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Ameliorative Effect Of *Smialx china* On Mercuric Chloride Persuaded Oxidative Stress In Testis: In Vitro Study.

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ABSTRACT

Smialx china L. is an herbal plant that belongs to China and Japan but it is imported to India and is used for various medicinal uses. It is commonly called Jin Gang Teng in Chinese, Chobchini in Hindi, Madhusnuhi in Sanskrit, and china root in English. It possesses anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic, and digestive properties. Free radicals scavenging and antioxidant enzyme promoting activities were seen in the juice of *Smialx china* L. foot mercury (HgCl2) induces various toxic effects in different organs of the body. The present work is to bring out the beneficial uses of *Smialx china* on mercuric chloride persuaded oxidative stress in rat testis. To analyze the AmeliorativeEffect of *Smialx china* on Mercuric chloride persuaded oxidative stress in Testis by Vitro Study. Male albino Wister rats were grouped into 9 groups, receiving mercuric chloride in various doses and *Smialx china* for different periods (Phase I – 30days & Phase II – 45days).The antioxidative indices assayed were superoxide Dismutase, catalase, and lipid peroxidase. Mercury exposure shows a decrease in the values of antioxidants such as SOD and CAT and increased levels of TBARS. Prophylactic administration of *Smialx china* (400mg /Kg/Bw)causes an increase in the level of SOD and CAT for animals treated with a low dose of Mercury (0.5mg/kg/Bw) and also a decrease in the levels of TBARS ($P<0.001$) significantly, was noticed. In animals on a High dose of Mercury chloride the reaction of *Smialx china* is less when compared to the low dose of the drug. Methanolic extract of *Smialx china* showed significant protection in antioxidant enzyme levels of SOD and catalase in low dose induced $HgCl_2$ albino rats, which could be due to its strong antioxidant properties.

Keywords: *Smialx china*, Mercuric Chloride, Oxidative stress, Testis

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INTRODUCTION

Male infertility is well documented as a result of exposure to many toxic substances, the consequence of inorganic mercury on Testis and potency are less well known. A 2008 study on the outcome of various heavy metals on semen quality reported data on humannonoccupational exposure to mercury (Hg), and its reproductive outcomes are very sparse [1]. An earlier review of the consequences of mercury hazards in the workplace on fertility and related reproductive outcomes found only three studies about male fertility [2], which were ambiguous at best. Two studies found effects by establishing the toxic influence on the fertility of organic mercury compounds within concentrations that can be seen at the workplace [3] and reduced concentration of testosterone in the serum of male workers, considered to be associated with exposure to inorganic mercury [4]. Male fertility can be impaired by various toxicants known to target Sertoli cells, which play an important role in spermatogenesis. Sertoli cells from male Sprague-Dawley rats were at high risk in vitro to mercury had severely inhibited inhibin production [5].

HgCl2 is the commonest form of toxin in the mercury group of chemicals because it easily forms organomercury complexes with proteins [6]. It is a terrible toxic substance that gets absorbed into the bloodstream once it is converted into oxidative form. The inorganic mercury joins with proteins in the plasma or enters the RBCs. The inorganic ionic mercury has a higher affinity for SH groups of fragment elements, such as glutathione (GSH) and sulfhydryl proteins, which may add to its toxicity [7]. Oxidative stress beings when the formations of ROS such as superoxide anion (O−2)hydrogen peroxide (H2O2), and the hydroxyl radical (-OH) outstrip the body's defense mechanism, leading to the damage of larger molecules such as DNA, proteins, and lipids [8] and trigger many pathological processes in the Testis and accessory sexual organs in male [9]. This confirms that the ROS may have a detrimental effect on carping substances of the steroidogenic pathway [10]. Moreover, many studies [11] have implied that a strong correlation exists between mercury causing toxicity and the formation of lipid peroxidation which is considered the most broadly studied expression of oxygen activation in biology. *Smilax chinensis* L (Liliaceae) is a deciduous climber with rounded leaves and red berries. The root tubes of which furnish the drug known as china root. It is found in the south Indian states namely Andra Pradesh, Tamil Nadu, and Karnataka [12]. Several species of Smilax are well-known Chinese traditional medicines used as an anti-inflammatory, anti-oxidant, anti-cancer, and analgesic agents. The root of Smilax Chinese has widely used in Chinese traditional medicines for the treatment of diverse diseases, especially for pelvic inflammation and chronic pelvic inflammation [13]. This study was proposed to investigate the ameliorative effect of Methanolic extract of *Smialx china* in albino rats induced by mercury toxicity.

