

***Spirulina fusiformis*: A Food Supplement against Mercury Induced Hepatic Toxicity**

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(Received January 28, 2005; Accepted April 18, 2005)

The protective effect of *Spirulina fusiformis* extract against mercury toxicity studied in Swiss albino mice. Animals treated with HgCl₂ (5.0 mg/kg b.wt. *i.p.*) showed a significant elevation in lipid peroxidation level (LPO), aspartate amino transferase (AST) and alanine amino transferase (ALT) activity. However, a marked decline in serum alkaline phosphatase activity and reduced glutathione (GSH) content was recorded. Whereas, animals treated with *Spirulina fusiformis* extract (800 mg/kg b.wt. orally) before and after mercury intoxication showed a significant decrease in LPO level, AST and ALT activity and increase in serum alkaline phosphatase activity and GSH content. *Spirulina fusiformis* alone treatment did not alter reduced glutathione, AST, ALT and alkaline phosphatase activity but significantly diminishes the LPO level. Thus, the results obtained from the present study suggest that oral administration of *Spirulina fusiformis* extract provides protection against mercuric chloride induced toxicity in Swiss albino mice.

Key words — liver, mercuric chloride, *Spirulina fusiformis*, lipid peroxidation, glutathione

INTRODUCTION

Mercury and its compounds widely used in variety of products and processes, including pressure sensitive devices (thermometers, barometers) electrical apparatus (wiring, switches, batteries), paints, pharmaceuticals and in the production of various chemicals, known to be one of the most highly toxic metal to man (ATSDR, 1989¹; Girardi and Elias, 1993²). It is a transition metal, promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxide. These ROS enhances the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell.

Several naturally occurring dietary or non-dietary constituents, and parts of several species of edible plants having pharmacological activity, influence the antioxidant enzymes and provide protec-

tion against free radical induced damage.

In recent years, *Spirulina* is gaining more attention from medical scientists as a nutraceutical and source of potential pharmaceuticals. It is blue green algae (mycobacterium) belonging to the family Oscillatoriaceae. *Spirulina fusiformis* possess potent antiviral activity (Hayashi *et al.*, 1996)³ anticancer effects (Mittal *et al.*, 1999),⁴ strengthens immune system (Qureshi *et al.*, 1995; 1996),^{5,6} radioprotective (Verma, 2000)⁷ and metalloprotective effects (Shastri, 1999⁸; Saxena and Kumar, 2004⁹).

Keeping in view the pharmacological properties of *Spirulina fusiformis*, present investigation was undertaken to assess the protective effect of *Spirulina fusiformis* extract against mercuric chloride induced hepatic toxicity in Swiss albino mice.

MATERIALS AND METHODS

Animals — Adult male Swiss albino mice (6–8 weeks old, weighing 23 ± 2 g) from an inbred colony (Procured from IVRI — Izzat Nagar) maintained at the animal house of the department were used for the present study. The animals were maintained on the standard mice feed and water *ad libitum*. Tetracycline water, once in fortnight was given as a preventive measure against infection.

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Spirulina fusiformis — *Spirulina fusiformis* in the form of powder was obtained from RECON Ltd., Bangalore, India. It was suspended in vehicle (olive oil) and 0.05 ml of *Spirulina* suspension was given to each mouse by oral gavage daily.

Mercuric Chloride — Mercury in the form of HgCl_2 was obtained from Merck India Ltd. (Mumbai, India). It was dissolved in 0.9% NaCl and administered *i.p.*

Experimental Design — The animals (Swiss albino mice) were divided into the following 4 groups.

Group I ($n = 30$): No treatment was given to these animals.

Group II ($n = 30$): The animals were given orally *Spirulina fusiformis* extract (800 mg/kg b.wt. in olive oil) for 30 consecutive days.

Group III ($n = 30$): These animals were administered HgCl_2 5.0 mg/kg b.wt. in 0.9% NaCl intraperitoneally.

Group IV ($n = 30$): In this group of animals, *Spirulina* extract 800 mg/kg b.wt. was given orally for 10 consecutive days, before mercuric chloride (5.0 mg/kg b.wt.) administration and until 30 days of mercuric chloride administration.

