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Research Article

Effect of Smilaxchina on Mercuric Chloride Induced Histopathological Alterations in Testis of Male Albinorats

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ABSTRACT

There is a growing problem of worldwide contamination of the environment with mercury. The fate and behavior of mercury in the environment depends on its chemical form, which affects the spermatogenesis of a man. Smilax china is an Traditional Herbal root tuber which is used for various medical allignments. The present study is to evaluate the Protective Effect of Smilax china on Merucric Chloride in testis of the Albinorats.The animals were divided in to 5 groups i)control ii) High dose Mercury (1mg/Kg/BW) iii) low dose Mercury (0.5mg/Kg/BW) iv) High doseMercury (1mg/Kg/BW) and Smilax china 400mg/Kg/BW v)Low dose mercury (0.5mg/Kg/BW) and Smilax china 400mg/Kg/BW) for period of 30days orally. The animals were sacrified and the Histopathological analysis were done.Animals treated orally with Mercury chloride shows Necrosis in lumen, intranuclear vaculoation on secondary spermatogonia, and irregular arrangement on the primary spermatogonia. Degenerative changes on the leydig cells, and occasional detachment of the epithelial cells are seen .Animals treated Prophylatically with Smilax china shows decreased intranuclear vaculoation in primary and secondary spermatogonia, tubules are uniform and there is no detachment of the epithelial cells, showing the Protective effect of Smilax china on Merucric chloride intoxication on Testicular TissuesHence Smilax china can be used as a drug of choice for increasing the Spermatogenesis. The study supports the ancient traditional review that Smilax china can be used in condition like infertility to increase the sperm count.

Key Words**:** Smilax china , Mercury chloride, Spermatogenesis

INTRODUCTION

Heavy metals have become one of many contaminants found inour environment. Many of these metals, including lead, mercury,cobalt, cadmium, and chromium are known to exert toxic effects on testicular function, while others such as zinc, manganese, andselenium have been shown to be essential for normal functioningof the testis[1,2,3]. Mercury is a ubiquitous element in the environment causing oxidative stress in the exposed individualsleading to tissue damage. Its contamination and toxicity has poseda serious hazard to human health. The release of mercury fromdental amalgam dominates exposure to inorganic mercury andmay have an acceptable risk among the general population[4]. Human exposure to mercury can occur by inhalation, ingestion and consumption via food chain. Adverse effects of metals onhuman reproduction and development continue to be a demandingchallenge for researchers. Mercury compounds are known to affect testicular spermatogenic and steroidogenic functions inexperimental animals[5]. Oral exposure of mercuric chlorideproduced adverse effects on the reproductive performance of mice[6]. The principal sources of exposure to inorganic mercury (Hg2+) are from water, food and air,while exposure to other forms of mercury are from dental amalgamin tooth filling (elemental Hg0) and from consumption of fishand other seafood (organic mercury Hg+)[7].

Smilax china L., popularly known as 'Jin Gang Teng' or 'Ba Qia', is widely used as a traditional Chinese medicine (TCM) for the treatment of diuretic and rheumatic arthitic conditions, as well as for detoxication, and to treat lumbago, gout, tumors, and inflammatory diseases; it is also used as a food in some areas of China[8]. The dried rhizome of Smilax china L. of the family Smilacaceae, known as Chobchini in Hindi, contains fat, sugar, glycoside, coloring matter, saponin, gum, tannin, cinchonin, smilacin and starch[9] and it exhibits anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic and digestive properties[10]. Steroidal saponins have been isolated from Smilax riparia and Smilax china L. and the anti-inflammatory activities of the isolated fractions have been investigated[11]. Steroidal saponins, isolated from Smilax china L. have been reported to possess anti-inflammatory activity[12]. Therefore the present studies to conform theprotective activity of smilax chinensis L. on testis of albino rats in mercuric chloride intoxication.

