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PROTECTIVE EFFECT OF *Spirulina* AGAINST PARACETAMOL-INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

The present investigation was designed to examine the possible potentials of *Spirulina* against hepatic intoxication induced by paracetamol in adult male rats. This study was conducted as an attempt to understand the mechanism of action, which may pave the way for the possibility to use it for therapeutic application. Oral administration of paracetamol (2g/kg.b.w.) induced liver damage in rats as an evidenced by the significant elevation in enzyme activities (AST, ALT and ALP), serum total lipids, total cholesterol, creatinine, total bilirubin and liver MDA, LDH. While a significant decrease in the levels of serum total protein and albumin and liver Pr-SHs, GSH, GST, GPx, SOD was recorded. Pre oral administration d of rats with *Spirulina* (500 mg/kg b.w. daily for 21 days) succeeded to modulate the effect of observed abnormalities caused by paracetamol, this fact was established by investigating biochemical and antioxidant parameters. These results substantiated the potential hepatoprotective and antioxidant activity of *Spirulina*.

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1 Introduction

Chemotoxic effects of chemicals or drug have been reported on the all body parts (organs) but some organs are more sensitive than the others and show higher toxicity. The liver is the largest internal organ in the body and played a vital role in the detoxification of harmful substances. It has regulatory effect on the many important metabolic functions and is responsible for maintaining homeostasis of the body (Mayuren et al., 2010). The concentration of the free radical is normally very low in the healthy organisms than the diseased person because they have capacity to neutralize, metabolize or subtract the toxic effects by free radical scavengers. The induction of excessive free radicals during metabolism may cause liver damage (Fridovich, 1983). Paracetamol is a commonly used analgesic-antipyretic drugs but excess use causes centriolbular liver necrosis, acute liver failure and death at high doses (Zhu et al., 2009).

Paracetamol can extensively metabolized by the liver via three main pathways; sulfonation, glucronidation and oxidation (Mitchell et al., 1974). The first two pathways are quantitatively more important than the last one, but the oxidative pathway is the culprit as far as toxicity is concerned (Jollow et al., 1973). Oxidation of paracetamol occurs in the hepatic microsomes and is primarily catalyzed by cytochrome P-450 (Potter et al., 1973). The process produces a highly reactive arylating compound called N-acetyl-p-benzoquinoneimine (NAPQI) (Dahlin et al., 1984). In human liver microsome P-4501A2 was shown to be principal catalysts of paracetamol activation (Raucy et al., 1989). NAPQI is rapidly conjugated with GSH and excreted eventually as the cysteinyl conjugate or the corresponding mercapturic acid (Cover et al., 2005). As long as the rate of formation of this toxin is not greater than the maximal rate of synthesis of GSH there will be no damage to the cell or organ (Sabina et al., 2009). Hepatic synthesis of GSH can be directly suppressed within the first few hours following ingestion of hepatotoxic does of paracetamol and manifestations of toxicity appear when GSH level falls below 30% of normal (Makin & Williams, 1997). If more NAPQI is formed it can be conjugated with GSH, the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins (Vermeulen et al., 1992; El- Banna et al., 2013).

Moreover, some investigators have suggested that hepatic macrophages may have a pathogenic role as the inhibition of their function status attenuates the degree of paracetamol-induced hepatic injury by a mechanism that is independent of NAPQI production (Jaeschke et al., 2003). So there is a worldwide trend to natural resources, which are culturally acceptable and economically viable. Among the important and effective drugs used to treat chronic diseases are derived from plants and certain species of cyanobacteria.