The animals from the above groups were autopsied at 1, 3, 7, 15 and 30th days after mercuric chloride administration. The liver was excised out and processed for histological and biochemical alterations. Blood from these animals was collected by cardiac puncture and serum was separated and processed for liver function tests.

Histopathological Studies — Liver from autopsied animals were excised out and fixed in Bouins fixatives for 24–48 hr. Five-micron thick histological sections were prepared and stained with hematoxyline and eosin. These stained slides were observed under light microscope for histological alterations.

Liver Function Tests — To assess the liver functions following biochemical parameters were done in the serum.

Serum Alkaline Phosphatase: Serum alkaline phosphatase activity was measured by the method of Kind and King (1954),¹⁰ using commercially accessible kits (Span Diagnostics Ltd., Surat, India).

Principle: Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol, at pH 10.0; phenol so formed reacts in alkaline medium with 4-amino antipyrine in the presence of an oxidizing agent, potassium ferricyanide, and forms an orange — red colored complex, which can be measured colorimetrically or spectrophotometrically at 510 nm. The color intensity is proportional to the enzyme activity, which is expressed as Kings and Armstrong Unit (KAU).

Serum Aspartate Amino Transferase (AST): The aspartate amino transferase activity was estimated by the method of Reitman and Frankel, (1959).¹¹

Principle: Aspartate amino transferase (AST) catalyses the conversion of α -ketoglutarate and aspartate into glutamate and oxaloacetate respectively. Oxaloacetate so formed is coupled with 2,4 dinitrophenyl hydrazine (DNPH) to give the corresponding hydrazone, which gives brown color in alkaline medium and this is measured colorimetrically.

Standard Curve: Standard curve was obtained using different amounts of pyruvate and enzyme activity was expressed as units/ml.

Serum Alanine Amino Transferase (ALT): The alanine amino transferase activity was measured by the method of Reitman and Frankel (1959)¹¹ using DNPH as a colour reagent.

Principle: Alanine amino transferase (ALT) catalyses the conversion of α -ketoglutarate and alanine into glutamate and pyruvate respectively. Pyruvate so formed is coupled with DNPH to give the corresponding hydrazone, which gives brown color in alkaline medium and this can be measured colorimetrically.

Standard Curve: Standard curve was obtained using different amounts of pyruvate and enzyme activity was expressed as units/ml.

Biochemical Studies in Liver —

Lipid Peroxidation (LPO) Assay: Lipid peroxidation (LPO) level in liver was estimated by the method of Ohkawa *et al.* (1979)¹² as thiobarbituric acid reactive substances (TBARS). The concentration of TBARS was exposed as n moles of malondialdehyde per mg of tissue using 1, 1, 3, 3-tetramethoxy propane (TMP) as the standard.

Principle: Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining the extent of the peroxidation reaction. Peroxidation of lipids generates MDA, which reacts with thiobarbituric acid to give a red species absorbing 532 nm.

Reduced Glutathione: The liver reduced glutathione (GSH) level as determined by the method of Moron *et al.* (1979).¹³

Principle: The Reduced Glutathione reacts with 5',5'-dithiobis(2-nitrobenzoic acid) (DTNB) and forms a yellow coloured complex with DTNB that