MATERIALS METHODS

Mercuric Chloride: Mercury in the form of HgCl2 was purchased from Fisher Scientifics (Mumbai), (Product no. 15564),

Plant Materials: The plant selected for present work was Smilax china (Family: Liliaceae). The root tuber was collected from Tirunelveli district, Tamilnadu which was

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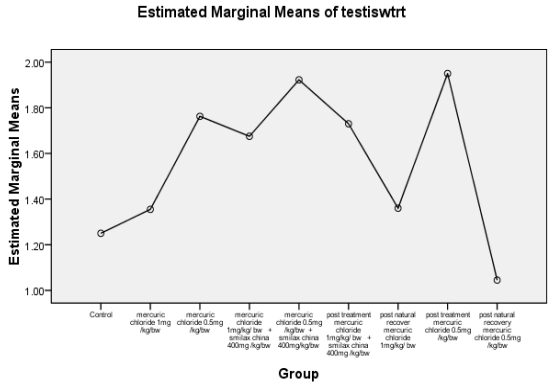
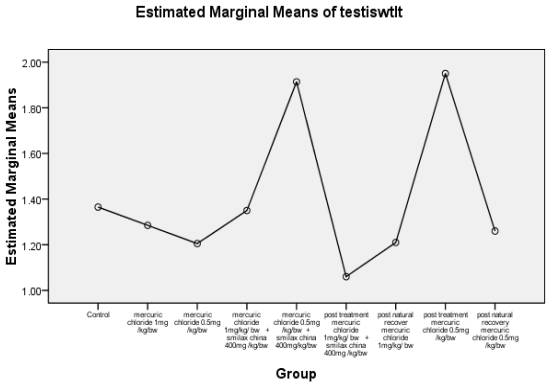


GRAPH.NO.1 showing the comparison of Right testis



GRAPH.NO.2 showing the comparison of Left testis

identified and certified from the National Institute of Siddha Medicine, Chennai.

Methods

Preparation of extract: The rhizomes of plants were dried in shade, separated and made to dry powder. It was then passed through 40 mesh sieve. A weighed quantity (125gm) of the powder was subjected to continuous cold extraction in soxhlet apparatus. The filtrates (methanol extract) obtained were evaporated under ceiling fan into a stainless steel tray until they had dried. They rendered a gummy concentrate of brown colour · Prelimnary Phytochemical screening , antioxidant property and HPTLC finger printing of the Herbal drug was performed. The methanoic extract dose was fixed as 400mg/bg/kg[13].

Animals: Male albino Wistar rats weighting 140±6 to 200±5 g were kept in an animal house under constant temperature conditions (24± 2^{o}C) for at least 1 week before and through the experimental work, being maintained on a standard diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture, and 5% vitamins. Water was available *ad-libitum*. All the experiments were done in compliance with the guide for the care and use of laboratory animals. The Institutional ethical committee clearance has been received prior to the experiment EC:46/IAEC/2011

Experimental Design**:** The Methanolic extract of whole plant of Smilax china was tested in this experimental part.

Animals were divided into 5 groups as follows containing 8 animals in each Group:

Group 1- control- Rats were fed on the standard diet

Group 2 – Receiving Mercuric chloride 1mg /kg/bw., orally

Group 3- Receiving Mercuric chloride 0.5mg /kg/bw ., orally

Group 4- Mercuric chloride 1mg/kg/ bw + smilax china 400mg /kg/bw ., orally

Group 5 – Mercuric chloride 0.5mg /kg/bw + smilax china 400mg/kg/bw., orally.

The Experimental procedure for conducted for period of 30days.

At the end of the experimental period, the animals fromboth control and experimental groups were dissected under ether anesthesia, and the reproductivesystem was exposed and testis were dissected out.

Out of 8 animals in Group2 and 3 only 4 animals were sacrificed, and other 4 animals in each of those group is taken for phase two of the study for a period of 15days.

Group 6 - Post treatment with Smilax china 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.

Group 7 - Natural recovery after receiving Mercuric chloride 1mg/kg/BW orally for 30days.

Group 8 - Post treatment with Smilax china 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 0.5mg/kg/BW orally for 30days.

Group 9 - Natural recovery after receiving Mercuric chloride 0.5mg/kg/BW orally for 30days.

Gravimetry: Morphological changes in the body of the treatedanimals were noticed every day by careful visual observation to see any physical changes in the body such as hair fall,necrosis, infection and overgrowth. The body weights of theanimals were recorded, prior to and after treatment.

Wet weight of the Testis : After dissection of the Testis , the wet weight of the testis Right and Left in all animals in each group were recorded and compared.

