Hepato-Protective Effects of Blue-Green Alga *Spirulina platensis* on Diclofenac-Induced Liver Injury in Rats

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ABSTRACT

Introduction: This research aimed at evaluating the hepato-protective effect of the alga *Spirulina platensis* against diclofenac-induced liver injury in rats. The study's ultimate aim was to understand whether *Spirulina* could be developed as a functional food for therapy and protective therapeutic use. **Methods:** Hepatic injury was induced by administering diclofenac sodium (50mg/kg i.p.) for 5 days. *S. platensis* dose (300mg/kg) was administered to rats (180-220 g) orally for 5 days. On day 3 and day 4 after *Spirulina* dosage, diclofenac was administered. The reference drug silymarin was used in the study. Antioxidant activities like superoxide dismutase (SOD) activity, catalase (CAT) activity, reduced glutathione (GSH) and lipid peroxidation (LPO), glutamate-pyruvate transaminase (GPT), glutamate-oxaloacetate transaminase (GOT), alkaline phosphatase (AlP) activities, and levels of total protein, total bilirubin, direct bilirubin, and lipid profiles including high density lipoproteins (HDL), low density lipoproteins (LDL), very-low-density lipoprotein (VLDL), triglycerides and total cholesterol in serum were determined and compared with animals treated with diclofenac alone. **Results:** Animals receiving *S. platensis* prior to the administration of diclofenac significantly counteracted the diclofenac-induced changes, decreasing GPT, GOT, and AlP activities, total bilirubin, LDL, and total cholesterol in serum, and lipid peroxidation in liver tissue. *Spirulina*-treated rats revealed similar results to those treated with silymarin. **Conclusion:** These results, combined with hepatic histopathological observations, demonstrated that *S. platensis* has potential hepato-protective effect against diclofenac-induced hepatic injury in rats. More studies should be conducted to confirm the hepato-protective properties of *S. platensis*, and its development as a potential functional food.

 Key words: Diclofenac, hepato-protection, liver, rat, spirulina

INTRODUCTION

Several agents are known to cause moderate to severe hepatic complications. Depending on the duration of injury and the histological location of damage, drug-induced liver injury is categorised as acute or chronic, and either as hepatic, cholestatic, or a mixed pattern of injury. Liver is a prime target for medication-induced damage. In addition, a wide range of traditional medical therapies and herbal remedies may also be hepatotoxic. Non-steroidal anti-inflammatory drugs (NSAIDs) have been implicated in causing liver injury, and diclofenac is more commonly associated with hepatotoxicity (Unzueta & Vargas, 2013).

Quite a few drugs available presently have been associated with a number of side effects. Hepatotoxicity is currently a class

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warning for NSAIDs, and frequent hepatic injury has been observed for nearly all NSAIDs currently dispensed in the market. Drugs that have been more commonly associated with liver disease are diclofenac, sulindac, and aspirin (Marija *et al*., 2012). Diclofenac and its metabolites undergo extensive conjugation with glucuronic acid and sulfates (Sarda *et al*., 2012).

In liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver and steatohepatitis, drug- and chemi-cally-induced hepatic toxicity), antioxidant medicines such as silymarin are the primary therapeutic choice (Blázovics & Fehér, 2001). Hepatoprotective activity of silymarin has been demonstrated by various researchers from all over the world against partial hepatectomy models and toxic models in experimental animals by using acetaminophen (Muriel *et al*., 2006), carbon tetrachloride (Mourelle *et al*., 2009), ethanol, D-galactosamine and *Amanita phalloides* toxin (Pradhan & Girish, 2006). Silymarin has also been found to protect liver cells from injury caused by ischemia, radiation and viral hepatitis (Pradhan & Girish, 2006).

Naturally occurring nutritional supplements have been studied for their beneficial effects in drug-induced tissue and organ damage. *Spirulina platensis* is a blue green alga, rich in proteins, lipids and carbohydrates. The antioxidant properties of *S. platensis* and its capacity to scavenge hydroxyl radicals and to inhibit lipid peroxidation (Karadeniz, Cemek & Simsek, 2009), together with its hepato-protective properties through decreasing the liver lipid profiles and lipoperoxidation products (Abd El-Baky, El-Baz & El-Baroty, 2009), have attracted the attention of many researchers.

