



## Protective Effect of Smilax china on Mercuric Chloride Induced Oxidative Stress in Testis

### KEYWORDS

Smilax china, Mercuric Chloride, Oxidative stress, Testis

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### ABSTRACT

Smilax china L. is indigenous to China and Japan but it is imported to India and is common in Indian bazaars. It is commonly known as Jin Gang Teng in Chinese, Chobchini in Hindi, and Madhusnuhi in Sanskrit and china root in English. It possesses anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic, digestive properties. Free radical scavenging and antioxidant enzyme promoting activities were observed in the extracts of Smilax china L. root. Mercury (HgCl<sub>2</sub>) induces various toxic effects in different organs of the body. The present work is to evaluate the beneficial effect of Smilax china on mercuric chloride induced oxidative stress in rat testis. The antioxidant indices assayed were superoxide dismutase, catalase and lipid peroxidase. Mercury exposure shows decrease in the levels of antioxidants such as SOD and CAT, increased levels of TBARS. Prophylactic administration of Smilax china (400mg /Kg/ Bw) causes increase in the level of SOD and CAT for animals treated with low dose of Mercury (0.5mg/kg/Bw) and also decrease in the levels of TBARS (P<0.001) significantly, was noticed. In animals on High dose of Mercury chloride the effect of Smilax china is less when compared to the low dose drug.

### INTRODUCTION

Although male infertility is well documented as a result of exposure to numerous toxicants, the effects of inorganic mercury on male reproduction and fertility are less well-known. A 2008 study on the outcome of various heavy-metals in relation to semen quality reported data on human occupational exposure to mercury (Hg), and its reproductive outcomes are very sparse<sup>1</sup>. An earlier review of the consequences of mercury exposure in the workplace on fertility and related reproductive outcomes found only three studies pertaining to male fertility<sup>2</sup>, which were ambiguous at best. Two studies found effects by establishing the toxic influence on fertility of organic mercury compounds within concentrations that can be seen at the workplace<sup>3</sup>

and reduced concentration of testosterone in the serum of male workers, considered to be associated with exposure to inorganic mercury<sup>4</sup>. Male fertility can be impaired by various toxicants known to target Sertoli cells, which play an essential role

in spermatogenesis. Sertoli cells from male Sprague-Dawley rats exposed in vitro to mercury had severely inhibited inhibin production<sup>5</sup>.

HgCl<sub>2</sub> is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins<sup>6</sup>. It is highly toxic and oxidative once absorbed into blood stream: inorganic mercury combines with proteins in the plasma or enters the red cells. The inorganic ionic mercury has great affinity for SH groups of biomolecules, such as glutathione (GSH) and sulfhydryl proteins, which may contribute to its toxicity<sup>7</sup>. Oxidative stress occurs when the production of ROS such as, superoxide anion (O<sup>-2</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and the hydroxyl radical (-OH) exceeds the body's defense mechanism, causing damage to macromolecules such as DNA, proteins and lipids<sup>8</sup> and trigger many pathological processes in the male reproductive system<sup>9</sup>. There is evidence that ROS may have a detrimental effect on critical components of the steroidogenic pathway<sup>10</sup>. Moreover, various studies<sup>11</sup> have suggested that a strong

correlation exists between mercury induced toxicity and the induction of lipid peroxidation which is considered as the most extensively studied manifestation of oxygen activation in biology.

Smilax Chinensis L ( Liliaceae ) is a deciduous climber with rounded leaves and red berries. The root tubers of which furnish the drug known as china root. It is found in the south Indian states namely Andhra Pradesh, Tamilnadu and Karnataka<sup>12</sup>, several species of Smilax are well known Chinese traditional medicines used as anti-inflammatory, antioxidants, anti-cancer and analgesic agents. The tubers of Smilax chinensis have widely used in Chinese traditional medicines for treatment of diverse disease, especially for pelvic inflammation and chronic pelvic inflammation<sup>13</sup>. This study was proposed to investigate the ameliorative effect of Methanolic extract of Smilax china in albino rats induced by mercury toxicity.

### MATERIALS AND METHOD

Male wistar strain Albino rats, weighing (150-200gms) were used for the study after getting the Institutional Ethical Clearance EC:46/IAEC/2011. The rats were fed on the standard commercial laboratory chow and distilled water ad libitum and were housed in the plastic cages with good ventilation. Light dark conditions as well as temperature was maintained (12h: 12h and 26±2°C respectively) throughout the seasons. Animals were assigned to 5 groups of 8 rats each.

Group I served as control and animals were provided with distilled water.

