

Conclusion

This study provides compelling evidence that Spirulina exerts potent antioxidant and nephroprotective effects against thiamethoxam-induced renal toxicity in rats. These findings suggest that Spirulina exhibits significant potential as a natural therapeutic agent for mitigating the adverse effects of thiamethoxam, a widely used insecticide. By effectively counteracting oxidative stress, preserving renal function, and ameliorating histopathological damage, Spirulina demonstrates promising renoprotective properties. To further advance our understanding, it is crucial to elucidate the underlying mechanisms of Spirulina's protective actions, with a particular focus on its potential to modulate key signaling pathways and inflammatory responses. Furthermore, translating these preclinical findings into potential therapeutic interventions for humans exposed to thiamethoxam and other nephrotoxicants warrants further investigation.

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Conflict of interests.

There are non-conflicts of interest.

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