Spirulina (Blue green algae) is a microscopic single cell alga which grows in fresh water and has a simple structure but a complex composition. It is a concentrated source of food containing nutraceutical, antioxidants, probiotics properties. *Spirulina* is an important source of the blue photosynthetic pigmented protein C-phycocyanin, which has strong antioxidant and anti-inflammatory properties. *Spirulina* is known for its wide ranging biological activities, like prevention of anemia because of high iron and vitamin contents (Hemalatha et al., 2012), inhibition of herpes simplex infection (Ferreira-Hermosillo et al., 2011), reduction in HIV replication velocity (Ayehunie et al., 1998), increased production of antibodies, prevention of proliferation of neoplastic cells (Premkumar et al., 2004), hypoglycemic (Parikh et al., 2001; Abdel-Daim et al., 2013), hypolipemic (Jarouliya et al., 2012) and antihypertensive properties in experimental animal and humans models (Ponce-Canchihuamán et al., 2010), furthermore, it shows hepatoprotective properties through decreasing of the liver lipid profiles and lipoperoxidation products (Abd El-Baky et al., 2009), antimutagenic, antiviral, immune enhancing, cardio protective and anticancer properties (Khan et al., 2005). It attracted attention due to its ability to stimulate mineral absorption by its effects on intestinal microflora, carbohydrates, polyunsaturated fatty acids, sterols (Rehab & Ibrahim, 2012) moreover the presence of some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium was also reported (Gini & Muraleedhara, 2010). Some evidence also suggests that *Spirulina* can act on bone metabolism but at present no experimental evidence are available on this interesting and important aspect (Saxena et al., 2004). Hence, an attempt has been made to investigate the chelating mechanism followed by *Spirulina* against the paracetamol toxicity in albino wister rat. *Spirulina* has a property of reducing heavy metals and nephrotoxic substances from the body (Deepti et al., 2011). The present investigation was designed to examine possible potentials of *Spirulina* against hepatic intoxication induced by paracetamol in adult male rats in an attempt to understand its mechanism of action, which may pave the way for the possibility to use it for therapeutic application.

2 Material & Methods

Adult male albino rats (*Rattus rattus*) weight between 150 to 180 gm were obtained from the Nuclear Research Center, Inshas, during the experimental period these were kept in a well ventilated animal house and under the control managerial and environmental conditions. These animals were fed to appetite on a stander laboratory animal diet according to the NRC (1977).

Twenty four animals were randomly divided into four groups (six animals in each). Animals of the first group served as untreated control and orally received distilled water (2ml/kg b.wt.). The second experimental group was given *Spirulina* dissolved in water by gastric gavages @ 500 mg/kg b.wt. daily for 21days.

Animals of the third group received a single oral dose of paracetamol (2g/ kg b.wt.), rats of this group were sacrificed 48h after paracetamol administration. Group four rats administrated *Spirulina* for 21 days and on day 21 they received paracetamol and were sacrificed 48h after administration.

At the end of experimental period, the rats were slightly anaesthetized by diethyl ether (Sigma Chem. Co., St Louis, Mo. U.S.A.) and blood was collected from the heart in clean dry test tubes. At the same time, liver was carefully excised from each rat and immediately immersed in a saline solution (0.9% NaCl). Liver homogenates (10%) were prepared in 0.01 M Tris-HCl buffers (pH 7.4). The homogenate were centrifuged at 860g for 20 min at 4ºC, and the resultant supernatants were frozen at -20º C for hepatic parameters assay.

2.1 Biochemical assays

Serum was separated for the assessment of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) kinetically. Total lipids, cholesterol, proteins, albumin, bilirubin and creatinine were evaluated colormetrically using kits purchased from Bio- Merieux Co. Marey- L EtoileChorbonnieres, Les- Bains, France.

2.2 Chemicals

All reagents were of the highest purity available. Paracetamol was purchased from Sigma-Aldrich (St. Louis, Mo, USA). Spirulina powder was purchased from DXN Company in Malaysia.

2.3 Markers of oxidative stress

Lipid peroxidation in liver was estimated colorimetrically by measuring Malondialdehyde (MDA) by the thiobarbituric acid assay procedure (Albro et al., 1986). Lactate dehydrogenase (LDH) (Buhl & Jackson, 1978), protein thiols (Pr-SHs) (Koster et al., 1986), reduced glutathione (GSH) was determined by the method of Ellman (1959). Glutathione peroxidase (GPx) was determined by the methods described by Rotruck et al. (1973). Superoxide dismutase (SOD) activity was measured according to the method of Oyanagui (1984) by the inhibition of nitric reduction rate by 50% with superoxide anions generated by xanthine / xanthine oxidase system. The reaction was monitored at 550 nm light absorbance. One activity unit (50% inhibition) was expressed as NU (nitric unit) 1mg protein / min. Catalase (CAT) was estimated colorimetrically by the method of Sinha (1972) using diachromate-acetic reagent.

2.4 Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's multiple Range Test (DMRT). The values are expressed as means ± S.E. for 6 rats in each group. *P-Values* < 0.05 were considered significant according to (Snedecor & Cochran, 1982).

