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Research Article

PROTECTIVE EFFECTS OF BLUE GREEN ALGAE *SPIRULINA FUSIFORMIS* AGAINST GALACTOSAMINE-INDUCED HEPATOTOXICITY IN MICE

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ABSTRACT

Objective: The present investigation was carried out to assess the protective properties of *Spirulina fusiformis* against galactosamine induced toxicity in swiss albino mice.
Methods: Evaluation of serum glutamate oxaloacetate transferase (SGOT), serum glutamate pyruvate transferase (SGPT), alkaline phosphatase (ALP), serum bilirubin (SBLN), antioxidant status and TNF-α was done and was compared with the standard reference drug silymarin.
Results: Galactosamine injection significantly increased the levels of SGOT, SGPT, SBLN and TNF–α in the serum and caused depletion in the antioxidant status in the liver. Administration of *Spirulina fusiformis* altered these parameters and brought them near to normal levels.
Conclusion: Hence results of this study clearly indicate that *Spirulina fusiformis* has hepatoprotective activity against galactosamine induced toxicity in mice.

Keywords: Spirulina, antioxidant, galactosamine, hepatoprotective, silymarin

INTRODUCTION

Liver is an important organ in the body as it performs metabolism of various exogenous as well as endogenous compounds, rendering it to be more susceptible to injury. Galactosamine (GalN) is known for inducing the features of acute hepatitis and its toxic effects are connected with an insufficiency of UDP-glucose and UDP-galactose and the loss of intracellular calcium homeostasis. This affects energy metabolism, cell membranes and organelles and the synthesis of proteins and nucleic acids [1, 2].

Spirulina is tiny, single celled, blue green alga which has been used as a source of potential pharmaceuticals as it is constituted of proteins, vitamins, essential amino acids, minerals and various essential fatty acids [3].Also, *Spirulina* contains a photosynthetic pigment phycocyanin (PC), which is found to posses antioxidant and anti-inflammatory properties and various pharmacological properties of *Spirulina* may be attributed due to it [4-6]. *Spirulina* is known to strengthen the immune system and is used for treatment of HIV and AIDS. [7] It also exhibits antiviral [8], anti-bacterial [9], anti-platelet [4], anti-cardiotoxic [5, 6], hypocholesterolemic [10] and anti-nephrotoxic effects [11].

Spirulina has been shown to prevent cataract [12], acute allergic rhinitis [13], cerebral ischemia [5, 6] and vascular reactivity [14] and has also been shown to be effective against cadmium [15] and arsenic induced-toxicities [16]. In recent years some of its properties have been confirmed through studies while additional pharmacological properties need to be proved. Thus, the present study was performed to evaluate the protective effects of *Spirulina fusifomis* against GalN induced liver injury in mice.

MATERIALS AND METHODS

Animals

Male Swiss albino mice weighing about 25-30 grams, were obtained from animal house, VIT University, Vellore. They were acclimatized for a week in a light and temperature –controlled room with a 12 hr dark-light cycle and were fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water *ad libitum*. The animals used in this study were treated in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of culture, Government of India, Chennai.

Drugs and Chemicals

The commercially available *Spirulina* tablets were obtained from the Acumen pharmaceutical private Ltd, Pondicherry. Silymarin, a standard hepatoprotective drug obtained from the (Micro labs, Goa, India) and galactosamine was obtained from SISCO Research Laboratories, Bangalore. Both, Silymarin and *Spirulina fusiformis* were dissolved in distilled water for use. All other reagents and chemicals used were standard reagents of analytical grade from SRLs, SD fine and other chemical companies.

Experimental Design

Mice were randomly divided into 5 groups consisting of six animals each. All animals were made to fast 24 h before the commencement of the study. The groups were as follows:

Group I: Control group, received saline (0.89 % NaCl, i.p.)

Group II: Galactosamine induced test group; hepatotoxicity was induced by the single dose of galactosamine (700 mg/kg body weight, i.p.)

Group III: Drug treated group (*Spirulina fusiformis* + galactosamine), *Spirulina* (100mg/ kg body weight, i.p.) was administrated after 30 min of the single injection of galactosamine (700 mg/kg body weight, i.p.)

Group IV: Positive control group (Silymarin + galactosamine), Silymarin (25 mg/kg body weight, i.p.) was administrated after 30 min of the single injection of galactosamine (700 mg kg body weight, i.p.)