MATERIALS AND METHOD

The study was conducted in the year 2021 at Meenakshi Medical College & Research Institute, Kancheepuram Male Wistar strain Albino rats, weighing (150-200gms) were used for the study after getting the Institutional Ethical Clearance EC:46/IAEC/2011. The albino rats were given a standard commercial laboratory diet and distilled water. The albino rats were housed in plastic cages with good ventilation. Light& dark conditions are maintained. The temperature for the albino rats was maintained (12h: 12h and26±2oC respectively) throughout the study. **Animal Grouping:** Male albino Wister rats were grouped into 9 groups, receiving mercuric chloride in various doses and *Smialx china* in different periods (Phase I – 30days & Phase II – 45days). **Phase-I – 30days.** Albino rats were divided into 5 groups as follows containing 12 animals in each group: Group 1 – control Rats were fed with the standard diet, Group 2 - Received Mercuric chloride 1mg /kg/BW. Orally, Group 3 - Receiving Mercuric chloride 0.5mg /kg/BW . orally, Group 4 - Mercuric chloride 1mg/kg/ BW + *Smialx china* 400mg /kg/BW orally, Group 5 – Mercuric chloride 0.5mg /kg/BW + *Smialx china* 400mg/kg/BW., orally. Out of 12 animals in Group2 and 3 only 4 animals were sacrificed, and the other 8 animals in each of those groups is taken for phase two of the study for a duration of 15days.**PHASE II – 15 days:** Group 6 - Post-treatment with *Smialx china* 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.Group 7 - natural recovery (15days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.Group8 - Post-treatment with *Smialx china* 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 0.5mg/kg/BW orally for 30days.Group 9 - natural recovery (15days) after receiving Mercuric chloride 0.5mg/kg/BW orally for 30 days. All the drugs were administered for a period of 1month and on the 31st day, the animals were weighed in the weighing machine, an autopsy was performed in the phase 1 group of animals and on the 45h day for the phase two animals. The testis was removed from the scrotal sac 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

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15 min; the supernatants were saved; and the protein concentration was measured, according to the method of Lowry et al [14], using bovine serum albumin as standard. **ANTIOXIDANT PARAMETERS:** The antioxidant parameters like superoxide dismutase (EC.1.15.1.1, SOD), Lipid peroxidation (TBARS), and catalase (EC.1.11.1.6, CAT) were analyzed by the spectrophotometric process which was framed by Kakkar et al. (1984), Ohkawa et.al., (1979) and Sinha et.al (1972) respectively [15-17].

RESULTS

Table 1: Comparison of in Vitro Antioxidant Activity of Phase I Animals

GROUP	Superoxide dismutase	Catalase	Lipid peroxidase
G1-Control	102.24±6.63	9.78±0.12	0.11±1.18
G2- High Hg	88.49±0.98	11.12±0.01	0.13±0.68
G3-Low Hg	59.43±0.86	9.19±0.01	0.18±0.65
G4- High Hg + SC	54.97±0.67	7.66±0.01	0.15±0.15
G5-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

Table 2: comparison of Invitro antioxidant activity of Albinorats Treated with HD HgCl2. HD HgCl2+ SC & HD HgCl2 on PT & NR.