3 Results and Discussion

3.1 Effect of *Spirulina* on serum hepatic enzymes

As shown in Table 1, activities of serum AST, ALT and ALP were markedly elevated in paracetamol treated animal groups compared to control group, indicating liver injury. Administration of *Spirulina* @ 500 mg/kg body weight, significantly ($p<0.05$) lowered the elevation of serum enzymes induced by paracetamol in relation to control group (Table 1). The present study revealed the hepatoprotective activity of the *Spirulina* against well known hepatotoxins produced by paracetamol. The liver is the most sensitive organ for peroxidative damage because it is rich in oxidizable substances. The improvement in the oxidative stress of liver cells and the consequently decrease in the antioxidant ability of the cells caused an aggressive cellular damage in those cells in which destruction of membranes occurred and enzymes were released into the blood stream. More severe liver damage releases the higher amount of liver enzymes (El-Khayat et al., 2009). The increased levels of serum enzyme such as AST and ALT indicated the increased permeability and damage or necrosis of hepatocytes (Pari & Suresh, 2008).

In present study, the activities of AST, ALT, ALP and total bilirubin level were increased after administration of paracetamol, these incidence indicates that liver damaged by paracetamol. The present results are in agreement with the results of Ibrahim et al. (2011) and Hurkadale et al. (2012) in which hepatic markers were elevated. The increased activities of serum hepatic enzymes; AST, ALT, ALP in paracetamol treated animal groups may be attributed to cellular leakage and loss of functional integrity of cell membrane in liver (Abraham, 2005). Moreover, Bhat & Madyastha (2001) reported that the presence of blue pigment phycocyanin in *Spirulina* reduced the hepatotoxicity caused by paracetamol – induced free radicals.

Reduction in the levels of liver enzymes induced by *Spirulina* attributed to the inhibition of reaction involved in the formation of reactive metabolites and its radical scavenging activity. The presence of β-carotene, enzyme superoxide dismutase, vitamins or selenium in *Spirulina* produced immunostimulant activities and protective effects against paracetamol – induced liver damage (Alam et al., 2013). Furthermore, Luxia et al. (1996) confirmed that *Spirulina* treated animal groups resulted in the stabilization of plasma membrane as well as the repair of hepatic tissue damage produced by paracetamol.

Paracetamol toxicity is due to the formation of toxic metabolites when a part of it metabolized by cytochrome P_{450} (Ibrahim et al., 2011; Hurkadale et al., 2012).

Table 1 Effect of *Spirulina* on the activities of serum hepatic enzymes in normal and paracetamol treated rats.

Groups Parameters	Control	*Spirulina*	Paracetamol	Spirulina + Paracetamol
AST(U/L) % change from control	141.33 ± 1.15	127.67 ± 1.31[a] (-9.665)	171.15±1.33[ab] (21.1)	138.35±0.85[bc] (-2.109)
ALT(U/L) % change from control	39.17 ± 0.67	37.15 ± 0.50 (-5.157)	69.28 ± 1.35[ab] (76.87)	43.83 ± 1.25[abc] (11.9)
ALP(U/L) % change from control	125.70 ± 0.60	110.47 ± 0.93[a] (-12.116)	180.88 ± 1.40[ab] (43.898)	112.30 ± 0.76[ac] (-10.66)

The results are presented as means ± SE of six rats.
[a]significant from control group, [b] significant from *Spirulina* group and [c]significant from paracetamol group. Level of significance is at P <0.05.

Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity. The present study evaluates the protective effect of *Spirulina* against liver damage induced by paracetamol in male rats, especially the damage to DNA molecules, thus playing a role in the repair of damaged liver cells.

The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage caused by paracetamol (Luxia et al., 1996). The protective role of *Spirulina* may be attributed to the presence of β-carotene, Enzyme superoxide dismutase or selenium and blue pigment phycocyanin (Bhat & Madyastha, 2001). Phycocyanin (major pigment of cyanobacteria) significantly reduced the hepatotoxicity caused by paracetamol which induce the formation of free radicals. The hepatoprotective effect of phycocyanin was therefore attributed to the inhibition of reaction involved in the formation of reactive metabolites and possibly due to its radical scavenging activity.

3.2 Effect of *Spirulina* on biochemical parameters

The effects of paracetamol in toxification as well as the preventive effects of *Spirulina* on serum biochemical analysis were shown in table 2. Paracetamol treated group show significantly (P< 0.05) higher activities of serum total cholesterol, total bilirubin, creatinine and total lipid (37.269, 52.459, 190.816and 28.771% respectively) compared to untreated control group. *Spirulina* pre-administration at a dose of 500 mg/kg significantly (P< 0.05) reduced the serum total cholesterol, total bilirubin, creatinine and total lipid (4.762, 27.869, 22.449 and 4.666 % respectively) while, serum total protein and albumin were increased (2.801 and 0.873 % respectively) in this group as comparison with control group.