Group V: The placebo control group, received *Spirulina fusiformis* (100mg/kg body weight, i.p) alone

Animals were decapitated after 4 hours of the last dose and blood was drawn from the trunk of the mice and plasma and serum were separated. Liver tissue of the control and experimental mice were isolated and were used for further biochemical and histopathological analysis.

Biochemical Parameters

SGOT [17], SGPT [17], ALP [18], SBLN (Autospan diagnostics, India) and TNF-α (ELISA, Cayman Chemicals, USA) were assayed in the serum of control and experimental mice. Lipid peroxidation in liver

tissue was determined by the procedure of Ohkawa et al. [19] by measuring the malondialdehyde (MDA) which is formed as an end product of the lipid peroxidation. Superoxide dismutase in the liver tissue was assessed according to the method of Marklund and Marklund [20].Catalase was assayed by the method of Sinha [21], glutathione reductase was assayed by the method of Bellomo et al. [22], reduced glutathione was evaluated using method of Moron et al. [23] in the liver tissue. Total protein was estimated using Lowry's et al. [24] using bovine serum albumin as standard.

Histopathological studies

Immediately after the sacrifice, a portion of the liver was fixed in 10% formalin, then washed dehydrated in descending grades of isopropanol and finally with xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5 µm thickness, stained with haematoxylin and observed under microscope.

Statistical Analysis

Results were expressed as mean ± S.D. and statistical analysis was performed using ANOVA, to determine significant differences between groups, followed by student's Newman-Keul's test.

RESULTS

Effect of *Spirulina fusiformis* on levels of liver functional markers in galactosamine treated mice

Liver functional markers i.e. SGOT, SGPT, ALP and bilirubin levels in serum were found to be significantly ($p<0.05$) higher in mice treated with galactosamine as compared to the control group (Figure 1-3). These were brought near to normal levels by *Spirulina fusiformis* treatment and similar effects were shown by the standard drug silymarin.

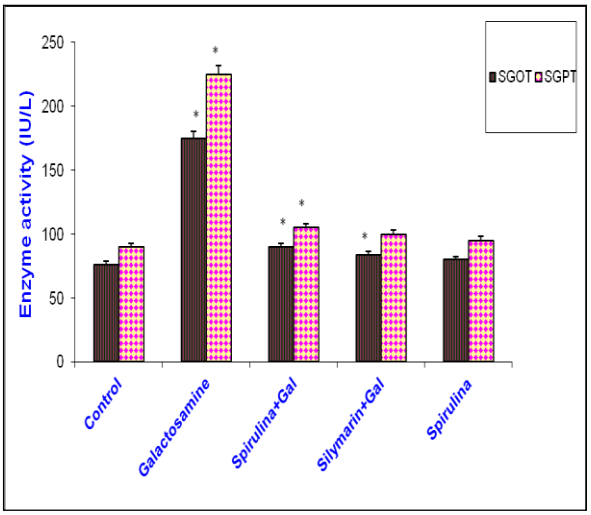

Fig. 1: Effect of galactosamine on the activity of SGOT and SGPT in the serum of control and experimental mice

For each group n=6, the values are mean ± SD. Comparisons were made as follows: group I vs. groups II, III, IV and V. Statistical analysis was calculated by one way ANOVA followed by Student's Newman–Keul's test. *p<0.05 (statistically significant)

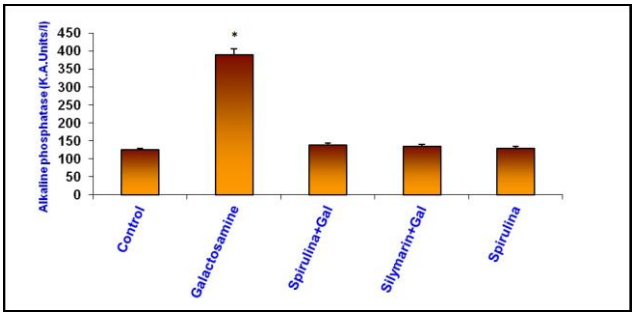

Fig. 2: Effect of galactosamine on the activity of alkaline phosphatase in the serum of control and experimental mice

For each group n=6, the values are mean ± SD. Comparisons were made as follows: group I vs. groups II, III, IV and V. Statistical analysis was calculated by one way ANOVA followed by Student's Newman–Keul's test. *p<0.05 (statistically significant)

