Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 9 (1): 57-64, 2014
ISSN 1815-9923 / DOI: 10.3923/ajb.2014.57.64

Ameliorative Effects of *Spirulina fusiformis* Against Bromobenzene Induced Nephrotoxicity in Rats: A Biochemical and Histoarchitectural Study

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ABSTRACT

In the current study, we investigated the protective role of *Spirulina fusiformis* against bromobenzene-induced nephrotoxicity and oxidative damage in rats. Bromobenzene is a commonly used industrial solvent which causes hepatic and renal necrosis. Nephrotoxicity was induced in rats by oral administration of bromobenzene (10 mmol kg^{-1}) and the protective effect of *Spirulina fusiformis* was evaluated by assessing changes in levels of the renal functional markers (Urea, uric acid and creatinine), glutathione, antioxidants and lipid peroxidation. Antioxidant status was found to be decreased whereas lipid peroxidation (0.32±0.06) increased depicting oxidative stress and histopathological examination of the tissue showed tubular necrosis due to the administration of bromobenzene. Pre-administration of *Spirulina fusiformis* to bromobenzene treated rats caused significant ($p<0.05$) restoration in the levels of renal functional markers, antioxidants, lipid peroxidation (0.93±0.10) and histological architecture in rats. The nephroprotective role of *Spirulina fusiformis* against bromobenzene-induced toxicity in rats can be attributed to its antioxidant effects and its free radical scavenging properties.

Key words: Nephroprotective, glutathione, silymarin, lipid peroxidation

INTRODUCTION

Bromobenzene is a well-known industrial solvent which has profound use in the manufacture of various drugs and chemicals, as flame retardant, as crystallizing agent and as an additive to motor oil. Living beings are exposed to it during its production or its use in various industries or as a result of exposure to motor oil. Since it is a hydrophobic compound, it is bio-transformed in liver and its secondary metabolites 2, 3-epoxybromobenzene and 2-bromohydroquinone oxidized to 2-bromoquinone, enter the kidney (Bruchajzer *et al*., 2002). At high doses of bromobenzene, the levels of glutathione (GSH) are reduced as a result of conjugation to these metabolites and thus the intracellular protection against Reactive Oxygen Species (ROS) is lost. This may lead to a number of secondary events that damage the cell, like lipid peroxidation (Benedetti *et al*., 1986), ATP depletion (Locke and Brauer, 1991; Wang *et al*., 1999) mitochondrial dysfunction, energy imbalance and altered intracellular calcium levels. This leads to necrosis in the kidney. It is a good model for toxicity studies as levels of detectable liver injuries are significantly higher and also it is a dose-dependent toxicant (Jollow *et al*., 1974).

Spirulina is a blue green algae of the Oscillateriaceae family and is known for its high content of essential amino acids, vitamins and minerals. In addition, several studies have shown that Spirulina species exhibit various biological activities such as anticancer (Amaro *et al*., 2013), anti-inflammatory (Vo *et al*., 2013), chemoprotective (Chamorro-Cevallos *et al*., 2008), hepatoprotective (Sabina *et al*., 2009) and anti-arthritic effect. From the previous studies, *Spirulina fusiformis* has been found to overcome nephrotoxic effects of cisplatin (Yaman and Balikci, 2010), gentamicin (Mohan *et al*., 2006), cyclosporin (Khan *et al*., 2006) and mercury (Sharma *et al*., 2007). Considering the previous beneficial effects, the present study was conducted to determine the protective effects of *Spirulina fusiformis* against bromobenzene induced nephrotoxicity in rats. For the purpose of comparison, a standard reference drug silymarin (25 mg kg^{-1}) was used. It was found that *Spirulina fusiformis* (400 mg kg^{-1}) was able to provide a significant ($p<0.05$) protection to the kidney against bromobenzene induced nephrotoxicity.

MATERIALS AND METHODS

Animals: Wistar albino female rats weighing 120-150 g were obtained from animal house, VIT University, Vellore. They were fed with commercial pelleted feed from Hindustan Lever Ltd., (Mumbai, India) and water was provided *ad libitum*. The experimental procedure was approved by the ethical committee of VIT University, Vellore, India.