Table 2 Serum biochemical parameters of rats under normal and experimental conditions.

Groups Parameters	Control	*Spirulina*	Paracetamol	Spirulina+ Paracetamol
T.C (mg/dl) % change from control	139.23 ± 0.21	128.53 ± 0.25[a] (-7.685)	191.12 ± 0.20[ab] (37.269)	145.86 ± 0.27[abc] (4.762)
T. protein (g/dl) % change from control	8.21± 0.14	8.55± 0.10 (4.141)	7.20 ± 0.12[ab] (-12.302)	8.44 ± 0.17[c] (2.801)
Albumin (g/dl) % change from control	4.58 ± 0.04	4.50± 0.09 (-1.747)	3.64± 0.01[ab] (-20.524)	4.62 ± 0.03[c] (0.873)
T. bilirubin (mg/dl) % change from control	0.61 ± 0.01	0.59 ± 0.01 (-3.279)	0.93 ± 0.01[ab] (52.459)	0.78 ± 0.01[abc] (27.869)
Creatinine (mg/dl) % change from control	0.98± 0.01	0.96±0.01 (-2.041)	2.85±0.01[ab] (190.816)	1.20±0.05[abc] (22.449)
T. lipids (g/dl) % change from control	6.43±0.12	6.33±0.09 (-1.555)	8.28±0.06[ab] (28.771)	6.73±0.09[abc] (4.666)

The results are presented as means ± SE of six rats.
[a]significant from control group,[b] significant from *Spirulina* group and [c]significant from paracetamol group. Level of significance is at P <0.05.

Pre-treatment with *Spirulina* (21 days prior to paracetamol treatment), reversed the changes caused by paracetamol in most of the studied parameters.

The present results revealed that serum total protein and albumin of rats were significantly decreased in paracetamol treated animal groups. The decreased level of total protein is in agreement with the observation of Thirunavukkarasu & Sakthisekaran (2003). The reduction level of serum total protein produced by paracetamol – induced hepatotoxicity may be attributed to an increase in amino acid deamination (Varely, 1987) and significant fall in protein synthesis (Kanchana & Sadiq, 2011) which could be due to the peroxidative damage of liver (Bharathi et al., 2011). In the present study, total protein and albumin concentrations were increased and restored nearly to the normal concentrations in rats treated with *Spirulina* which may be attributed to its antioxidant properties.

According to Lyanda et al., (2010), estimation of total protein level in serum an important way to assess paracetamol-induced hepatic damage. The present results demonstrated that serum total protein and albumin of rats were significantly decreased in response to paracetamol administration. The decreased level of serum total protein is concomitant with those observed by Thirunavukkarasu & Sakthisekaran (2003) and may be attributed to an increase in amino acid deamination and / or significant fall in protein synthesis (Kanchana & Sadiq, 2011), which could be due to the peroxidative damage of liver (Bharathi et al., 2011). The present study disclosed the protective effect of *Spirulina* against the paracetamol induced damage, since they ameliorated the changes caused by paracetamol treatment. Similarly, Sadeghi et al. (2008) reported that total protein and albumin concentrations were decreased in CCl_4 treated animals, in comparison with the control group and they were restored almost to be near the normal values in groups treated with Cichoriumintybus leaves extract.

Creatinine is an important biochemical parameter for diagnosis of renal failure (Simeon et al., 1995). The present study observed that administration of paracetamol caused a significant increase in serum creatinine as compared with the control. This results are in agreement with the result of Ojiako & Nwanjo (2006) those are reported that administration of prostaglandin inhibiting drugs like paracetamol, ibuprofen exacerbated renal function. The nephroprotective effect of *Spirulina* appeared as it caused a reduction in the elevated creatinine level in paracetamol treated rats. Findings of the present study are in accordance with the Adeneye & Benebo (2008) those reported that elevation of serum creatinine was significantly attenuated by *Phyllunthus amarus* treatment. The nephroprotective ability of *Spirulina* recorded in the present study may be due to the presence of antioxidant compounds, as flavonoids and triterpenes or because of its free radicals scavenging effects (Annie et al., 2005).

Serum cholesterol level was significantly decreased in rats treated with *Spirulina* as comparison with paracetamol treated animal groups. Reduction in total cholesterol produced by *Spirulina* may be attributed to the presence of alkaloid, flavonoid and glycoside components (Vinuthan et al., 2007) and its ability to increase of lecithin-cholesterol acyl transferase activity (Shigemastsu et al., 2001).

