Modulatory potential of *Spirulina fusiformis* on testicular phosphatases in Swiss albino mice against mercury intoxication

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Administration of mercuric chloride (HgCl$_2$; 5.0 mg/kg body weight) to male Swiss albino mice resulted in significantly higher levels of testicular acid phosphatase (ACP) and alkaline phosphatase (ALP) activities as compared to control. In combination group where *S. fusiformis* (800 mg/kg body weight) was given before and after HgCl$_2$ treatment, the mercury induced toxicity reduced in terms of decreased levels of ACP and ALP activities in the testis. The animal treated with only *Spirulina* did not show any alteration in ACP and ALP values. It is suggested that oral administration of *Spirulina* can modulate mercury induced testicular toxicity.

**Keywords:** Mercury intoxication, Modulatory potential, *Spirulina*, Testicular phosphatases

The World Health Organization reported the industrial use of mercury and its general toxic effects on human and animal systems. Exposure to elemental mercury vapour results in an accumulation of mercury in the pituitary, thyroid and testis. Administration of mercuric chloride (HgCl$_2$) to immature and adult male rats gradually reduced testicular weight. Lee and Dixon stated that HgCl$_2$ primarily affected spermatogonial and premeiotic cells and statistical analysis indicated significant antifertility effects. Sager et al. postulated that spermatogonia and premeiotic spermatocytes are sensitive to both inorganic and organic mercury. HgCl$_2$ treatment caused degeneration of Leydig cells and diminished the activity of steroidogenic enzymes and also resulted in the intracellular accumulation of mercury in the interstitial and Sertoli cells of the seminiferous tubules in the rats. Mercury in any chemical form denatures proteins, inactivates enzymes and causes severe disruption of any tissue with which it comes into contact in sufficient concentration. Mercuric mercury (Hg$^{2+}$), a form that shows primarily epithelial toxicity, can inhibit Na$^+/K^+$-ATPase at low concentration. Mercury can bind to phosphate, sulphydryl and imidazole groups in proteins and disrupt the activity of a number of enzymes.

It may also affect the transport of ions across cell and mitochondrial membranes. Histoenzymological analysis revealed toxic effects of HgCl$_2$ on hydrolytic enzymes of testicular tissue. Keck et al. reported that occupational exposure to mercury could lead to a significant impairment of male reproductive potency. Therefore, there is a need to provide protection against mercury induced toxicity. In recent years plant products have been used as modulators against heavy metal toxicity.

*Spirulina fusiformis*, a blue green algae (cyanobacterium; Oscillatoriaaceae) is the most cultivated algae for its high proteins, vitamins and mineral contents. The *Spirulina* is a rich source of provitamin A or β-carotene and super oxide dismutase (SOD) enzyme. Presence of these two antioxidants makes a very effective system for prevention of various harmful effects of heavy metals and chemicals.

Therefore, this study has been undertaken to investigate the effect of HgCl$_2$ on testicular phosphatases and to evaluate the modulatory potential of *S. fusiformis* on mercury induced testicular toxicity.

**Materials and Methods**

Young healthy and sexually mature (6-8 week old) male Swiss albino mice (120) procured from JNU, New Delhi, were used. They were fed with balanced food pellets and water ad libitum. The animals were kept in highly hygienic conditions, maintained at...
22±2°C with 12:12 h L : D photoperiods. Inorganic mercury in the form of HgCl₂ was obtained from Merck (India) Ltd., Mumbai. It was dissolved in 0.9% saline and administered intraperitoneally. *Spirulina fusiformis* was obtained in powder form from Recon Ltd., Bangalore, India. A homogenous suspension of *Spirulina* was made in olive oil (vehicle) and given orally to the animals. *Spirulina fusiformis* was given orally (in Olive oil) for 30 consecutive days at various doses (200, 400, 800 and 1200 mg/kg body weight) to see the toxicity of drug on the animal. This drug did not show any sign of toxicity and mortality up to 1200 mg/kg body weight dose. The 800 mg/kg body weight dose was found more suitable for this investigation as it was less in the amount and was easily taken up by the mice with satisfactory response. The percentage mortality following HgCl₂ administration was very high at the dose of 6 and 7.5 mg/kg body weight whereas 5 mg/kg body weight of HgCl₂ treatment did not show any significant mortality within 30 days, therefore this dose was chosen for modulation at enzymatic level/to carry out further studies.