Group II animals received (high dose) HgCl<sub>2</sub> (1mg/kg /Bw) orally.

Group III was administered with 0.5mg mg/kg /BW (low dose) of mercuric chloride.

Group IV received (high dose) HgCl<sub>2</sub> (1mg/kg/BW) with Smilax china 400mg /kg/Bw

Group V received (low dose)HgCl<sub>2</sub> (0.5mg/kg/Bw) along with Smilax china 400mg/kg/Bw

All the treatments were administered for 1month and on the 31st day the animals were weighed and necropsy was performed. The testis was dissected carefully and weighed. testicular tissues frozen at -80 °C were thawed and homogenized in 2 ml of lysis buffer (50mM Tris, 150mM NaCl adjusted to pH 7.4); the homogenates were centrifuged at 9000rpm for 15 min; the supernatants were saved; and the protein concentrations were measured, according to the method of Lowry et al.<sup>14</sup>, using bovine serum albumin as standard.

#### ANTIOXIDANT PARAMETERS

The antioxidant enzyme activities like superoxide dismutase (EC.1.15.1.1, SOD), Lipid peroxidation (TBARS), and catalase (EC.1.11.1.6, CAT) were analysed by the spectrophotometric method of Kakkar et al. (1984), Ohkawa et al., (1979) and sinha et al. (1972) respectively<sup>15-17</sup>.

#### STATISTICAL ANALYSIS

Data were statistically analyzed by Student's t-test and ANOVA.

#### RESULTS

GROUP	Superoxide dismutase	Catalase	Lipid peroxidase
Control	102.24±6.63	9.78±0.12	0.11±1.18
High Hg	88.49±0.98	11.12±0.01	0.13±0.68
Low Hg	59.43±0.86	9.19±0.01	0.18±0.65
High Hg + SC	54.97±0.67	7.66±0.01	0.15±0.15
Low Hg + SC	75.74±0.76	9.22±0.03	0.17±0.96

Values are expressed as Mean ± SEM. Data were Analyzed by one way ANOVA

**Abbreviation :** Hg - Mercuric chloride , SC - Smilax china

In the present study antioxidant enzymes such as SOD and Catalase were significantly (P<0.001) decreased in animals treated with both doses of HgCl<sub>2</sub> (Table.No.1). The Prophylactic administration of Smilax china causes increases in the level of antioxidant followed by a depletion in total -SH Groups in low dose affected group (P<0.001). Administration of Smilax china along with high dose HgCl<sub>2</sub> doesn't reveal significant difference. The Levels of Lipid peroxidase also increased in groups treated with Mercury and only on low dose group with smilax china had shown marked elevation in TBARS level (P<0.001).

#### DISCUSSION

SOD is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide (O<sub>2</sub><sup>-</sup>) to hydrogen peroxide. CAT is a peroxisomal haem protein that catalyses the removal of H<sub>2</sub>O<sub>2</sub> formed during the reaction catalysed by SOD. Thus SOD and CAT acts mutually supportive antioxidative enzymes, which provide protective defense against reactive oxygen species. These ROS are very unstable and highly reactive. They become stable by acquiring electron from nucleic acids, proteins, carbohydrates and lipids, there by a cascade of chain reaction are initiated resulting in cellular damage and causes lipid peroxidation<sup>18</sup>. Thus in the present study chronic administration of HgCl<sub>2</sub> causes decreases in the levels of SOD and Catalase. Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFAs) and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of a several membrane bound enzymes<sup>8</sup>. Thus, it is plausible to speculate that mercury treatment may result in peroxidation of PUFAs, leading to the degradation of phospholipids and ultimately result in cellular deterioration in the testis.

Various studies also suggested that a strong correlation exists between mercury-induced toxicity and the induction of LPO<sup>11</sup>. The present data revealed significant increase in lipid peroxidation level which may due to oxidative stress resulting in cellular damage. Co-administration of Smilax china with low dose of HgCl<sub>2</sub> exposed groups exerted amelioration effects. This antioxidant and ROS scavenging effects of curcumin is only due to its phenolic (-OH) group, which would inhibit the -SH group oxidation and block thiol depletion and thus it protects the oxidation of protein. Further it also enhances the activities of some antioxidant enzymes such as SOD and catalase is in agreement with the previous findings<sup>19</sup>.

#### CONCLUSION

Methanolic extract of Smilax china showed significant protection in antioxidant enzyme levels of SOD and catalase in low dose induced HgCl<sub>2</sub> albino rats, which could be due to its strong antioxidant properties.

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