Total lipids and total cholesterol levels were increased significantly in the serum of paracetamol intoxicated rats group. The possible explanation of the observed hyperlipidemia might reflect the impairment of liver cells to metabolize lipids or lipid peroxidation (El-Sayeda et al., 2012). The increase in serum lipids may be attributed to the increased hepatic synthesis and / or reduced of lipoprotein lipase (Mathur et al., 2005). *Spirulina* pre-treat rats prevented the paracetamol induced rise in serum total lipid and total cholesterol. These findings demonstrated its protective action on hepatic injury induced by paracetamol. Similarly, treatment with silymarin (Ramachandra Setty et al., 2007; Hsu et al., 2009) significantly lowered serum cholesterol content in animals received paracetamol or CCl_4, respectively.

3.3 Indices of oxidative stress

Data presented in Table (3) concluded that there were significant decreases in the activities of liver GSH, GPx, SOD and CAT in the paracetamol treated animals as compared with their corresponding control values. Treatment with *Spirulina* significantly restored these enzymatic activities to be approximately near the normal limits in most of the cases.
Administration of paracetamol (2g/kg. body weight) to animals led to the saturation of conjugation pathway leading to glutathione, caused marked depletion and an increase in the formation of toxic reactive metabolites, mitochondrial dysfunction and oxidative stress (Gini & Muraleedhara, 2010). The C-phycocyanin has an excellent antioxidant property and scavenging free radicals like superoxide and hydroxyl radicals.

The generation of ROS, free radicals and decreased GSH levels significantly increased oxidative stress (Nagaraj et al., 2011).The overall effect of depletion of glutathione with concomitant increases in lipid peroxide levels provides evidences about the vital role of glutathione during oxidative stress. Some of the active constituents of *Spirulina* such as flavonoids, β-carotene and phycocyanin have been reported to possess strong antioxidant activity and provokes free radical scavenging enzyme system. The significant protection offered by C-phycocyanin demonstrated the radical scavenging activity and its inhibitory effect on lipid peroxidation chain reaction (Abdel-Daim et al., 2013). The observed increase in circulating lipid peroxides of paracetamol treated animals in the present study, correlates with the decline of circulatory antioxidants such as SOD, CAT, GPx and GSH.

Table 3 The activities of antioxidant enzymes in the liver of control and experimental rats.

Groups Parameters	Control	*Spirulina*	Paracetamol	Spirulina + Paracetamol
GSH (mg/g protein) % change from control	23.19 ± 0.66	24.25 ± 0.77 (4.571)	10.92 ± 0.55[ab] (-52.911)	20.41 ± 0.88[abc] (-12.009)
GPx (Units/mg protein) % change from control	11.41 ± 0.27	11.24 ± 0.22 (-1.490)	8.14 ± 0.15[ab] (-28.659)	10.49 ± 0.28[abc] (-8.014)
SOD (Units/mg protein) % change from control	15.37 ± 0.76	16.22 ± 0.65 (5.530)	6.96 ± 0.45[ab] (-54.717)	13.88 ± 0.81[bc] (-9.722)
CAT (Units/mg protein) % change from control	72.32 ± 0.63	73.08 ± 0.57 (1.051)	41.67 ± 0.67[ab] (-42.381)	65.48 ± 0.52[abc] (-9.458)

The results are presented as means ± SE of six rats.
[a]significant from control group,[b] significant from *spirulina* group and [c]significant from paracetamol group. Level of significance is at P < 0.05.

Table 4 Activity of lactate dehydrogenase (LDH), malondialdehyde (MDA) and protein thiols (Pr-SHs) in liver of control and experimental groups.

Groups Parameters	Control	*Spirulina*	Paracetamol	Spirulina + Paracetamol
LDH (U/g protein) % change from control	302.53 ± 1.18	283.76 ± 1.78[a] (- 6.204)	552.51±6.41[ab] (82.629)	324.86±2.97[abc] (7.381)
MDA (nmole/mg protein) % change from control	12.95 ± 0.72	13.92 ± 1.45 (7.490)	27.72 ±2.53[ab] (114.054)	16.22±1.63[abc] (25.251)
Pr-SHs (µmol/g protein) % change from control	75.71 ± 1.39	84.23 ± 2.38[a] (11.253)	49.52 ± 1.57[ab] (-34.593)	86.21 ± 2.38[abc] (13.869)

The results are presented as means ± SE of six rats.
[a] significant from control group,[b] significant from *Spirulina* group and [c]significant from paracetamol group. Level of significance is at P < 0.05.