The animals were divided into following 4 groups of 30 each: Group I : control, this group was further divided into 2 sub-groups. Group IA : control for HgCl₂ treated group: the animals in this sub-group were received only vehicle (0.9% saline, ip) on day zero. Group IB : control for *Spirulina* treated group: the animals of sub-group IB received only olive oil (50 µl, po) continuously for 30 days. Group II : *Spirulina* treatment. Animals of this group were given *Spirulina* suspension (800 mg/kg body weight of *Spirulina* in olive oil ) orally (50 µl to each mouse) continuously for 30 days. Group III : HgCl₂ treatment. The animals in group III received 5 mg/kg body weight of HgCl₂ in 0.9% saline (ip) on day zero. Group IV : Combined treatment of *Spirulina* and heavy metal. In this group *Spirulina* was administered orally for 10 days and on day 11ª animals were injected 5 mg/kg body weight of HgCl₂ (ip) after 30 min of *Spirulina* administration; this day was considered as day zero (0). After that *Spirulina* was given continuously up to 30 days. The whole experiment was conducted for 40 days. At each autopsy interval (1, 3, 7, 15, and 30th day) overnight fasted six animals from each group were autopsied in the morning hours. The testes were quickly dissected out, blotted and weighed immediately for the biochemical estimation of acid phosphatase (ACP) and alkaline phosphatase (ALP). The data were analyzed with the help of t test and expressed as mean±SE with the level of significance.

### Results and Discussion

The results are presented in Fig. 1.

The enzyme system is very important as it plays a major role in maintaining normal cellular physiology. There is a possible relationship between the dynamic changes in the germ cells leading to sperm formation and the consequent modification in the enzyme system. Acid and alkaline phosphatases are important biochemical markers for assessing the functional status of the reproductive organs.

**ACP** a lysosomal enzyme, serves as a biochemical marker for specific androgen-dependent steps in spermatogenesis and is very important for tissue reorganization and tissue repair. In this study, increased ACP activity in the testes of HgCl₂ treated mice could be due to an increase in the leakage of the enzyme (Fig 1a). Passia *et al.* have ascribed increased ACP in Sertoli cells with severe cell damage and in germ cells with heavy exfoliation. Gill *et al.* also postulated that the exposure of HgCl₂ (181 µg/l for 48 hr) to Rosy barb (*Puntius conchonius*) stimulated the ACP activity in the testes. Therefore, it may be stated that higher levels of ACP are related with germ cell loss, arrest of spermatogenesis and testicular damage.

**ALP** is known to be associated with the plasma membrane of the non-germinal cells. ALP is involved in the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters and in mobilizing carbohydrates and lipid metabolites to be utilized either within the cells of the accessory sex structures or by the spermatozoa in the seminal fluid. Nun *et al.* reported a significantly higher ALP levels in the semen of azoospermic men. Gill *et al.* also reported that intoxication of HgCl₂ (181 µg/l for 48 hr) to Rosy barb (*Puntius conchonius*) resulted in a increase in ALP activity in the testes. It is postulated that after administration of inorganic mercury to rabbits, the ALP activity increased in liver by 77%. This result favours present findings. It was postulated that oral administration of HgCl₂ (2 mg/kg/day) resulted in a highly significant increase in ALP activity in the testes of rat. Since, the HgCl₂ (5 mg/kg body weight) inhibited the spermatogenic process, the unutilized ALP concentration was contributing to an increase in the testes of the HgCl₂ treated mice (Fig 1b).
El-Damerdash\textsuperscript{31} reported that selenium administered as sodium selenite in combination with mercury, partially or totally alleviated the toxic effects of mercury on ACP and ALP in rats. He concluded that selenium could be able to antagonize the toxic effects of mercury. Rao \textit{et al.}\textsuperscript{32} also reported the antidotal effects of selenite on reproductive tissues of mercuric chloride fed mice. \textit{Spirulina} contains selenium\textsuperscript{19,33}. Rao and Sharma\textsuperscript{34} reported that the levels of testicular ACP and ALP did not differ from control levels when vitamin E was coadministered with HgCl\textsubscript{2} in mice. Vitamin E, considered as a heavy metal protector\textsuperscript{35}, has a protective effect against mercury toxicity\textsuperscript{36} and is involved in cellular repair\textsuperscript{37}. Vitamin E is a well known antioxidant which protects the cell from free radical attack\textsuperscript{38}. \textit{Spirulina} contains vitamin E\textsuperscript{18,33}. Administration of \(\beta\)-carotene concurrent with cadmium ameliorate cadmium induced testicular toxicity\textsuperscript{39}. \textit{Spirulina} has the highest content of \(\beta\)-carotene, the provitamin A – an antioxidant\textsuperscript{19}. Vitamin A and \(\beta\)-carotene are unique toxic metal protective agents\textsuperscript{40}. Rana \textit{et al.}\textsuperscript{36} postulated defensive role of vitamin B-complex, vitamin C and vitamin E in liver and kidney functions in mercury and cadmium fed rats. \textit{Spirulina} is a whole source of vitamin B-complex\textsuperscript{41}. Therefore it can be suggested that \textit{Spirulina} can ameliorate mercury induced toxic changes in the ACP and ALP activities.

It is evident from the present investigation that HgCl\textsubscript{2} has a definite influence over ACP and ALP of
the testis. Eventually, this may have an impact on testicular functions and fertility of the animal. Further, the cotreatment of Spirulina with HgCl₂ may effectively reduce the mercury induced testicular changes.

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