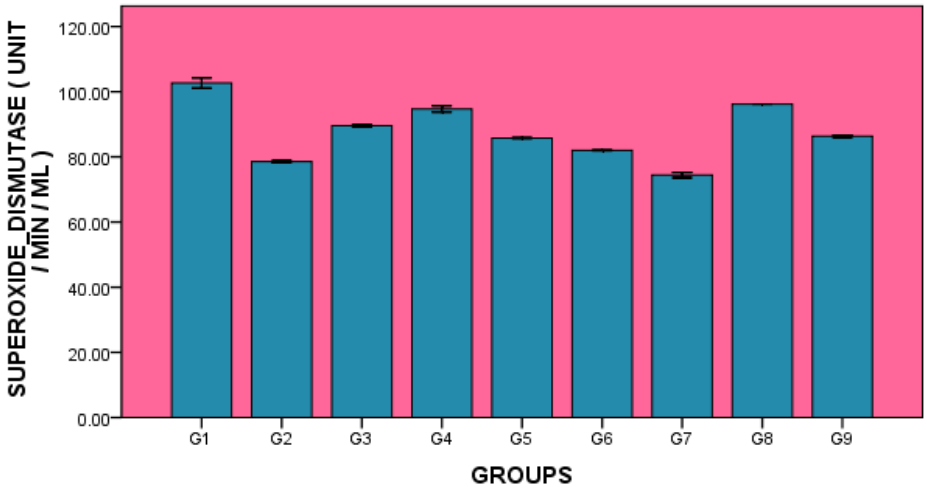
GROUPS	Superoxide dismutase	Catalase	Lipid peroxidase
G1	102.69±0.77 [a]	9.7525±0.03 [a]	0.105±0.01 [a]
G2	78.546±0.15 [b]	8.1125±0.01 [b]	0.1825±0.01 [b]
G4	94.705±0.48 [c]	8.6325±0.02 [c]	0.15±0.01 [c]
G6	81.992±0.05 [d]	8.49±0.04 [d]	0.1225±0.01 [d]
G7	74.417±0.39 [e]	7.54±0.01 [e]	0.145±0.01 [c]

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

Table 3: Comparison of Invitro antioxidant activity of Albinorats Treated with LD HgCl2. LD HgCl2+ SC & LD HgCl2 on PT & NR.

GROUPS	Superoxide dismutase	Catalase	Lipid peroxidase
G1	102.69±0.77 [a]	9.7525±0.03 [a]	0.105±0.01 [a]
G3	89.558±0.16 [b]	7.1725±0.03 [b]	0.1225±0.01 [b]
G5	85.776±0.07 [c]	9.2125±0.02 [c]	0.0925±0.01 [a]
G8	96.205±0.02 [d]	9.565±0.03 [d]	0.0925±0.01 [a]
G9	86.335±0.16 [c]	9.0125±0.10 [e]	0.13±0.01 [b]

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



Graph 1: Comparing Values of Superoxide Dismutase in all Phases of Animals

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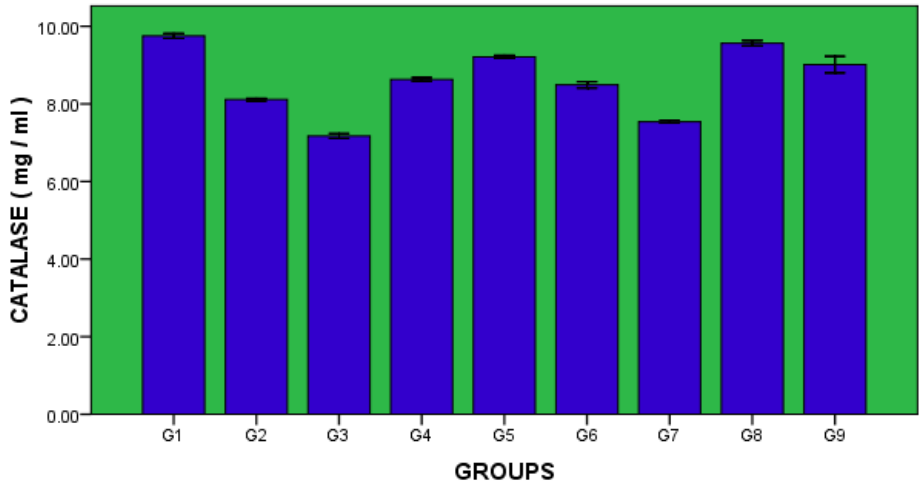
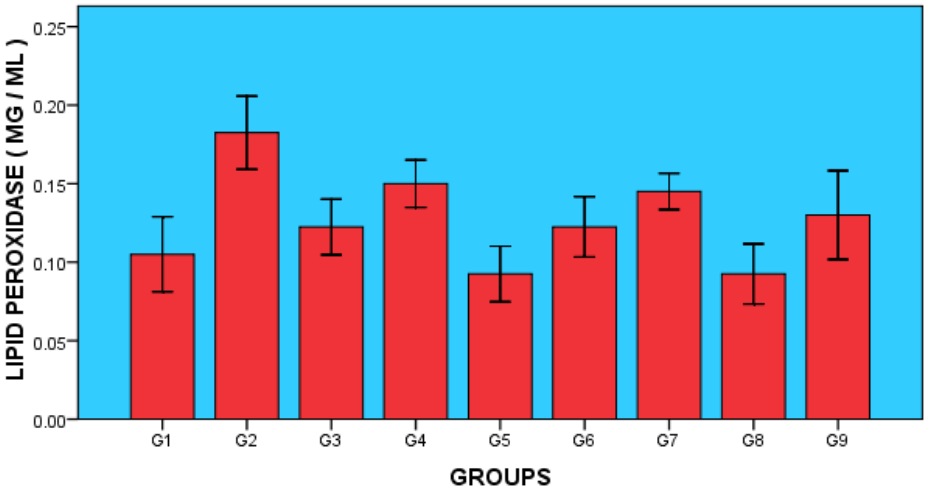