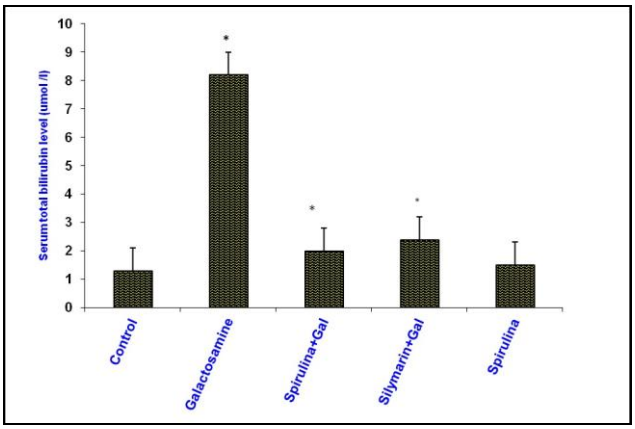

Fig. 3: Effect of galactosamine on the activity of total bilirubin in the serum of control and experimental mice

For each group n=6, the values are mean ± SD. Comparisons were made as follows: group I vs. groups II, III, IV and V. Statistical analysis was calculated by one way ANOVA followed by Student's Newman–Keul's test. *p<0.05 (statistically significant)

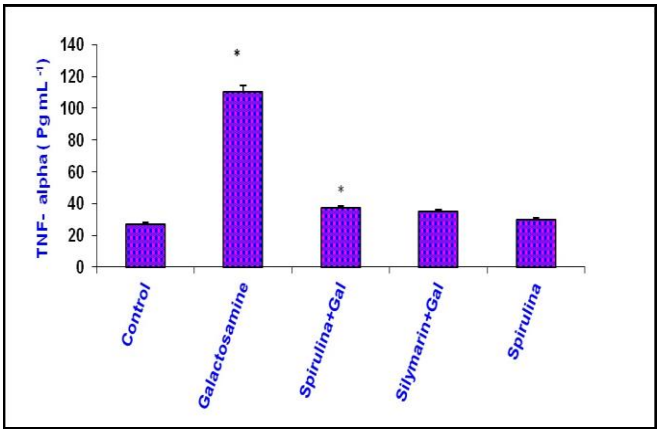

Fig. 4: Effect of galactosamine on the levels of TNF-α in the serum of control and experimental mice

Comparisons were made as follows: group I vs. groups II, III, IV and V. Statistical analysis was calculated by one way ANOVA followed by Student's Newman–Keul's test. *p<0.05 (statistically significant)

Effect of Spirulina fusiformis on levels of inflammatory mediator TNF-α in galactosamine treated mice

The levels of inflammatory mediator TNF-α were also found to be increased as a result of cell injury in the serum of Group II i.e., galactosamine treated mice. *Spirulina fusiformis* was able to provide significant ($p<0.05$) protection from the injury and thus the levels of TNF-α were found to be normal in Group III (Figure 4).

Effect of Spirulina fusiformis on antioxidant status in galactosamine treated mice

There was a significant reduction in the levels of antioxidants ($p<0.05$) and an elevation in lipid peroxidation in galactosamine treated mice (Table 1). *Spirulina fusiformis* was able to alter the levels of antioxidants and was able to inhibit lipid peroxidation as observed in group III (Table 1).

Table 1: Effect of galactosamine on lipid peroxidation and antioxidant status in the plasma of control and experimental mice

Parameter	Group I	Group II	Group III	Group IV	Group V
Superoxide dismutase(SOD)	240.83±31.0	146.0±18.9*	223.80±18.2	217±13.4*	255.67±12.1
Catalase(CAT)	160.33±7.92	86.66±8.76*	152±16.3*	138.67±15.3*	157.0±8.49
Glutathione reductase (GR)	43.0±7.16	16.16±1.03*	40.50±7.97	38.33±5.92*	41.66±4.93
Reduced glutathione(RG)	33.5±6.89	15.66±3.78*	26.33±5.35*	25.0±4.47*	29.66±4.97*
Lipid peroxidation(LPO)	1.68±0.33	4.75±0.93*	1.88±0.48	2.06±1.88*	1.51±0.41