Chemicals: Commercially available *Spirulina fusiformis* was obtained from Acumen pharmaceutical private Ltd., Pondicherry. *Spirulina fusiformis* was found to be most effective when administered at 400 mg kg^{-1}. This dosage was chosen to conduct all further studies. Bromobenzene was obtained from Sigma Aldrich, India and silymarin, a standard drug, was obtained from the Micro Labs Ltd., (Goa, India). All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

Experimental procedure: Animals were allocated randomly in five groups of six animals each. In this study, all groups of rats except group I and group V received bromobenzene dosage (intragastric intubation) only once. Suspension of silymarin and *Spirulina fusiformis* were made in double distilled water for administration to rats. Group I was normal control and received 0.1 mL coconut oil (orally); group II received bromobenzene (10 mmol in 0.1 mL coconut oil, intragastric intubation); group III was administered with *Spirulina fusiformis* (400 mg kg^{-1}, orally) for 8 days and then bromobenzene (10 mmol in 0.1 mL coconut oil, intragastric intubation) on the last day; group IV received silymarin (25 mg kg^{-1}, orally) for 8 days and bromobenzene (10 mmol in 0.1 mL coconut oil, intragastric intubation) on the 8th day; group V was given only *Spirulina fusiformis* (400 mg kg^{-1}, oral) for 8 days. After 19 h of the last dosage, all the animals were sacrificed under ether anesthesia. After the collection of blood, kidney tissues (approximately 0.05-0.1 g) were homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate. This homogenate was centrifuged at 3000 g and 4°C for 10 min; the supernatant was stored at -20°C until analysis.

Serum analysis: Levels of kidney functional markers (urea, uric acid and creatinine) and total cholesterol were determined in the serum of control and experimental rats using commercially available kits (Autospan diagnostics, India) according to the manufacturer's protocol.

Antioxidant status in kidney: Lipid peroxidation was determined by the procedure of Ohkawa *et al.* (1979) and malondialdehyde (MDA) formed as the end product of the peroxidation of lipids, which served as an indicator for evaluation of oxidative stress, was measured. The procedure of Marklund and Marklund (1974) was used to determine the levels of superoxide dismutase (SOD). The unit of enzyme activity is defined as the enzyme required giving 50% inhibition of pyrogallol auto-oxidation. Catalase (CAT) was measured by the method of Sinha (1972) and chromic acetate, which is produced in the end, was measured colorimetrically at 610 nm. Glutathione Peroxidase (GPx) was assayed according to the method of Rotruck *et al.* (1973) based on the reaction between glutathione remaining after the action of Gpx and 5,5'-dithiobis-(2-nitrobenzoic acid) to form a complex and the absorbance was measured at 412 nm. Glutathione-s-transferase or GST (Habig *et al.*, 1974), reduced glutathione (Moron *et al.*, 1979) and total protein (Lowry *et al.*, 1951) were also evaluated.

Histopathological studies: After the sacrifice, a portion of kidney was fixed in formalin (10%) and was washed and then dehydrated in descending grades of isopropanol and finally rinsed with xylene. The tissues were then embedded in molten paraffin wax and were cut in sections at 5 mm thickness. These were stained with haematoxylin and eosin and were observed microscopically for histopathological changes.

Statistical analysis: Results were expressed as Mean±SD and statistical analysis was performed using ANOVA, to determine the significant differences between the groups, followed by Student Newman-Keul's test; $p<0.05$ implied significance.

RESULTS

Effect of *Spirulina fusiformis* on serum profile: Urea, uric acid and creatinine levels are the most important clinical parameters for evaluating abnormality in renal function. As shown in Table 1, there was a significant ($p<0.05$) increase in the levels of urea, uric acid and creatinine in group II rats which were treated with only bromobenzene, indicating that the kidney function was affected. Pre-treatment of rats with *Spirulina fusiformis* was able to restore the levels of urea, uric acid and creatinine and brought them near to normal levels, thus proving its protective effects on renal function. Also, there was elevation in the levels of total cholesterol in the bromobenzene

Table 1: Effect of administration of bromobenzene (BB) on urea, uric acid, creatinine and total cholesterol with or without prior administration of *Spirulina fusiformis* (Spi) in serum of control and experimental rats