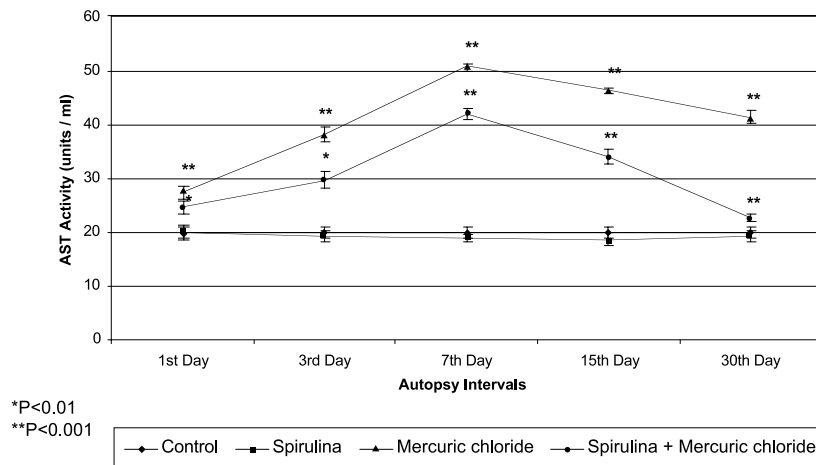


Fig. 1. Variation in AST Activity in Different Experimental Groups

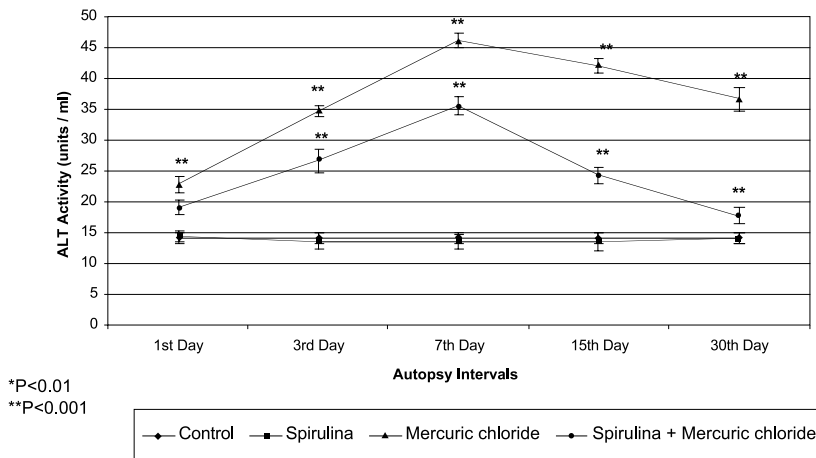


Fig. 2. Variation in ALT Activity in Different Experimental Groups

absorbs at 412 nm.

The tissue homogenates (10% w/v) were immediately precipitated with 0.1 ml of 25% tri chloro acetic acid (TCA), and the precipitate was removed after centrifugation. Free endogenous GSH was assayed in a total 3 ml volume by the addition of 2 ml of 0.5 mM DTNB prepared in 0.2 M phosphate buffer (pH = 8.0) to 0.1 ml of the supernatant, and the absorbance was read at 412 nm using a UV-visible (UV-VIS) Systronic Spectrophotometer. GSH was used as a standard to calculate μ mole GSH/g tissue.

Statistical Analysis — The values were expressed as mean \pm SEM. The data were subjected to student's *t*-test for comparison between the groups.

RESULTS

Histological Studies

Hg intoxication produced various pathological lesions in the liver such as cytoplasmic vacuolisation, karyorhexis, karyolysis, pycnosis and centrilobular necrosis. On day 1st hepatocytes showed fragmented or lysed nuclear material. Congestion of sinusoidal spaces was also prominent. From day 3rd to 15th pathological signs were more prominent, hepatocytes showed massive centrilobular as well as peripheral necrosis. Cytoplasmic vacuolisation, enucleation, karyorhexis, karyolysis and pyknosis were also evident. At the day 30th the liver showed slight recovery as compared to day 15th. Pre and post treatment of *Spirulina* with mercury showed prominent recovery. There was reduced cytoplasmic vacuolisation and centrilobular necrosis observed as compared to

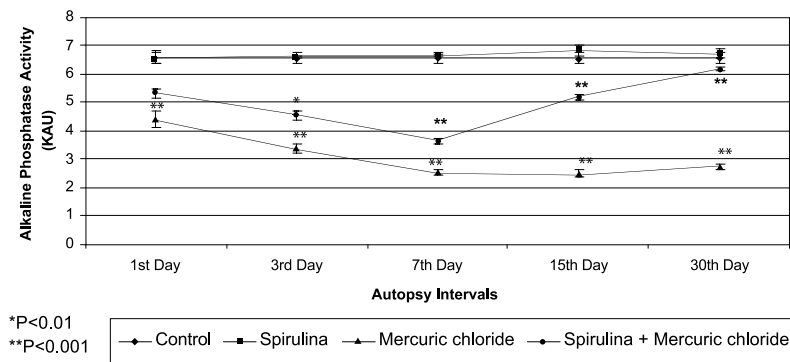


Fig. 3. Variation in Serum Alkaline Phosphatase Activity in Different Experimental Groups