These results may be due to their extensive utilization to scavenge the products of lipid peroxidation as well as sequestration by injury cells. Antioxidant enzymes such as super oxide dismutase (a Cu^{2+} dependent enzymes), catalase (in which NADPH protect it against inactivation by its substrate H_2O_2) and glutathiones-transference (glutathione related enzymes affected by ROS) play a major role in the intracellular defense against oxygen radical damage to aerobic cells (Hemalatha et al., 2012). Furthermore *Spirulina* is considered as a valuable additional food source of some macro and micronutrient including high quality protein, iron, gamma linolenic fatty acids, carotinoids and vitamins (Mazo et al., 2004).

Table 4 showed the concentration of liver LDH, MDA and Pr-SHs of control and experimental groups of rats. Oral administration of paracetamol (2g/kg. body weight), to rats markedly increased the lipid peroxidation in the tissue of liver as indicated by increased ($p< 0.05$) level of malondialdehyde (MAD) in comparison with the control value in paracetamol rats. Also, the levels of hepatic tissue lactate dehydrogenase (LDH) activity in paracetamol treated rats were significantly higher than control rats, whereas rats treated with *Spirulina* before paracetamol restored the altered values to the near normal values. The decreased concentration of hepatic Pr-SHs was observed in paracetamol intoxicated rats. Administration of *Spirulina* before paracetamol treatment tends to bring the Pr-SHs level to near normal.

For estimating the level of lipid peroxidation, MDA was elevated in hepatic tissues demonstrated in the present study after administration of paracetamole could be expected owing to the depletion in GSH stores and reduced GPx activity. It is well documented that liver tissue contains a relatively high content of polyunsaturated fatty acids, which are sensitive to peroxidative damage (Galal et al., 2012), another reason that could explain the marked effects of paracetamol on lipid peroxidation that has been observed in the liver in this study (Table 4). Gini & Muraleedhara (2010) reported that C-phycocyanin present in *Spirulina* inhibited paracetamol-induced lipid peroxidation in rats liver cells.

The present conducted preliminary study concludes the non-toxic nature of spirulina on normal rats. Paracetamol causes acute centrilobularhepatic necrosis in rats and other animal species. Hepatic necrosis following massive paracetamol administration is well documented (Waters et al., 2001). Drastic elevation in the activity of liver cytosolic LDH after administration of paracetamol was reported in the study. Paracetamol toxicity was reported to associate with increased amount of LDH in the experimental animals (Blazka et al., 1996).

The increase in cytosolic LDH activity by paracetamol might be due to the intracellular accumulation of $Ca^{2+,}$ which results in activation of phosphofructokinase and anaerobic glycolysis leading to lactate formation. Loss of Ca^{2+} homeostasis as a result of oxidative damage and the increase in intracellular Ca^{2+} has been reported to a late and perhaps irreversible final stage in the process of cell death for paracetamol(Strubelt & Younes, 1992). *Spirulina* administration controlled hepatic LDH. However, it did not normalize the LDH level completely as it remained lesser than paracetamol rats.

On the other hand, hepatic protein thiols (Pr-SHs) content was markedly decreased after paracetamol administration, as observed in the current investigation. Similar results were reported for paracetamol (Kyle et al., 1990). The loss of Pr-SHs is held to be a critical event in the genesis of lethal injury by an acute oxidative stress (Di Monte et al., 1984). Such depletion is presumed to be a direct oxidation of the thiol groups of contiguous amino acids with the formation of protein-protein disulphides. The cytotoxic effects of paracetamol have been attributed to depletion of Pr-SHs, (Kyle et al., 1990). The covalent binding of NAPQI accounts for only a part of Pr-SHs depletion induced by paracetamol by NAPQI itself.

In addition, the metabolism of paracetamol also generates GSSG, a product that reacts with Pr-SHs to form GSH mixed disulphides. In the present study, the elevation of Pr-SHs levels in liver was observed in the *Spirulina* rats. This indicates that the *Spirulina* can either increase the biosynthesis of Pr-SHs or reduce the oxidative stress.

Conclusion

The findings of the present study confirmed the role for ROS activation in the pathogenesis of paracetamol-induced liver damage, and indicate that a blockade of their formation/activation by *Spirulina* has a potentate-inflammatory effect. *Spirulina* can be used as a model for the biotechnological production of antioxidant compounds. This study showed that *Spirulina* has hepatoprotective effect, powerful antioxidant action and free radicals scavenging activity.

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