Parameters (mg dL^{-1})	Group I (Control)	Group II (BB 10 mmol kg^{-1})	Group III (Spi-400 mg kg^{-1} BB)	Group IV (Silymarin 25 mg kg^{-1}+BB)	Group V (Spi-400 mg kg^{-1})
Urea	10.19±0.53	22.56±0.62a*	13.24±0.56b*	14.72±0.43b*	13.98±0.560b*
Uric acid	1.12±0.01	2.98±0.09a*	1.53±0.08a*b*	1.34±0.10 b*	1.22±0.050b*
Creatinine	0.73±0.01	2.15±0.04a*	1.03±0.06a*b*	1.22±0.05a*b*	0.98±0.040b*
Cholesterol	72.07±2.36	109.64±3.07a*	82.81±1.06a*b*	79.40±.720b*	83.69±10.22a*b*

Each value represents the Mean±SD of six rats. Comparisons were made as follows: a-group I vs groups II, III, IV, V, b-group II vs group III, IV, V. The symbols represent statistical significance at *$p<0.05$. Statistical analysis was calculated by one-way ANOVA followed by the student Newman-Keul's test

Table 2: Effect of administration of bromobenzene (BB) on antioxidant status and lipid peroxidation with or without prior administration of *Spirulina fusiformis* (Spi) in kidney homogenate of control and experimental rats

Parameters	Group I (Control)	Group II (BB-10 mmol kg^{-1})	Group III (Spi-400 mg kg^{-1}+BB)	Group IV (Silymarin-25 mg kg^{-1}+BB)	Group V (Spi-400 mg kg^{-1})
Lipid peroxidation (nmol mg^{-1} protein)	0.32±0.06	0.93±0.100a*	0.47±0.21a*b*	0.64±0.07a*b*	0.30±0.03b*
Catalase (Units min^{-1} mg^{-1} protein)	61.42±0.86	28.76±1.320a*	52.86±1.04b*	51.42±4.32b*	59.87±4.21b*
SOD (U mg^{-1} protein)	14.22±6.32	8.57±2.260a*	13.43±5.13b*	13.74±4.23b*	14.38±4.62b*
GST (nmol min^{-1} mg^{-1} protein)	6.28±0.06	2.03±0.230a*	4.54±0.41b*	4.58±0.29b*	4.41±0.26b*
Reduced glutathione (mmol mg^{-1} protein)	8.57±0.33	4.21. ±0.76a*	8.43±0.45b*	8.15±0.59b*	8.16±0.26b*
Glutathione peroxidase (µg of GSH utilized min^{-1} mg^{-1} protein)	5.69±0.43	2.89±0.590a*	4.98±0.41b*	4.18±0.45b*	4.85±0.62b*

Each value represents the Mean±SD of six rats. Comparisons were made as follows: a-group I vs groups II, III, IV, V; b- group II vs group III, IV, V. The symbols represent statistical significance at *$p<0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman-Keul's test

treated group of rats. On the other hand, prior administration of *Spirulina fusiformis* attenuated the increase in cholesterol in a significant manner (Table 1). Silymarin, which is the standard drug for reference, showed similar results.

Effect of *Spirulina fusiformis* on antioxidant activity in kidney: Bromobenzene significantly induced oxidative stress through the depletion of glutathione in the renal tissue of rats. This was evidenced by a significant ($p<0.05$) increase in the levels of lipid peroxidation and decrease in the levels of antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione and glutathione-s-transferase, as compared to the normal control group of rats (Table 2). Prior administration of *Spirulina fusiformis* was able to reduce the levels of MDA significantly as compared to only bromobenzene treated group of rats. *Spirulina fusiformis* pre-treatment of rats for 8 days before bromobenzene administration was able to increase the levels of superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione and glutathione-s-transferase significantly ($p<0.05$) as compared to only bromobenzene treated group as shown in Table 2.

Histopathological results: The renoprotective effect of *Spirulina fusiformis* was established by histopathological examination of kidney tissues of the control and experimental group of rats. The histology of the kidney cells of control group (Fig. 1a) was found to be normal. In group II (Fig. 1b) rats, which were treated with bromobenzene, the sections showed congestion of the blood vessels of the renal parenchyma and dilation of the renal tubules. The histological architecture of rats in group III (Fig. 1c), which were treated with *Spirulina fusiformis* and bromobenzene, showed congestion of the renal parenchyma. Congestion of the glomeruli and interstitial blood vessels along with the dilation of the renal tubules was observed in the sections of group IV animals (Fig 1d), which were treated with silymarin along with bromobenzene. Minimal congestion of blood vessels of the renal parenchyma and dilation of a few renal tubules were seen in the cross section of kidney of group V animals (Fig. 1e), which were treated with only *Spirulina fusiformis* (400 mg kg^{-1}).

