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Biochemical and histopathological evaluation of the potential ameliorating effects of *Spirulina platensis* against acute hepatorenal toxicity of diclofenac sodium in male rats

Rania A. A. Faraag, Omnia M. Abdelhamid, Yakout A. El-Senosi, Afaf D. Abdelmagid, Alshaimaa M. Said*

Biochemistry and Molecular Biology Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac sodium (DFS) are used to treat pain, inflammatory conditions, and dysmenorrhea; however, the primary concerns with this medication are liver and kidney issues. The purpose of this study is to demonstrate the potential hepatoprotective and nephroprotective benefits of *Spirulina platensis* (SP) in rats exposed to DFS. Four equal groups of forty male rats were randomly assigned (10 each). The control group: rats received normal saline; SP group: rats were given SP orally 200 mg/kg b.wt dissolved in distilled water for 7 days; the DFS group: rats were received DFS orally 5 mg/kg b.wt dissolved in distilled water for 7 days; the DFS + SP group rats were received 5 mg /kg b.wt DFS with 200 mg/kg SP for 7 days. After the experiment was completed, serum samples were taken for evaluation of liver and kidney functions, and liver and kidney tissues were collected for histopathological examination. The results revealed that DFS administration for one week was followed by a significant increase in ALT and AST enzyme activities, a significant decrease in albumin concentration, and a significant increase in the levels of creatinine, urea, and uric acid when compared to the control group. However, concurrent oral administration of SP with DFS exhibited a moderate ameliorating effect against DFS hepatotoxicity and a powerful nephroprotective effect, as shown in the biochemical evaluations and confirmed with the histopathological examination. Therefore, it was concluded that *Spirulina platensis* had hepatonephroprotective effects against NSAID as diclofenac sodium used in the current study.

1. INTRODUCTION

Non-steroidal anti-inflammatory medicines (NSAIDs) are frequently used to treat acute pain, postoperative pain, rheumatoid arthritis, osteoarthritis, and spasms. They are also frequently used as analgesics, antipyretics, and anti-inflammatory therapies. They are sometimes referred to as cyclooxygenase inhibitors (Bindu et al., 2020). In addition, there have been several recent speculations regarding the use of NSAIDs, such as indomethacin, to treat COVID-19 by preventing the production of coronavirus RNA. It is also shown that it is possible to use celecoxib and diclofenac for inhibiting cyclooxygenase-2, which is promoted by SARS coronavirus (FitzGerald, 2020). Despite the NSAIDs' extensive therapeutic use, they have detrimental effects on a variety of organs, including the gastrointestinal tract, brain, cardiovascular system, liver, and lungs. These effects can increase the risk of organ damage, particularly if the NSAIDs are taken regularly at high doses for an extended period (Bindu et al., 2020; Dong et al., 2018). Consequently, diclofenac sodium is the NSAID that is most widely and regularly used, most readily available, and cheapest. Numerous investigations have demonstrated their toxicities in rats, which include immediate pulmonary toxicity, serious injuries in the liver and brain, gastric damage, hepato-renal failure, and gastrointestinal ulcers (Aljuhani et al., 2019; Mostafa et al., 2020). use of NSAIDs causes unwanted side effects, including kidney, heart, and gastrointestinal problems (Baker, 2018; Bjarnason et al., 2018; El-Yazbi et al., 2018) .

A common non-steroidal anti-inflammatory drug (NSAID) recommended due to its analgesic, anti-inflammatory, and antipyretic extended properties is diclofenac (Gan, 2010). Diclofenac sodium (DFS) is conjugated with glucuronic acid after its hydroxylation at sites 3, 4, and 5 in the liver. Although some of the metabolites are active, they have relatively little therapeutic value. Merely negligible amounts are eliminated as unaltered medications (Simon, 1994). Tissue damage is caused by 5-OH-diclofenac and 4-OH-

Correspondence to: alshaimaa.said@fvtm.bu.edu.eg

diclofenac, which are the intermediate products of diclofenac metabolism .