Graph 2: Comparing Values of Catalase Dismutase in all Phases of Animals



Graph 3: Comparing Values of Lipid Peroxidase Dismutase in all Phases of Animals

In this current study, antioxidant enzymes such as SOD and Catalase were significantly (P<0.001) decreased in albino rats administered with both doses of $HgCl_2$. (Table.No.1). The Prophylactic administration of *Smialx china* causes increases in the level of antioxidants, due to depletion in total -SH Groups in low dose affected group (P<0.001). Administration of *Smialx china* together with a huge dose of $HgCl_2$(1mg /kg/BW., orally), doesn't reveal a significant difference. The values of Lipid peroxidase also increased in groups treated with Mercury and only in low dose group with *Smialx china* had shown marked elevation in TBARS level (P<0.001).

DISCUSSION

The early antioxidant enzyme that deals with oxy radicals are SOD. The SOD escalates the dissimulation of superoxide (O2-) to hydrogen peroxide**.** CAT is a peroxisomal haem protein that catalyzes the removal of H202 formed during the reaction catalyzed by SOD. Thus, SOD and CAT act as mutually supportive antioxidative enzymes, which provide protective defense against reactive oxygen species. The ROS are ambiguous and extremely effective. They become stable by acquiring electrons from nucleic acids, proteins, carbohydrates, and lipids, thereby a cascade of chain reactions are initiated resulting in cellular damage and causing lipid peroxidation [18]. Thus, in the current study chronic administration of $HgCl_2$ causes decreases in the levels of SOD and Catalase. Lipid peroxidation is the mechanism by which the oxidative deterioration of polyunsaturated fatty acids (PUFAs). The presence of lipid peroxidases in the cell membrane, impaired its function and anatomical architecture**,** decreases in membrane fluidity, and inactivation of several membrane-bound enzymes[8]. Thus, it is evident that mercury chloride exposure leads to the peroxidation of PUFAs, leading to the disintegration of phospholipids and resulting in cellular

changes in the testis**.** Various studies also recommended that a close relationship endure between mercury persuaded toxicity and the formation of LPO[11]. The present data revealed a significant increase in lipid peroxidation levels is because of the oxidative stress that leads to cellular damage. Co-administration of *Smialx china* with a low dose of $HgCl_2$ exposed groups exerted amelioration effects. This antioxidant and ROS scavenging effects of *Smialx china* is due to the phenolic (-OH) group, which would inhibit the -SH group oxidation and block thiol depletion thus it protects the oxidation of protein. Further, it also enhances the activities of some antioxidant enzymes such as SOD and catalase agrees with the previous findings [19].

CONCLUSION

Methanolic extract of *Smialx china* showed significant protection in antioxidant enzyme levels of SOD and catalase in low dose induced $HgCl_2$ albino rats, which is due to higher antioxidant properties.

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