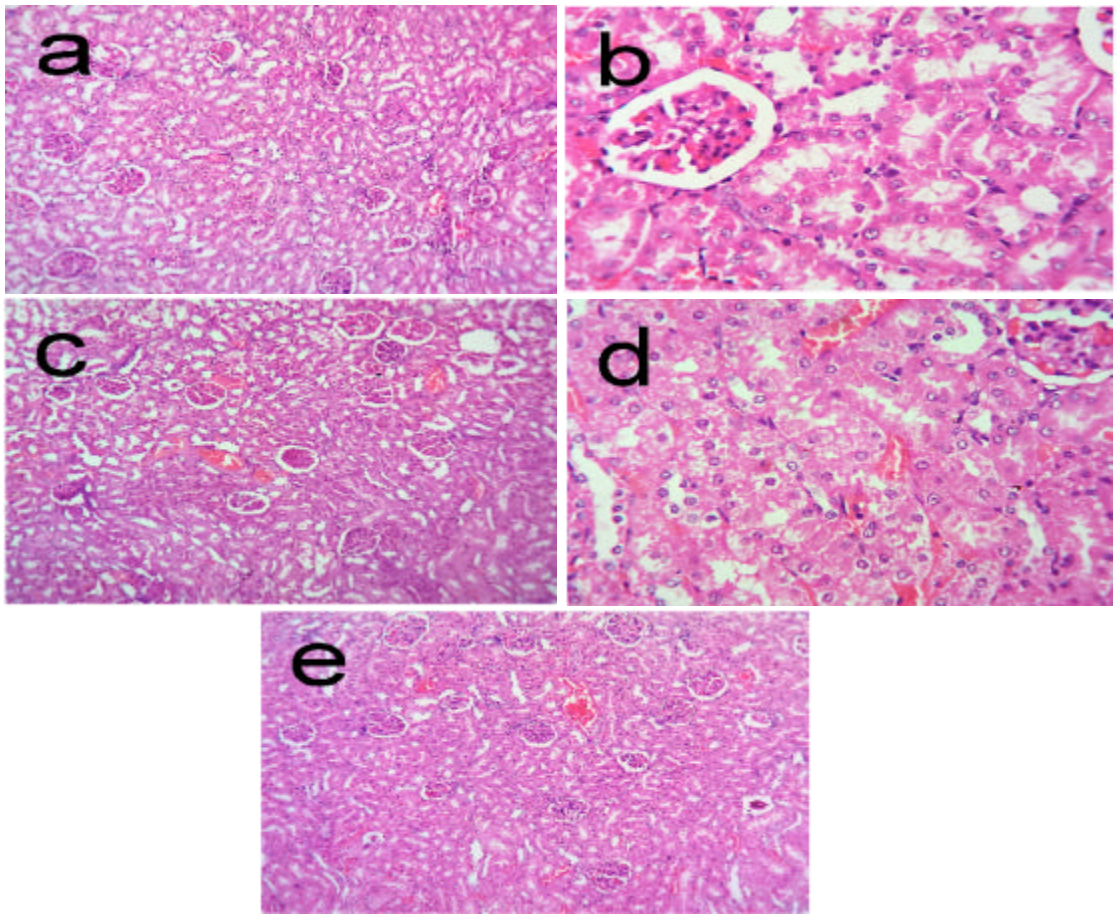

Fig. 1(a-e): Kidney sections (stained with H and E) from rats (a) Normal control group, (b) Bromobenzene only (10 mmol kg^{-1}) treated group, (c) *Spirulina fusiformis* (400 mg kg^{-1}) +Bromobenzene (10 mmol kg^{-1}) treated group, (d) Silymarin (25 mg kg^{-1}) +Bromobenzene (10 mmol kg^{-1}) treated group and (e) Only *Spirulina fusiformis* (400 mg kg^{-1}) treated group

DISCUSSION

Kidney is a target organ for toxicity resultant from bromobenzene. In kidney, metabolites of bromobenzene such as 2, 3-epoxybromobenzene and 2-bromohydroquinone get oxidized to 2-bromoquinone which gives various mono and di-substituted derivatives when combined with glutathione (Vedi *et al*., 2013; El-Sharaky *et al*., 2009). This leads to depletion in glutathione levels and subsequently causes nephrotoxicity. In the present study protective effect of *Spirulina fusiformis* against bromobenzene induced nephrotoxicity was evaluated by assessing the alteration in the levels of kidney functional markers (urea, uric acid and creatinine), lipid peroxidation and antioxidant status in the control and experimental rats.