There are a lot of herbal plants on the globe right now. Utilizing these natural remedies can reduce the harmful effects of a variety of illnesses at a small cost. (Hamidah et al., 2009). Blue-green algae, or Spirulina platensis, is a filamentous cyanobacterium that is frequently employed as a single-cell protein. The World Health Organization (WHO) classified these microalgae as a very healthy diet in terms of nutrition. S. platensis is a great resource of vital amino acids, fatty acids, minerals, and vitamins, making it one of the finest alternative treatments for food fortification and production. Moreover, it offers antioxidant pigments, the most significant of which are beta-carotene, chlorophyll, and phycocyanin. The National Institutes of Health (NIH) states that S. platensis can be used to treat illnesses of the neurological system and problems of the metabolism, such as diabetes and dyslipidemia. It also has anti-inflammatory, antiviral, antibacterial, antioxidant, antianemic, and anticancer effects. Consequently, spirulina is referred to be a "superfood" and a "miracle from the sea." (Bitam and Aissaoui, 2020; Jung et al., 2019). Proteins, minerals, vitamins (particularly the B12 family), and different antioxidants (such as flavonoids, carotenoids, and phycocyanins) are abundant in spirulina, which has been a key source of feed for humans (Al-Qahtani and Binobead, 2019; Gargouri et al., 2016) .

Therefore, the goal of the current investigation was to evaluate the probable hepatonephroprotective effects of Spirulina platensis (SP) against NSAIDs such as diclofenac.

2. MATERIALS AND METHODS

2.1 .Experimental animals

In the present investigation, forty male albino rats, weighing 180±40 g, of 7–8 weeks old were utilized. The Laboratory Animal Research Center, Faculty of Veterinary Medicine, Benha University, provided the rats. Normal food was provided, fresh water was available, and the animals were housed in cages with bedding made of hardwood shavings under appropriate living conditions (21 to 24 °C, 12 h light/dark cycle, not exceeding 60% humidity) for a week for acclimatization. The experimental methods were authorized by the Faculty of Veterinary Medicine's Animal Care and Use Committee at Benha University in Egypt (BUFVTM 06-07-23) .

2.2 .Drugs and algae

Diclofenac sodium (DFS) is a non-steroidal anti-inflammatory drug that was purchased from PHARCO company. Diclofenac sodium is an oral tablet used to treat pain and inflammatory disorders. The tablets were crushed, suspended in distilled water, and given to male rats at a dose level of 5 mg/kg body weight daily for 7 days (Al-Hayder et al., 2022).

Spirulina platensis: known as blue-green algae, was purchased from the local herbal market in Cairo, Egypt, and is available in powder form. It is considered a source of vital vitamins, minerals, and fatty acids. It was suspended in distilled water and given to male rats at a dose level of 200 mg/kg body weight (Raghuvanshi et al., 2022).

2.3 .Experimental Design :

Four equal groups of rats were randomly assigned (10 rats /group).

Control group: rats were received orally normal saline; SP group: rats orally administered SP 200 mg/kg b.wt dissolved in distilled water for 7 days (Raghuvanshi et al., 2022); DFS group: rats were received DFS 5 mg/kg b.wt dissolved in distilled water per os for 7 days (Al-Hayder et al., 2022); DFS + SP group: rats were received 5 mg/kg b.wt from DFS and 200 mg/kg b.wt from SP for 7 days orally.

2.4 .Sampling

2.4.1 .Blood samples

A capillary tube was used to collect the blood samples from the medial canthus of the eye. To separate the serum, the collected samples were centrifuged for 15 minutes at 3000 rpm. and maintained at -20 °C to assess the biochemical parameters.

2.4.2 .Tissue specimens (liver and kidney tissue)

After the collection of blood samples, rats were euthanized, and the liver and kidney were quickly removed, washed, and preserved in 10% formalin solution for histological examinations after the tissue specimens were dissected out and rinsed with sterile physiological saline to eliminate any blood or clots.

2.5 .Biochemical analysis

ALT and AST enzyme activities were measured using commercial kits, according to Huang et al. (2006). Additionally, the albumin concentration was measured according to spectrophotometry using the procedure outlined by (Doumas et al., 1971). The concentrations of serum creatinine and urea were measured using the techniques described by (Kaplan, 1984; Schirmeister et al., 1984). Additionally, the uric acid concentration was measured using spectrophotometry according to the method described by (Young et al., 1975).

2.6 .Histopathological analysis

The histological preparation process was carried out in accordance with (Bancroft and Layton, 2013). small tissue specimens were collected from both kidneys and liver and immediately preserved in 10% neutral buffered formalin (10% NBF), dehydrated in ascending strengths of ethanol concentrations, cleaned in xylene, and embedded in paraffin. In order to analyze general tissue structure, the paraffin blocks were sectioned using a microtome at a thickness of 4-6µm and then stained with hematoxylin and eosin. A Leica microscope (CH9435 Hee56rbrugg) (Leica Microsystems, Switzerland) was used for histopathological examination of these sections .