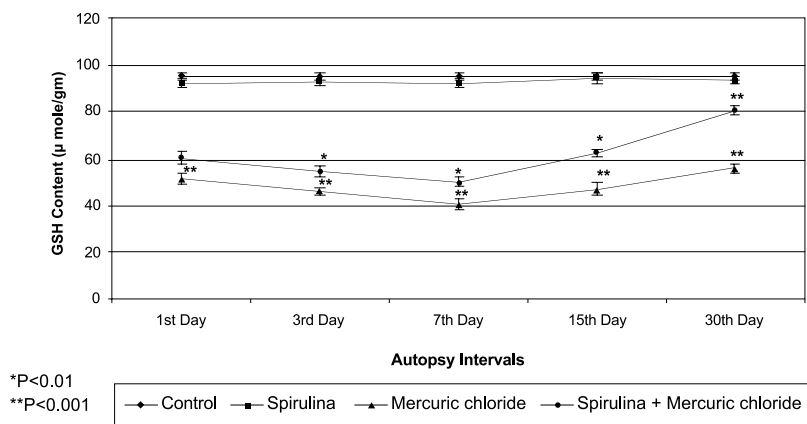


Fig. 4. Variation in Hepatic GSH Content in Different Experimental Groups

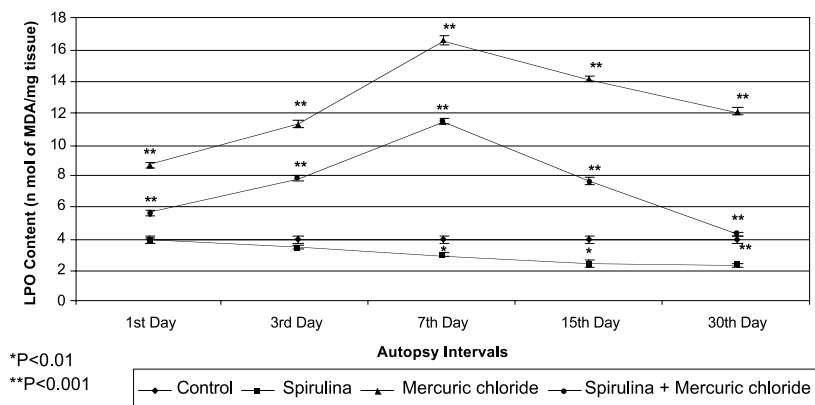


Fig. 5. Variation in Hepatic LPO Content in Different Experimental Groups

Hg intoxicated mice. Upto day 30th hepatocytes were become normal with prominent nuclei and well-marked granulated hepatocytolasm.

Biochemical Studies

Spirulina Treated Animals: The animals treated with *Spirulina* extract (Group II) alone did not ex-

hibited any significant alteration in hepatic glutathione, alkaline phosphatase, ALT and AST activity during the entire period of study and values remained near normal. A significant decrease in LPO level was observed (Figs. 1–5) throughout the experimental period as compared to normal animals.

HgCl₂ Treated Animals: Animals treated with

HgCl₂ (Group III) showed a significant elevation in AST, ALT and LPO level ($p < 0.001$). A maximum increase was observed at day 7th and afterwards a decline was observed (day 15th and 30th). However, a marked decrease in serum alkaline phosphatase activity and GSH content was recorded at all autopsy intervals and a maximum decline was noticed at day 15th ($p < 0.001$) and day 7th respectively (Figs. 1–5).

Combination Group: In the combination group (Group IV) where animals were treated with the *Spirulina* and HgCl₂, significant decline in activity of AST, ALT, and LPO level was noticed throughout all autopsy intervals as compared to HgCl₂ treated animals (Group III) and the values returned to normal on day 30th. Whereas, a significant elevation in serum alkaline phosphatase activity and GSH content was observed throughout the experimentation period as compared to HgCl₂ treated animals. (Group III) (Figs. 1–5).