The rationale for this work is that there has been no report available on the effect of *S. platensis* on diclofenac-induced hepatotoxicity. The potential of *S. platensis* to confer protective effects against diclofenac-induced liver injuries is yet to be completely explored. The current work was initiated to investigate the effects of *Spirulina* and the extent to which it protects the liver from diclofenac-induced hepatotoxicity.

METHODS

All experiments and protocols described in the present study were carried out after approval by the Institutional Animal Ethics Committee (IAEC) of Babaria Institute of Pharmacy, Vadodara and with permission from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, IAEC approval number M.Pharm Sem- IV/11-12/15.

Procurement and maintenance of experimental animals

Healthy adult Wistar rats weighing 200-250g were used in this study. The rats were housed in polypropylene cages, maintained under standardised conditions (12-h light/dark cycle, 23±1°C, 50 to 80% relative humidity) and provided free access to normal diet and pure drinking water *ad libitum.*

Chemicals

All the laboratory reagents used in the assays were of analytical grade, and were procured from Qualigens Fine Chem Pvt Ltd, Sulab laboratory, Sisco Research Laboratory Pvt Ltd, Himedia Lab Pvt Ltd and Chemdyes Corporation. Laboratory diagnostic kits for biochemical estimations were procured from Erba Diagnostic, Mannheim GmbH, IFCC Method, Kinetic, and Seimen's Ltd, Vadodara.

- Spray-dried *Spirulina platensis* was obtained as a gift sample from Smit Health Care, Vadodara.

- Silymarin was procured from Microlabs, Bangalore.

• Diclofenac sodium was procured from Chemdyes Corporation, Vadodara.

Treatment protocol

Rats were divided into four groups of 6 rats each.

Group 1:   Control (rats received standard diet and normal saline)

Group 2:   Rats received diclofenac sodium (DFS) (50mg/kg i.p. on day 3 and day 4)

Group 3:   Rats received *S. platensis* pretreatment at 300 mg/kg body weight p.o. per day, for 5 days. However, on day 3 and day 4, the rats were given diclofenac dose (50 mg/kg; i.p.) 1 h after treatment with *S. platensis*.

Group 4:   Rats received standard silymarin (100mg/kg; p.o.) for 5 days. However, on day 3 and day 4, the rats received diclofenac dose (50 mg/kg; i.p.) 1 h after the treatment with silymarin .

Isolation of tissues

Collection and processing of blood for estimation of biochemical parameters were carried out on day 1 to obtain baseline values. On day 6, after the animals were fasted overnight, blood was collected under light ether anesthesia from the retro-orbital plexus without any anticoagulants. The serum was separated by centrifuging the sample at 5,000 rpm for 10 min, and analysed for biochemical parameters using biochemical kits (diagnostic kits).

On completion of the treatment, rats belonging to different groups (normal control, diclofenac sodium control, *S. platensis* group and reference standard silymarin group) were sacrificed on day 6 of the treatment schedule, under high dose of ether (as per CPCSEA guidelines). A part of the liver tissue was isolated, washed with ice-cold saline and stored at -20°C until further analysis.

Histopathology

The liver was collected after the rats were sacrificed. After blotting free of blood and tissue fluids, the liver was washed with ice-cold saline and used for histopathological studies. The liver tissue was fixed for 48 h in 5% formalin and dehydrated by passing successively in different mixtures of ethyl alcohol–water, cleaned in xylene, and embedded in paraffin. Then,15 µm thick sections were cut on a microtome and mounted on a glass slide. The liver sections were then stained with 10% hematoxylin for 3-5 min and washed in running water to intensify the staining. The sections were then counterstained with 10% eosin for 2 min. The sections were observed and desired areas photographed using a photomicroscope under 40X magnification.

Analytical procedures

Isolation of subcellular organelles like mitochondria and peroxisomes was done by using previously established methods (Davies *et al*., 1994). These fractions were used for the assay of marker enzymes. All the activities of antioxidant enzymes were estimated in the mitochondrial fraction, except for catalase which was estimated in the peroxisomal fraction.

Marker enzymes for liver function, like glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were estimated in serum using standard kits.