2.7 .Statistical analysis

A one-way analysis of variance (ANOVA) was utilized to analyze the data, and Duncan was employed as the post-hoc test by SPSS 25 (SPSS Inc., Chicago, USA). The data was shown as Mean ± SE. P values ≤ 0.05 indicated statistical significance in the data.

3 .RESULTS

3.1 .Biochemical results:

The obtained data demonstrated that oral administration of DFS for one week showed a significant increase in ALT, AST enzyme activities, urea, creatinine, and uric acid levels with a significant decrease in albumin level compared to a control group. On the other hand, oral administration of SP with DFS showed a significant decrease in these liver enzyme activities and serum biomarkers of the kidney with a significant increase in albumin level compared to the DFS group, as shown in (Figure 1). Moreover, oral daily administration of SP to rats showed non-significant changes in all tested parameters compared with the control group.

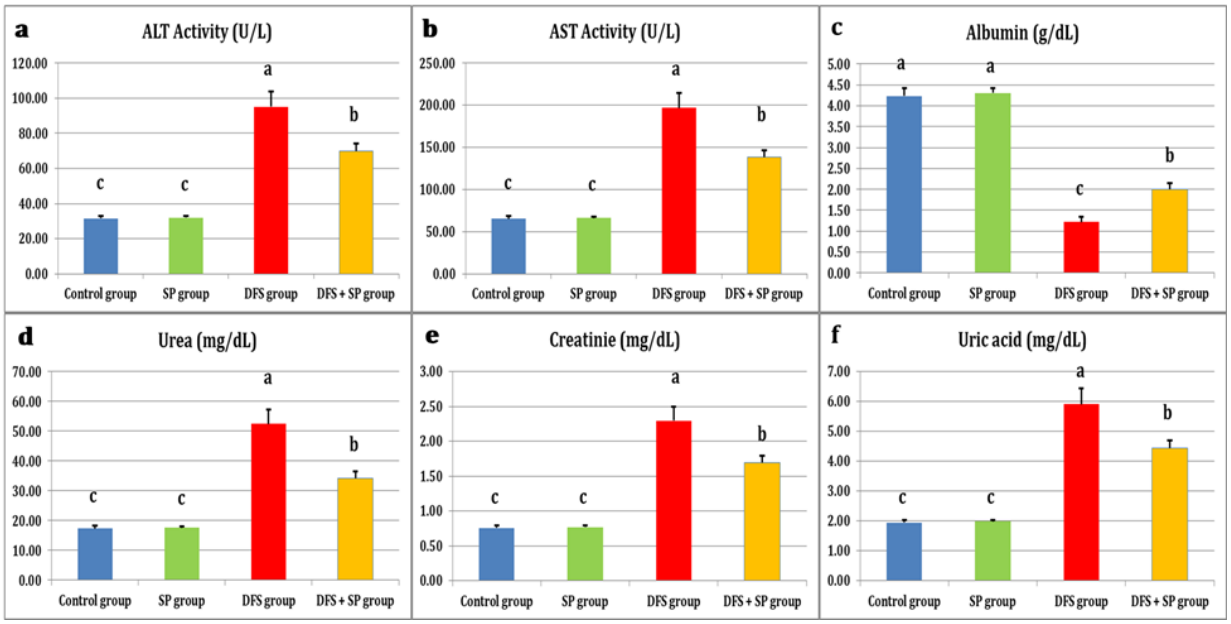

Figure 1:- Effect of DFS and /or SP on liver and kidney functions in male rats. a: serum ALT activity (U/L), b: serum AST activity (U/L), c: serum albumin (g/dl), d: serum urea level (mg/dL); e: serum creatinine level (mg/dL); f: serum uric acid level (mg/dL).

3.2. Histopathological results:

3.2.1. Histopathological changes in the liver tissue

The histological examination of the liver sections in the control and SP groups exhibited the typical structure of hepatic tissue with intact central veins. Hepatic cords existed from the central veins with regular organization and homing hepatocytes as light, vesicular, and spherical central nuclei. Between hepatic cords, hepatic sinusoids merged with standard structures (Fig. 2 a, b). Conversely, the DFS-treated rats highlighted severe substantial hepatic cell degeneration as well as hepatic cord disorganization. Most hepatocytes showed highly eosinophilic cytoplasm and pyknosis of their nuclei. Dilatation and congestion of the central veins and hepatic sinusoids were found. Furthermore, multifocal micro-vesicular hepatic steatosis was also detected (Fig. 2c). On the other hand, oral administration of DFS with SP demonstrated an obvious attenuation in the hepatic lesions induced by DFS where the majority of the liver sections showed well-organized hepatic cords organization with normal hepatocytes. However, a few of these hepatic cells showed mild cytoplasmic vacuolation. Moreover, mild congestion of the hepatic sinusoids with moderate micro-vesicular steatosis was infrequently seen (Fig. 2d).