Due to necrosis there is a decrease in the glomerular filtration rate leading to reduced renal clearance of urea, creatinine and uric acid and this leads to increased concentration of these substances in blood (Hamed *et al*., 2013). In the present study, a significant ($p<0.05$) increase was

seen in the serum levels of urea, uric acid and creatinine in the bromobenzene induced group as compared to the control group of rats (Table 1). *Spirulina fusiformis* (400 mg kg^{-1}) pre-treatment was able to restore their levels, showing its protective effect in restoring the glomerular filtration and similar effects were seen in rats which were pretreated with silymarin (Table 1). Also there was an increase in the levels of serum cholesterol showing its reduced metabolism in the bromobenzene treated rats (Table 1) which was then restored to normal levels in rats which were pre-treated with *Spirulina fusiformis*.

Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function. Lipid peroxidation and depletion in antioxidant levels are one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics and also it alters physiological and biochemical characteristics of biological systems (Anane and Creppy, 2001; Abdel Moneim *et al*., 2011). Superoxide dismutase diminishes the superoxide toxicity by converting it to hydrogen peroxide while catalase and glutathione peroxidase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Glutathione acts as a non-enzymatic antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes that reduces hydrogen peroxides and hydroperoxides by its redox and detoxification reaction (Vedi *et al*., 2013). Glutathione-s-transferase enzyme catalyzes the redox reaction via the thiol (BSH) group of glutathione, thereby neutralizing and rendering the products more water-soluble. Since there is a mutual relation between levels of gltuathione-s-transferase and reduced glutathione in the redox system as reduced glutathione is a substrate for gltuathione-s-transferase, the simultaneous decrease in both their concentration may suggest that the decrease in renal glutathione-s-transferase concentration might result, at least partly, from the decrease in reduced glutathione levels (Cervello *et al*., 1992). These observations support the hypothesis that the mechanism of nephrotoxicity is related to glutathione depletion and free radical generation. Levels of antioxidant enzymes such as SOD, catalase, glutathione peroxidase, glutathione-s-transferase and reduced glutathione showed a marked decrease in the bromobenzene treated group of animals (Table 2). However, in the groups pre-treated with *Spirulina fusiformis* (400 mg kg^{-1}), there was a predominant increase in the antioxidant status. Lipid peroxidation levels were significantly increased ($p<0.05$) in the kidney of bromobenzene treated group whereas prior administration of *Spirulina fusiformis* was able to bring the levels to near normal as seen in Table 2. This shows the free radical scavenging and antioxidant enhancing activity of *Spirulina fusiformis* which is probably due to the presence of its various active components, such as phycocyanin (Vedi *et al*., 2013). Silymarin (25 mg kg^{-1}), which is the standard reference drug, showed similar results.

Histological investigations revealed that bromobenzene administration caused progressive glomerular and tubular alterations along with congestion in parenchymal cells. In rats which received *Spirulina fusiformis* for 8 days prior to bromobenzene administration, the alterations in histological architecture were found to be reduced and it appeared to be similar to the normal control group of rats. Silymarin was found to be less effective in minimizing the damage as the congestion of renal parenchyma and glomeruli and dilation of tubules was visible.

CONCLUSION

In summary, *Spirulina fusiformis* has received considerable attention for its use as a food supplement. However, its role in attenuation of nephrotoxicity is still under keen research. It may be hypothesized that the active components present in *Spirulina fusiformis* may scavenge the free

radicals produced due to the abnormal metabolism and may enhance the production of nonenzymatic and enzymatic antioxidants, which may possibly lead to decreased damage due to the reactive oxygen species thereby reducing the oxidative stress and lipid peroxidation. Hence, our work opens a novel window to manage bromobenzene-induced nephrotoxicity. Further elucidation of the mechanisms may provide insights into nephrotoxicity resulting due to various xenobiotic compounds and may help in preventing it by the use of *Spirulina fusiformis*.

ACKNOWLEDGMENTS

The authors are thankful for to VIT University, Vellore (India) for the facilities and infrastructural support.

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