Protein profiles in this study were assayed using serum from the rats. Serum bilirubin (TBIL) was estimated using the method described by Otsuji *et al*. (1988), and the results were compared with those obtained with the commercially available Iatro T-Bil kit. Albumin-A in serum was estimated using the enzymatic kit (BCG

Table 1. Effect of diclofenac (DFS), spirulina (SPI) and silymarin (SLM) on serum glutamate-pyruvate transaminase (SGPT), serum glutamate-oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) enzyme activities in hepatic tissue of normal and experimental rats.

Group	Treatment	SGPT activity(U/L)	SGOT activity (U/L)	AlP activity (U/L)
1	Control	26.96±0.96	33.26±2.33	87.35±1.70
2	DFS	75.40±0.97***	90.04±1.32***	147.3±1.28***
3	SPI + DFS	54.93±1.80***	64.12±1.32***	128.7±1.86***
4	SLM + DFS	44.10±1.64***	51.10±1.76***	117.3±1.59***

Note: Values are means ± SEM of 6 separate observations. Statistical significance (*** *p*< 0.001) is calculated as follows: Against control for DFS and against DFS for SPI + DFS and SLM + DFS (Newman Keul's multiple comparison tests).

method) and total protein was estimated by the biuret method.

Lipid profiles were estimated in the serum collected from the blood of control and exposed rats. All the estimations were done using standard kits. Cholesterol in serum was estimated using the enzymatic kit (CHOD-PAP, end method), triglycerides using the GPO-TRINDER end point method, and HDL-C by using the PEG precipitation method.

Hepatic superoxide dismutase (SOD) activity was assayed by the method of Wysowski *et al*. (1993) at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. The activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1U per milligram of protein. Catalase (CAT) activity was determined by the method of Michelson (1991), and the absorbance of the sample was measured at 240 nm for 1 min in a UV-spectrophotometer. The concentration of reduced glutathione (GSH) in liver sub-cellular fractions was measured as described by Blum (1991). The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of Planas *et al*. (1990). All enzyme activities were expressed per mg protein and the tissue protein was estimated according to the method of Quinn *et al*. (1994) using bovine serum albumin (BSA) as the standard.

Statistical analysis

The values were expressed as mean ± SEM from six animals. The results of serum and tissue parameter analyses were expressed as Mean ± SEM. All the statistical analyses were carried out using the SPSS statistical tool (SPSS for windows, release 17.0.1, 2008, SPSS Inc., Chicago, IL). The Newman Keul's multiple comparison test and one-way analysis of variance (ANOVA) were used to assess the differences. P values of at least < 0.05 were considered statistically significant.

RESULTS

Effect of *Spirulina platensis* on serum glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (AlP) activities

The effects of various treatments on marker enzymes of liver function are shown in Table 1. Diclofenac sodium (DFS) (50mg/kg i.p.) elevated GOT, GPT and AlP activity levels when compared to the control. However, a significant decrease was observed in GOT and GPT and AlP activity levels in rats treated with *S. platensis* (300mg/kg) or silymarin (100mg/kg; p.o.) prior to diclofenac treatment, when compared to the DFS group. The effects of *S. platensis* on these enzyme activities were lower when compared with those of silymarin.

Table 2. Effect of diclofenac (DFS), spirulina (SPI) and silymarin (SLM) on total protein (TP) and serum albumin-A levels in hepatic tissue of normal and experimental rats

Group	Treatment	TP (g/dL)	Albumin-A (g/dL)
1	Control	6.60±0.20	92.40±1.25
2	DFS	5.33±0.16***	62.60±0.87***
3	SPI + DFS	5.85±0.06**	76.60±0.93***
4	SLM + DFS	6.43±0.16***	84.80±0.86***

Note: Values are means ± SEM of 6 separate observations. Statistical significance (*** *p*< 0.001 or ** *p*< 0.01) is calculated as stated in Table 1.

Table 3. Effect of diclofenac (DFS), spirulina (SPI) and silymarin (SLM) on total bilirubin (TBIL), direct bilirubin (BC) and indirect bilirubin (BU) levels in hepatic tissue of normal and experimental rats

Group	Treatment	TBIL (mg/dl)	BC (mg/dl)	BU (mg/dl)
1	Control	0.72±0.023	0.33±0.007	0.39±0.018
2	DFS	1.10±0.060***	0.25±0.003***	0.85±0.061***
3	SPI + DFS	0.91±0.022**	0.29±0.007**	0.62±0.024***
4	SLM + DFS	0.81±0.016***	0.29±0.008**	0.51±0.015***

Note: Values are means ± SEM of 6 separate observations. Statistical significance (*** *p*< 0.001 or ** *p*< 0.01) is calculated as stated in Table 1

Effect of *S. platensis* on total protein (TP) and serum albumin-A (ALB-A)
DFS significantly decreased TP and ALB-A when compared to the control. When treated with *S. platensis* or silymarin, the levels reverted towards the control. The effect of *S. platensis* was lower when compared to silymarin (Table 2).