3.2.2. Histopathological changes in the kidney tissue

The histological examination of the renal tissue in the control and SP groups demonstrated the normal microscopic structure of renal cortex tissue with intact renal corpuscle, proximal and distal convoluted tubules (Fig. 3 a, b). In contrast, the DFS-treated rats showed severe histopathological changes represented by hypertrophy of renal corpuscle, cellular vacuolation, and apoptosis along glomerulus damage. Interstitial hemorrhage, as well as congestion of the renal blood vessels, was seen. Furthermore, the renal tubules lost their regular structure and presented severe tubular dilatation with flattening of their nuclei. Epithelial desquamation and pyknosis of the nuclei, as well as cytoplasmic vacuolation, were also seen (Fig. 3 c). In contrast, the administration of SP with DFS demonstrated moderate enhancement in renal cortex structure. Some renal capsules appeared atrophied but with declined vacuolation, whereas the bowmen's capsules appeared intact, and in some examined sections, mild interstitial hemorrhages were observed. Almost all renal tubular epithelium seemed intact, and only some appeared degenerated and apoptotic, and other tubules displayed epithelial desquamation (Fig. 3 d).

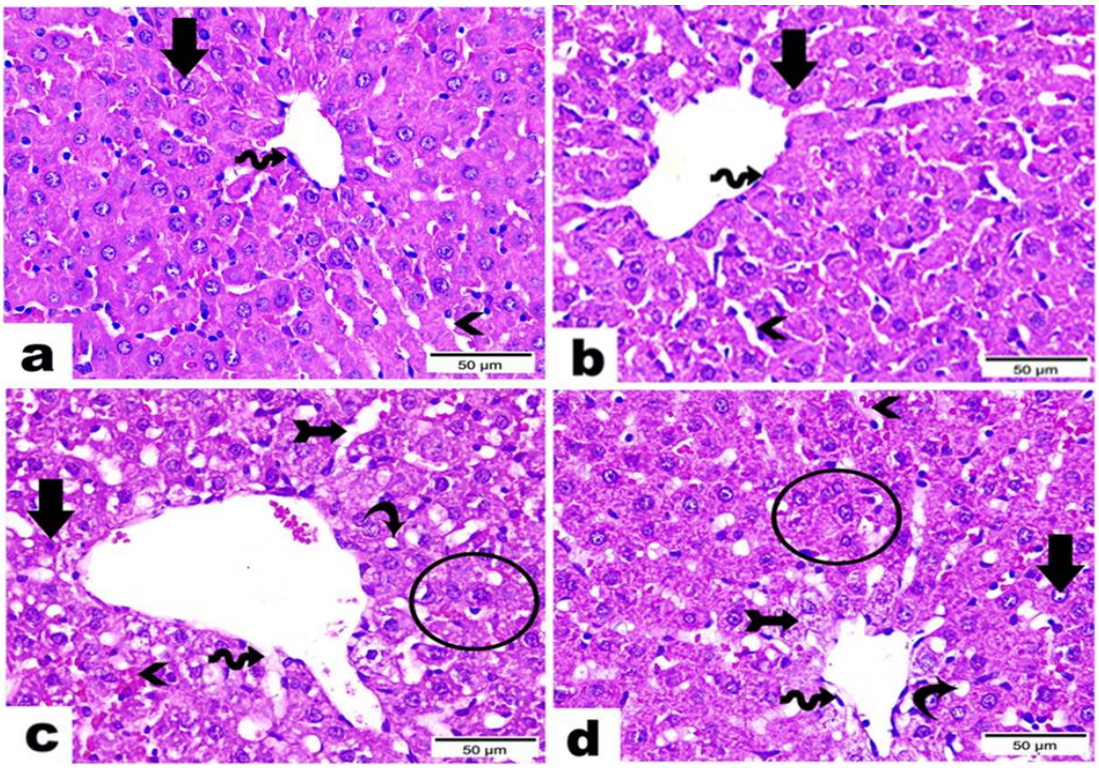

Figure 2:- Photomicrographs of histopathological changes in the liver tissue after oral administration of DFS and/or Spirulina to rats.a: control group, b: SP group, c: DFS group, d: DFS + SP group (wave arrows: hepatic tissue, arrows: hepatic cords, arrowheads between hepatic cords and hepatic sinusoid, arrow with tail: hepatic sinusoids, curved arrow: microvesicular steatosis)