For each group n=6, the values are mean ± SD. Comparisons were made as follows: group I vs. groups II, III, IV and V. Statistical analysis was calculated by one way ANOVA followed by Student's Newman–Keul's test. Units: SOD—units/milligram protein (1 U= amount of enzyme that inhibits the autoxidation of pyrogallol by 50 %), CAT—micromoles of H_2O_2 consumed/minute/ milligram protein, Glutathione reductase—nmol of NADPH oxidized/min/mg protein, reduced glutathione—nanomoles/milligram/protein, lipid peroxidation—nanomoles of MDA formed/milligram protein. *p<0.05 (statistically significant)

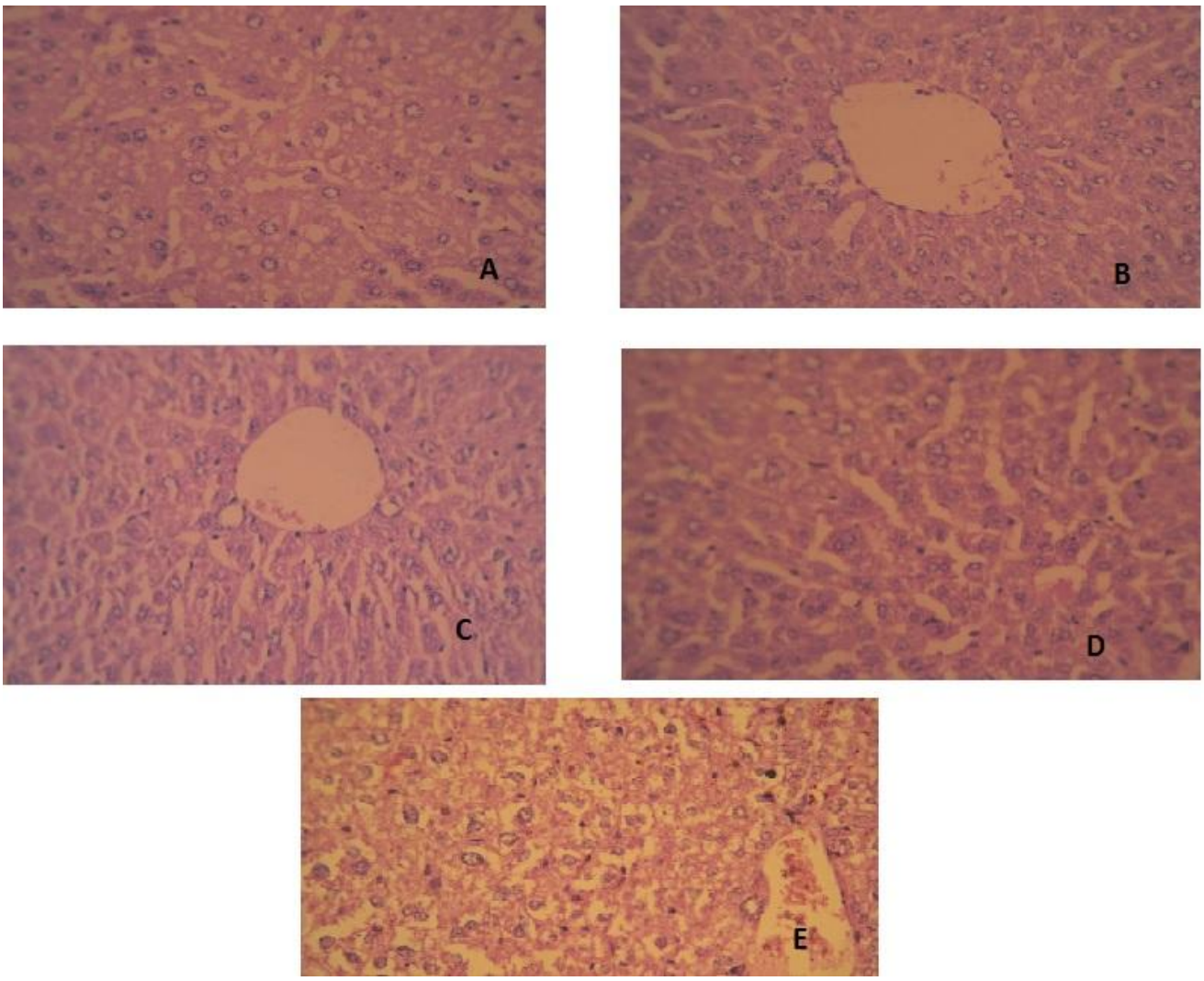

Fig. 5: Photomicrographs of liver sections

(A) Group I, (B) Group II, (C) Group III, (D) Group IV and (E) Group (V). (Haematoxylin & eosin stain, 400X magnification).