Effect of *S. platensis* on total bilirubin (TBIL), direct bilirubin (BC) and indirect bilirubin (BU) in serum
DFS caused significant elevation in TBIL and BU (Table 3) compared to the control. However, there was a decrease in the BC level compared to the control. The TBIL level decreased by *S. platensis* and silymarin compared to DFS. An increase was found for BC while BU decreased with *S. platensis* and silymarin compared to DFS. The ameliorative effect of silymarin was higher than that of *S. platensis*.

Effect of *S. platensis* on triglycerides (TGL), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels in serum
The effect of *S. platensis* and silymarin on DFS-induced changes in TC and TG is shown in Table 4. DFS hepatotoxicity was associated with a significant elevation in TC and decrement in TGL levels compared to the control. The levels of TC were reduced and TG levels were elevated when treated with *S. platensis* and silymarin as compared to DFS. *S. platensis* caused a lower elevation in TC than silymarin but silymarin caused higher elevation of TGL than *S. platensis*. Thus, silymarin showed a greater ameliorative effect than *S. platensis* in the case of TGL, while *S. platensis* showed a greater ameliorative effect than silymarin in the case of TC, as seen from Table 4.

Table 4. Effect of diclofenac (DFS), spirulina (SPI) and silymarin (SLM) on triglycerides (TGL), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels in hepatic tissue of normal and experimental rats

Group	Treatment	TGL (mg/dl) (Mean ± SEM)	TC (mg/dl) (Mean ± SEM)	HDL(mg/dl) (Mean±SEM)	LDL (mg/dl) (Mean ±SEM)	VLDL (mg/dl) (Mean ± SEM)
1	Control	96.00±1.67	98.09±1.70	61.86±2.14	18.35±2.24	19.20±0.33
2	DFS	52.80±1.85***	145.5±1.33***	45.70±0.84***	89.28±2.12***	10.56±0.37***
3	SPI + DFS	61.00±1.41**	119.3±1.93***	52.13±0.36**	54.98±2.13***	12.20±0.28***
4	SLM + DFS	71.20±1.02***	125.3±1.03***	52.49±0.70**	58.60±1.21***	14.24±0.20***

Note: Values are means ± SEM of 6 separate observations. Statistical significance (*** $p < 0.001$ or ** $p < 0.01$) is calculated as stated in Table 1.

Table 5: Effect of diclofenac (DFS), spirulina (SPI) and silymarin (SLM) on superoxide dismutase (SOD) and catalase (CAT) activities, reduced glutathione (GSH) and lipid peroxidation (malonyldialdehyde, MDA) levels in hepatic tissue of normal and experimental rats

Group	Treatment	SOD (Units/ mg protein)	CAT (μmol H2O2 /mg protein)	GSH (μg/mg protein)	MDA (nmol of MDA / mg protein)
1	Control	14.96±0.52	239.8±4.65	88.54±2.06	6.226±0.21
2	DFS	13.28±0.94***	148.4±2.12***	43.95±2.18***	15.12±0.37***
3	SPI + DFS	25.58±0.63***	170.5±1.77***	56.77±2.55***	7.97±0.28***
4	SLM + DFS	24.78±0.87***	172.5±1.45***	54.57±1.95***	8.156±0.23***

Note: Values are means ± SEM of 6 separate observations. Statistical significance (*** $p < 0.001$) is calculated as stated in Table 1.

The effects of *S. platensis* and silymarin on DFS-induced changes in all the three lipoproteins are shown in Table 4. HDL and VLDL were reduced significantly with DFS treatment. LDL levels were elevated compared to the control. *S. platensis* and silymarin elevated the HDL and VLDL levels in contrast to DFS. Conversely, LDL levels were reduced by *S. platensis* and silymarin compared to DFS. Only VLDL showed significant elevation with *S. platensis* compared to silymarin, and no comparable effect was seen on HDL and LDL.