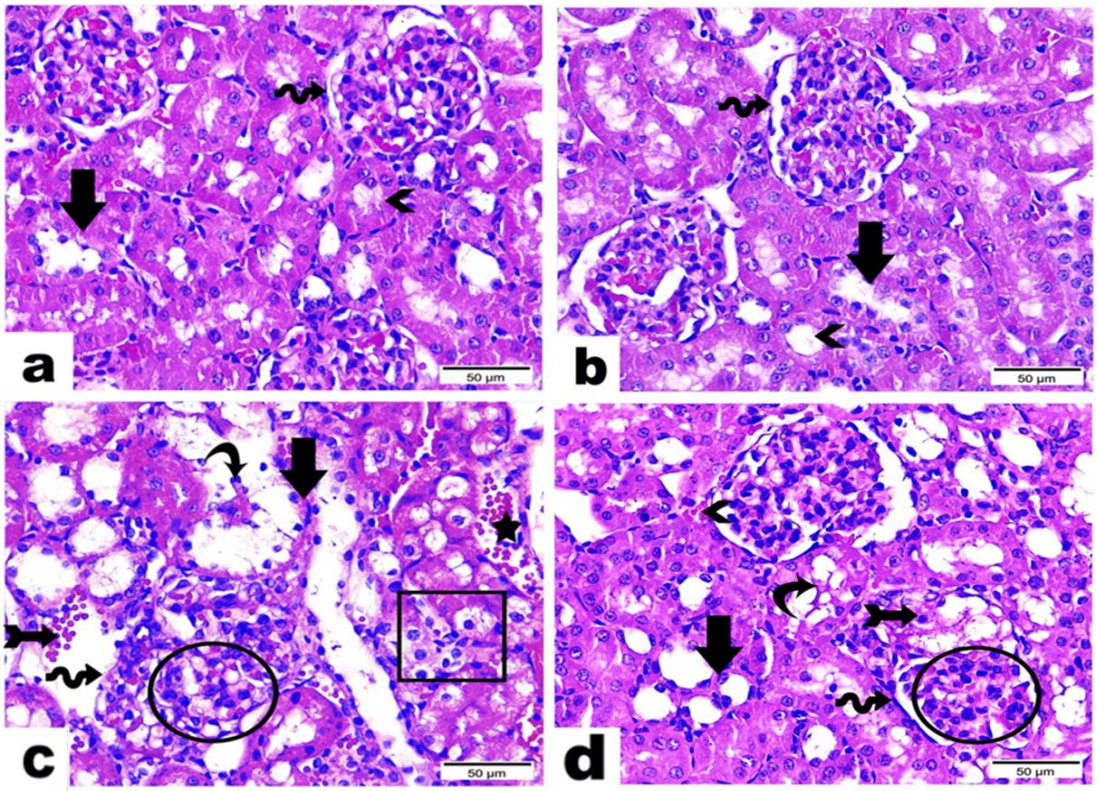

Figure 3:- Photomicrographs of histopathological changes in the kidney tissue after oral administration of DFS and/or Spirulina to rats (a: control group, b: SP group, c: DFS group, d: (DFS + SP) group. (wave arrows: renal cortex, arrowhead: proximal convolute tubule, arrows: distal convoluted tubule, arrow with tail: interstitial hemorrhage, star: congestion of blood vessel, curved arrow: epithelial desquamation, rectangle: apoptotic nuclei with perinuclear vacuolation)

4. DISCUSSION

The liver is a vital organ in the metabolism of several drugs, and it becomes vulnerable to oxidative stress from NSAID metabolites, which have significant therapeutic benefits in the treatment of arthritic and musculoskeletal pain, but large doses may damage liver cells. Diclofenac, a non-steroidal anti-inflammatory drug, has undesirable consequences on liver and kidney functions. (Siva et al., 2019). Diclofenac is metabolized in the liver and excreted in bile (35%) and urine (65%) (Vane and Botting, 1996). It has been reported that these metabolites can cause both nephrotoxicity and hepatotoxicity (Castell et al., 1997). In this study, we evaluated the influence of spirulina that can protect the liver and kidney against DFS-induced hepatorenal toxicity.