Histopathological findings

From the histopathological studies(Figure 5), we can see that the representative sections from control rats(Fig.5A) show central vein surrounded by normal hepatocytes, whereas in galactosamine treated group(Fig. 5B) liver sections show hepatocytes with enlarged nuclei with condensed and dispersed chromatin, Spirulina + galactosamine treated group showed central vein and hepatocytes with reactive and normal cells, silymarin and galactosamine treated group showed reactive hepatocytes(binucleate)and normal with degenerative hepatocytes, and the group which received only *Spirulina* showed central vein and normal hepatocytes with feathery cytoplasm(Fig 5E).

DISCUSSION

Galactosamine induced experimental model system in mice is recognized to be much like viral hepatitis in humans from both morphological and functional points of view [25]. The hepatoprotective effect of *Spirulina fusiformis* against galactosamine induced liver injury was evaluated and it was found to exhibit significant protection. Liver enzymes are membrane bound and due to cellular injury there is a leakage of these enzymes. So, their assessment in the serum is a useful quantitative marker of the hepatocellular damage [26].The increased levels of SGOT, SGPT and ALP in this study may be interpreted as a result of the liver cell destruction or changes in the membrane permeability and indicate the extent of hepatocellular damage caused by galactosamine. Treatment with *Spirulina fusiformis* attenuated the increased activities of these enzymes in serum caused by galactosamine as observed in the Figure 1 and 2. It can be suggested that the hepatoprotective action of *Spirulina fusiformis* might be due to presence of several active components.

Quantification of serum bilirubin is an evidence for the assessment of liver function and unusual increase in the levels of bilirubin in the serum indicates severe perturbation of hepatocellular function [27]. Increased levels of bilirubin in this study are in accordance with previous reports showing that GalN induced hepatitis is characterized by increment in levels of bilirubin in serum [28, 29] . *Spirulina* mediated suppression of the increased bilirubin level in Group III mice suggests the possibility of the *Spirulina* being able to strengthen biliary dysfunction (Figure 3).

Oxidative stress is known to play a key causative role in many diseases including liver damage [30]. Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against galactosamine induced liver damage. Antioxidant enzymes such as superoxide dismutase, catalase and glutathione provide protection against oxidative tissue damage [31].Superoxide dismutase is an effective defense enzyme that converted the dismutation of superoxide anions into peroxides [32]. Catalase is a hemeprotein in all aerobic cells that metabolize peroxides to oxygen and water. Glutathione reductase is a cytosolic hepatic enzyme involved in the detoxification of a range of xenobiotic compounds by their conjugation with reduced glutathione. These enzymes constitute a mutually supportive team of defense against reactive oxygen species. In the present study, the hepatic MDA level was increased in galactosamine-intoxicated mice, whereas a significant decrease in antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase) and total reduced glutathione was observed (Table 1). *Spirulina fusiformis* was able to restore the levels of these antioxidants showing its free radical scavenging action.

Macrophages, hepatocyte stress and/or damage could result in the secretion of signals that cause activation of other cells and are responsible for the production of proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver. It has been proved that inflammatory cytokines, such as tumor necrosis factor TNF-α, is involved in promoting tissue injury [33].Pretreatment with *Spirulina fusiformis* decreases the liver damage and inflammation and thus stabilizing levels of TNF-α (Figure 4).

It can be observed from the histopathological studies (Figure 5) that the liver tissue of mice that were treated with *Spirulina fusiformis* were normal as compared to the mice that were treated with the galactosamine.

CONCLUSION

Thus, the present study confirms the hepatoprotective action of *Spirulina fusiformis* against GalN induced hepatotoxicity in mice. The protective efficacy of *Spirulina fusiformis* is very promising as evidenced by the reversal of the altered values following administration probably by promoting regeneration of hepatocytes that restore integrity and it was confirmed by the histopathological studies. The hepatoprotective property of the extract may be attributed to the presence of various constituents which are present in *Spirulina fusiformis*. Still extensive research is required for understanding of the mechanism of action of *Spirulina fusiformis* for providing protection against galactosamine induced hepatotoxicity.

Conflict of interest statement

We declare that we have no conflict of interest between us.

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