Effect of *S. platensis* on superoxide dismutase (SOD) and catalase (CAT) activities, reduced glutathione (GSH) and lipid peroxidation (LP) levels
The effects of *S. platensis* and silymarin on DFS-induced changes in biomarkers of oxidative stress are shown in Table 5. Oxidative stress associated with DFS hepatic toxicity was ameliorated following treatment with both *S. platensis* and silymarin. Significant reduction was seen in SOD and CAT activities and in GSH levels, and increment in MDA levels with DFS-induced hepatic toxicity when compared to the control. SOD and CAT activities, and GSH level were elevated after treatment with *S. platensis* and silymarin, while MDA level decreased compared to the DFS group. *S. platensis* demonstrated comparatively higher effect on SOD, CAT, GSH and MDA when compared with the silymarin group.

Histopathological changes
Normal liver histopathological sections showed that hepatocytes were well preserved, with uniform cytoplasm and sinusoidal spaces. Compared with the normal control group, liver tissue in
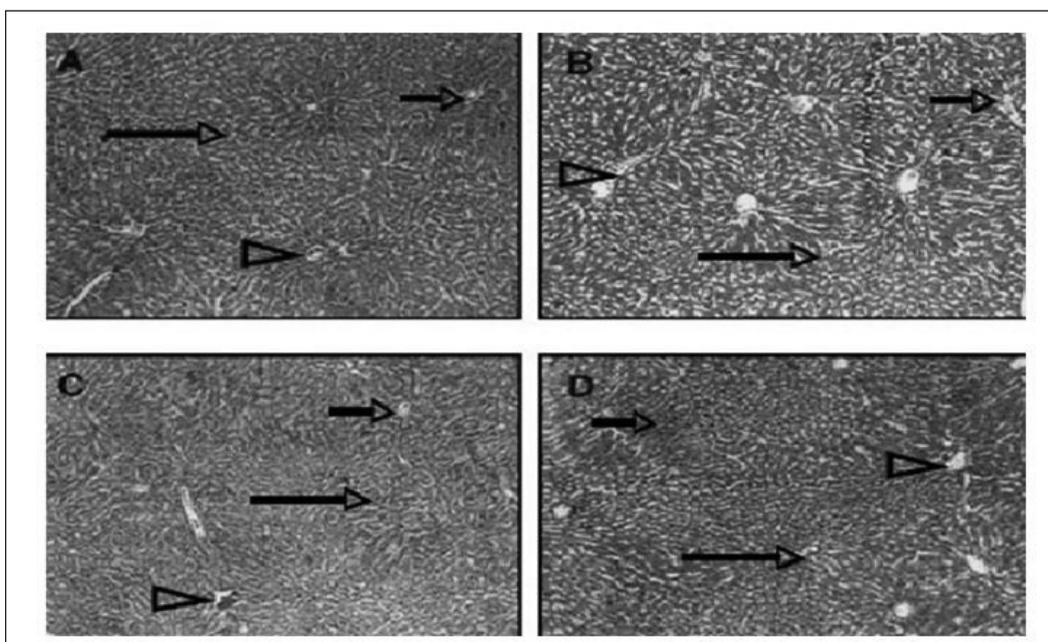
Figure 1. Rat liver histopathological sections of normal control (A), effects of diclofenac sodium (DFS) 50 mg/kg i.p. (B), *Spirulina platensis* 300 mg/kg p.o. + diclofenac sodium 50 mg/kg i.p. (C), and silymarin 100mg/kg p.o. + diclofenac sodium 50 mg/kg i.p. (D). Note the hepatic lesion caused by DFS-treatment, and the ameliorative effect of spirulina and silymarin.
Explanatory Note: A. Normal architecture of liver; B. Sinusoidal congestion, dilatation of blood vessels, enlargement of portal areas, and infiltration of mixed inflammatory cells around the necrotic hepatocytes and the portal area; C. Restoration of liver architecture to normal. Mild occasional congestion is observed. Inflammatory cells infiltration and necrotic hepatocytes are absent; D. Similar effect with Silymarin as in C with *Spirulina platensis*.

the rats treated with diclofenac sodium revealed liver injury, characterised by hepato-cellular degeneration, necrosis, dilatation of blood vessels, congestion in the lobules, enlargement of portal areas, and infiltration of mixed inflammatory cells around the necrotic hepatocytes and the portal area. The histopathological hepatic lesions induced by the administration of diclofenac sodium were improved by the treatment with *S. platensis*, and showed protective effect by decreasing hepato-cellular degeneration and necrosis. Similar protective effect was also observed in silymarin-treated animals (Figure 1).