Histopathological studies: Testis removed from the control and experimental rats,were washed thoroughly in physiological saline, cut intopieces of desired size and fixed in Bouin`s fluid fixativeimmediately after autopsy, and fixation proceeded at roomtemperature for 24 h, after which the tissues were transferredto 70% alcohol[14]. The tissues were then dehydrated bypassing through ascending grades of alcohol, cleared inxylene, infiltrated with molten paraffin, and finally embedded in paraffin wax (58°C MP). 5 µm thicknesssections were obtained using a rotary microtome (Leica,Germany). The sections were stained in Harris`hematoxylene and eosin, dehydrated using alcohol, cleared inxylene and mounted using dihydroxy phthalate xylol (DPX).The stained slides were observed in a research microscope and images were captured.

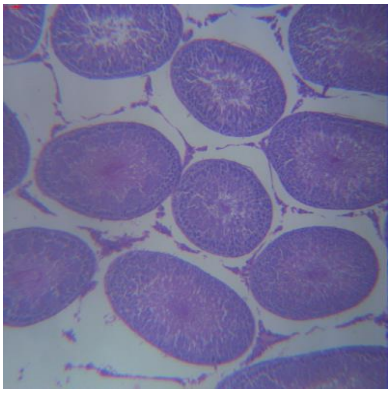


Fig. 1: Control, compact, well-differentiated seminiferous tubulesshowingactive spermatogenesis. 100X, H & E Stain

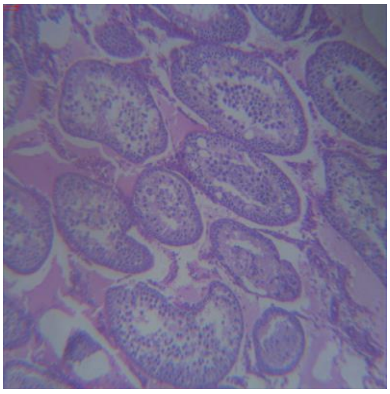


Fig. 2: hgcl2 (1mg/kg/bw) treated for 30 days showing necrosis in lumen, decrease in primary spermatocytes, intranuclcearvaculoation on secondary spermatocytes. 100x, h & e stain

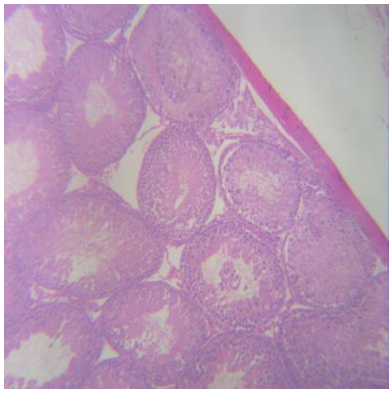


Fig. 3: hgcl2 (0.5mg/kg/bw) treated for 30 days showing intact basement membrane, regular primary spermatocytes , occasional intranuclcearvaculoation on secondary spermatocytes. 100x, h & e stain

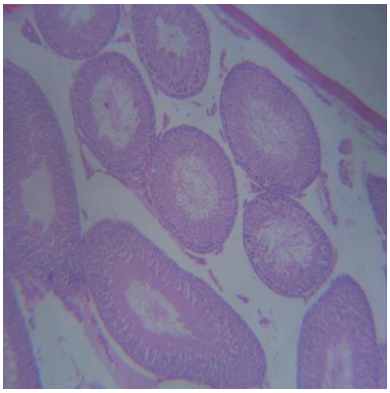


Fig. 4: hgcl2 (1mg/kg/bw) + smilax china (400mg/kg/bw) treated for 30 days showing intact basement membrane, typical mitosis.uniform spermatogenic cells and decreased intranuclcearvaculoation on secondary spermatocytes. 100x, h & e stain

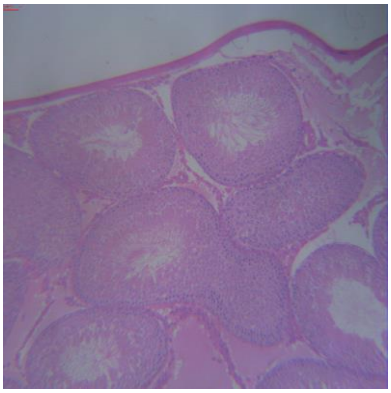


Fig. 5: hgcl2 (0.5mg/kg/bw)+ smilax china (400mg/kg/bw) treated for 30 days showing intact basement membrane, regular primary spermatocytes & secondary spermatocytes. 100x, h & e stain