According to the current study's biochemical findings, oral DFS administration for one week induced severe alteration in hepatic and renal functions when compared to the control group. These results came in line with those of (Ogbe et al., 2022), who recorded that the intramuscular injection of 10 mg/kg bw diclofenac produced liver injury. The mechanism of DFS-induced hepatotoxicity has been partially attributed to the generation of reactive oxygen species (ROS), which may cause oxidative stress (Adeyemi and Olayaki, 2018), the alteration of protein integrity, immune-mediated processes, and mitochondrial damage (Masubuchi et al., 2002). Moreover, (Orabi et al., 2020) attributed the hepatic damage produced after diclofenac administration to the activation of cytochrome P450 and the following oxidative stress, which alters the mitochondrial and cellular permeability. This was expressed as an elevation in hepatic enzymes and a decrease in serum albumin level, which is synthesized mainly in the liver. Also, the histopathological findings of the current study proved the hepatic damage induced by diclofenac, which is consistent with the previous report of (Esmaeilzadeh et al., 2020).

Concerning renal function, previous studies by (Izak-Shirian et al., 2022) and (Elbaz et al., 2022) reported obvious nephrotoxicity after diclofenac administration to rats at a dose (50 mg/kg) for 5 days and (10 mg/kg) for 7 days. These results were consistent with the current study. These alterations in renal function after diclofenac administration could be attributed to the oxidative stress elicited by the hydroxy metabolites of diclofenac. Naidoo and Swan, (2009) also connected diclofenac nephrotoxicity to the reactive oxygen species produced and the subsequent DNA and cellular damage (Aycan et al., 2018). Another explanation was presented by (Prince, 2018), who linked the decrease in prostanoids and the inhibition of the cyclooxygenase enzyme. Since prostanoids affect hemodynamics (Näslund et al., 2017), the reduced renal blood flow may disclose the resultant necrosis (El-Maddawy and El-Ashmawy, 2013).

On the other hand, concurrent oral administration with spirulina significantly improved hepatic and renal functions compared to the DFS group. The previous record by (Raghuvanshi et al., 2022) supported the results of the current study. They found that oral administration of spirulina in 4 doses (50,100, 200, and 400 mg/kg/day) for 7 days showed a significant decrease in ALT, AST enzyme activities, and bilirubin concentration and a significant increase in total protein concentration compared to the group which injected i.p with beryllium. In addition, Sayed et al., (2022) observed that oral administration of spirulina in a dose (300 mg/kg) for 4 weeks to rats showed a significant decrease in ALT, AST enzyme activities, and bilirubin concentration compared to rats administrated Titanium dioxide nanoparticles.

This noticeable improvement in liver function may be due to the presence of flavonoids and β-carotene in SP as well as their potent antioxidant properties and ability to activate free radical-scavenging enzymes (Althobaiti, 2023). Since the main cause of the hepatic function alterations in the current study was the reactive hydroxy metabolites of diclofenac it is acceptable that boosting the antioxidant system with a dietary supplement as spirulina constitutes powerful antioxidant properties markedly ameliorates diclofenac hepatotoxicity.

According to the results of the biochemical analysis the serum kidney biomarkers were declined in the current study compared to DFS group. These results were nearly similar to the reported studies of (Gargouri et al., 2020) and (Mokhbatly et al., 2020), who proved that spirulina constitutes a nephroprotective effect against lead acetate and chlorpyrifos, respectively. This efficacy may be due to its antioxidant content as polyphenols, b-carotene, vitamins C and E, etc. (Martins et al., 2016), which assists in scavenging free radicals (Sayed et al., 2022). Therefore, it is advisable to include spirulina in the daily routine, especially for those who use diclofen or any NSAIDs to avoid or minimize the undesirable side effects. Further studies are recommended to elucidate the possible mechanism of action through which spirulina exerts its action, which will be included in the subsequent studies.

5. CONCLUSIONS

This study provided more evidence that diclofenac sodium-induced pronounced hepatic and renal damage, as presented in the biochemical and histopathological findings. Furthermore, from the aforementioned biochemical and histopathological findings, we can conclude that spirulina constitutes hepatoprotective and nephroprotective effects against diclofenac sodium, probably via its powerful antioxidant and scavenging free radical activities.

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