DISCUSSION

The liver is an organ involved in many metabolic functions, and is prone to xenobiotic injury because of its central role in xenobiotic metabolism. Hepatotoxic drugs like paracetamol can cause damage to the liver (Buraimoh, Bako & Ibrahim, 2011). Diclofenac sodium (DFS) was used in this study to induce liver damage as it has been reported to be hepatotoxic (Gupta *et al*., 2004). The hepato-protective effects of *S. platensis* against DFS-induced liver injury in rats were assessed.

The hepatotoxic effects of DFS in both humans and experimental animals have been well-documented (Aydin *et al*., 2003). DFS has been shown to produce liver damage leading to alterations in biomarkers of liver function, lipid profile and endogenous antioxidant status of liver tissue. One of the most sensitive and dramatic indicators of hepatocyte injury

is the release of intracellular enzymes such as transaminases and serum alkaline phosphatase into the circulation (Aydin *et al*., 2003). Induction of hepatic injury with diclofenac in rats resulted in severe hepatotoxicity as reflected by an increase in the serum levels of GOT, GPT and AlP (Ahmed & Khater, 2001). It was observed that DFS elevated all these enzymes significantly, indicating severe hepatic cell necrosis. The rise in serum levels of GOT and GPT could be attributed to the damaged structural integrity of the liver as confirmed by histopathological results, which displayed cellular degeneration and loss of characteristic configuration. Notable restoration of all these parameters to the normal levels was possible with pretreatment with *S. platensis* and silymarin.

Pretreatment with *S. platensis* restored all the enzymes studied, and bilirubin levels as well. Bilirubin is the conventional indicator of liver diseases (Achliya, Wadokar & Dorle, 2004). The marked changes observed in serum bilirubin levels due to DFS-intoxication were reverted by *S. platensis* and silymarin. An increase in indirect bilirubin is most likely caused by destruction of too many red blood cells (Ahmad *et al*., 2013), which could have possibly resulted as a course of DFS toxicity. The decrease in total bilirubin and indirect bilirubin values indicate that the liver function was restored to near normal after pre-treatment of DFS treated rats with *S. platensis* and silymarin. This trend is in accordance with earlier findings (Gupta *et al*., 2004).

Drug-induced hepatotoxicity has been shown to be associated with high cholesterol levels and triglycerides (Pejic & Lee, 2006; Iweala, Obichi & Omotosho, 2011). The present study demonstrated a significant elevation in lipid profiles of rats treated with DFS. A marked increase in serum triglycerides and serum cholesterol was observed in DFS-treated rats (Baravalia, Vaghasiya & Chanda, 2011). In the present study, the levels of LDL and cholesterol were significantly higher in DFS-treated rats compared to the control rats, showing a drug-induced hypercholestiamic condition. These findings concur with earlier investigations on the elevation of lipid levels in rats due to DFS (Das & Roy, 2011). *S. platensis* and silymarin partially restored the triglycerides, total cholesterol levels, HDL-C and LDL-C. The results of our study showed a partial improvement in serum lipid profile in the DFS group pretreated with *S. platensis*. Although a gradual decrease was observed in the serum LDL, triglyceride and total cholesterol levels, restoration to normal was not possible with *S. platensis* alone as compared to the ameliorative effect produced by silymarin. The rise in protein and albumin levels suggests the stabilisation of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by *S. platensis* was similar to that of silymarin.

One of the main targets of ROS is the hepatic cells (Chetan *et al*., 2012). The induction of experimental liver injury in the rat using chemicals which selectively destroy hepatocytes is a simple method of demonstrating liver injury. This could possibly be related to one of the possible means involved in drug-induced hepatic injury which is to induce oxidative stress on cells that can cause an increase in the production of free radicals.