DISCUSSION

Mercury intoxication showed a significant increase in transaminases (AST and ALT) activities. The increase in AST and ALT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream (Vandenbergh, 1995¹⁴; Rana *et al.*, 1996¹⁵; Sharma *et al.*, 2002¹⁶). This confirms our earlier report on histopathological changes in liver induced by HgCl₂ intoxication (Sharma *et al.*, 2000; 2001; 2002).^{16–18}

Further, there was a significant decrease in the serum alkaline phosphatase activity after HgCl₂ intoxication. In the liver, it is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra hepatic or intra hepatic leads to decrease in serum alkaline phosphatase activity (Vandenbergh, 1995; Sharma *et al.*, 2002).¹⁶ Mercury causes cell membrane damage (lipid peroxidation), which leads to the imbalance between synthesis and degradation of enzyme protein, thus lowering the enzyme activity (Hardonk and Koudstaal, 1976).¹⁹ Present findings are in agreement with the findings of El-Demerdash, (2001)²⁰ and Sharma *et al.* (2002).¹⁶ They observed that HgCl₂ intoxication (0.5 $\mu\text{mol/ml}$ and 5.0 mg/kg body weight) significantly decreases the alkaline phosphatase activity.

GSH is the major thiol, which binds electrophilic

molecular species and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances. (Ketterer *et al.*, 1983²¹; Meister and Andersen, 1983²²). Mercury is a transition metal and has high affinity with GSH and causes the irreversible excretion of up to two GSH tripeptides (Zalups and Lash, 1996).²³ The metal-GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. As a result of the binding of mercury to glutathione and the subsequent elimination of intracellular glutathione, levels of reduced glutathione are lowered in the cell and thus decrease the antioxidant potential of the cell. In the present investigation it was observed that Hg intoxication significantly depletes the GSH content in the liver and thus reducing the antioxidant potential and accelerating the lipid peroxidation, resulting in hepatocytes damage.

It was observed that an extract of *Spirulina* when given in combination with mercuric chloride, significantly elevated liver GSH content and decline lipid peroxidation and reduces the mercury toxicity which in turn is reflected by significantly decrease in activity of serum transaminases (AST and ALT) and increase in serum alkaline phosphatase activity and reduces liver damages.

The protective efficacy of *Spirulina fusiformis* may be due to presence of several active components. The active component found in *Spirulina* may provokes the activity of free radical scavenging enzyme systems and renders protection against mercury induced liver damages. The metallo-protective role of *Spirulina* may be attributed to the presence of β -carotene (Prescott 1978²⁴); Seshadri *et al.*, 1991²⁵), vitamin C, E (Mathew *et al.*, 1995)²⁶ enzyme superoxide dismutase (Ben Amotz, 1987²⁷); Henrikson, 1989²⁸) and selenium (Henrikson, 1989²⁸).

β -Carotene acts as powerful quencher of singlet oxygen and a scavenger of free radicals (Foote *et al.*, 1970²⁹); Krinsky and Deneke, 1982³⁰). Luxia *et al.* (1996)³¹ reported that β -carotene of *Spirulina* may reduce cell damage, especially the damage to DNA molecules, thus playing the role in the repair of regeneration process of damaged hepatocytes cells.

Vitamin E of the *Spirulina fusiformis* prevents mercury induced lipid peroxidation and maintains intracellular thiols and ascorbic acid levels in damaged tissue by inhibiting free radical formation and oxidative damage (Duval and Poelman, 1994³²); Kulkarni and Bycz Kowski, 1997³³); Patil and Rao,

1999³⁴). According to Rao and Sharma (2001),³⁵ vitamin E showed protective effect against HgCl₂ through impaired absorption of mercury in the gastrointestinal tract. Rana *et al.* (1996)¹⁵ also postulated that vitamin E has a protective effect against mercury toxicity.

Selenium component in *Spirulina* induces selenium containing enzyme GSH peroxidase, proteins or compounds such as selenodiglutathione, selenocysteine and dimethylselenide, which are known to modulate the toxic effects of heavy metals (Henrikson, 1989²⁸; Lindh *et al.*, 1996³⁶). Superoxide dismutase is a mitochondrial enzyme, which is found to quench free radicals and prevents tissue damage (Henrikson, 1989²⁸; Girardi and Elias, 1995³⁷).

Spirulina also induces the activity of immune system. It builds up both the cellular and humoral arms of the immune systems and thus improving their ability to function inspite of stresses from environmental toxins and infectious agents (Hayashi *et al.*, 1994³⁸; Qureshi *et al.*, 1995⁶).

Acknowledgements Financial assistance provided by CSIR, New Delhi to M. K. Sharma is highly acknowledged. Authors are also thankful to Recon Limited, Bangalore for providing *Spirulina fusiformis*.

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