Lipid peroxidation has been implicated in the destructive process of liver injury due to DFS-administration (Darbar *et al*., 2010). In the present study, elevation in the levels of end-products of lipid peroxidation in the liver of rat treated with diclofenac was observed. An increase in lipid peroxidation and a decrease in the antioxidant enzyme activity in the DFS-treated group is indicative of the enormous oxidative stress, which is suggestive of the possibility of one of the factors for the occurrence of drug-induced hepatotoxicity.

The increase in malondialdehyde (MDA) level in liver suggests that

enhanced lipid peroxidation leads to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals, which occurred after diclofenac administration. Treatment with *S. platensis* significantly reversed these changes. Therefore, it is suggested that inhibition of lipid peroxidation by *S. platensis* may be attributable to its free radical scavenging activity (Amr *et al.*, 2006). This is in confirmation with earlier reports on the antioxidant effect of *S. platensis* (Karadeniz *et al.*, 2009). Similar results as stated above have been obtained in the present study, reflecting a significant increase in MDA content, and a lower activity of the enzymatic antioxidants SOD, CAT, and GSH in DFS-treated rats.

SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system (Chetan *et al.*, 2012). Decrease in SOD activity is a sensitive index in hepato-cellular damage. It scavenges the superoxide anion to form hydrogen peroxide, thus diminishing the toxic effect caused by this radical. A significant elevation in SOD activity was observed in the *S. platensis*-treated group as compared to the disease control group. This elevation might lead to a much more efficient removal of superoxide radical anions generated due to severe oxidative stress. As established by earlier reports regarding the antioxidant capacity of *S. platensis*, this could be possibly correlated to its effect on the liver injury, thus reducing the reactive free radical-induced oxidative damage of the liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver (Dash *et al.*, 2007). It decomposes hydrogen peroxide (H_2O_2) and protects the tissue from the highly reactive hydroxyl radicals (Anbarasu, Rajkapoor & Kalpana, *et al.*, 2011), and is thought to be the first line of defense against oxidative damage caused by H_2O_2 and other free radical-induced cellular damage

(Alanivel *et al.*, 2008). The reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radicals and hydrogen peroxide. The up-regulation of catalase activity was found to be slightly greater in the same treatment group (*Spirulina* + diclofenac) in comparison with the disease control group; subsequent treatment with *S. platensis* increased the level of CAT as in the case of silymarin.

Reduced glutathione (GSH) is an abundant tripeptide and non-enzymatic biological antioxidant present in the liver. DFS and its metabolised product, N-acetyl-*p*-benzoquinoneimine (NAPQI), can alkylate and oxidise intracellular GSH and protein thiol groups, which results in the depletion of GSH and subsequently leads to an increase in lipid peroxidation and hepatic damage as seen above.

The present study recorded data that clearly show decrements in the endogenous antioxidant levels in concurrence with earlier studies using NSAIDs such as diclofenac sodium (Nashwa & Abu Aita, 2014). A decreased level of GSH associated with an enhanced peroxidation in DFS-treated rats is an indication of toxicity. A significant increase in the GSH-Px activity was also observed with *S. platensis* treatment, indicating an active participation of the enzyme in scavenging the hydro-peroxides that are generated due to drug-induced liver damage. Antioxidant supplementation provided is one of the important means to reverse the deleterious effects (Sandrasegaran *et al.*, 2006). Supplementation of *S. platensis* restored the serum levels of liver enzymes and the lipid profile to a significant extent and increased the activity levels of SOD and CAT, and the level of GSH when compared to the DFS-treatment group.

In the present study, the lesions caused in rat liver by treatment with diclofenac sodium could be attributed both to biochemical and histopathological effects. The primary effect presumably is

the direct inhibition of lipid peroxidation and scavenging free radicals or indirect inhibition through impairment of the activity of catalase superoxide dismutase and disruption in reduced glutathione levels and the related biochemical machinery. The present study showed that diclofenac administration could also cause structural damage in liver tissue, and this damage could be ameliorated at least partially by treatment with *Spirulina vis-à-vis* silymarin.

CONCLUSION

The protective effect of *S. platensis* therapy against diclofenac sodium-induced oxidative stress in the present study may provide the much desired therapeutic benefit without prejudice to the therapeutic potential of a drug that carries with it the burden of undesirable and adverse effects.

Conflict of interest
The authors declare that they do not have any conflict of interest.

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