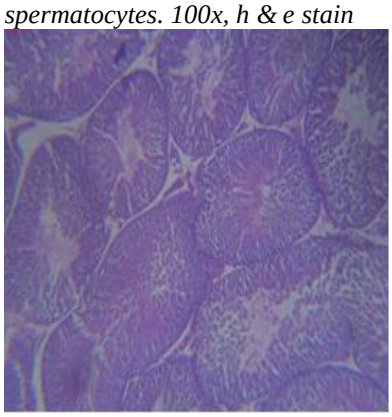


Fig. 6: post treatment with smilax china (400mg/kg/bw) for animals affected with hgcl2 (1mg/kg/bw) treated for 15 days showing sloughi8ng of spermtocytes with in the lumen of semiferous tubules . 100x, h & e stain

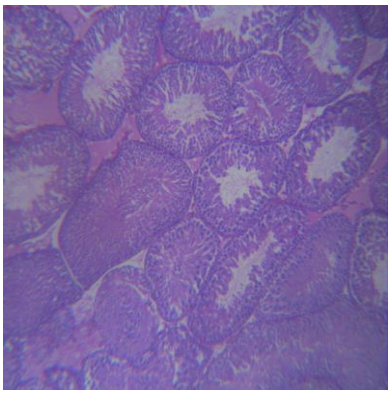


Fig. 7: natural recovery treated with high dose mercury chloride (1mg/kg/bw) showing intact basement membrane, regular primary spermatocytes , occasional intranuclcearvaculoation on secondary spermatocytes. 100x, h & e stain

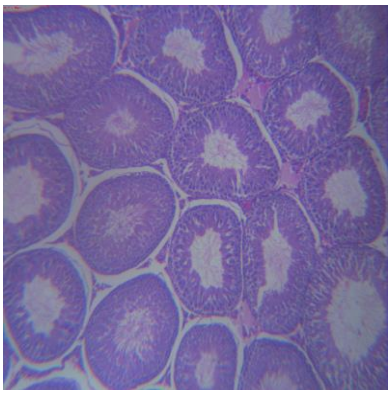


Fig. 8: post treatment with smilax china (400mg/kg/bw) for animals affected with hgcl2 (0.5 /kg/bw) treated for 15 days showing normal tubulues without necrosis. 100x, h & e stain

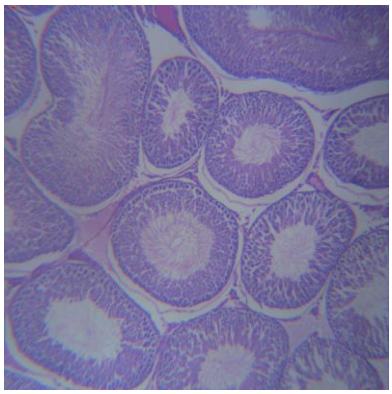


Fig. 9: : natural recovery treated with low dose mercury chloride (0.5mg/kg/bw) showing intact basement membrane, regular primary spermatocytes &secondary spermatocytes. 100x, h & e stain

Statistical Analysis: The results were expressed as Mean± SD , and statistical analysis was performed using Student "t"test.

RESULT

Effects of Hgcl2 on the body weight of the Albinorats: Their exists no change in the weight of the animals among all the groups throughtout the phase of the experiment (Graph.NO.1)

Effects of Hgcl2 on the wet weight of testis Albinorats: It shows difference in the right and left side testis and also among the group. Animal shows increased in testicular weight in Low dose mercury on treatment and also in Post treatment groups (Graph 2 & 3). This shows the prophylactic effect and therapeutic effect of smilax china in low doses is well exhibited

Histopathology Findings

Control and Merucry Treated Groups: Control Animal showed the normal architecture of the seminiferous tubules (fig.no.1). The animals treated with Mercury chloride for 30days on two comparatively doses 1mg/kg/bw and 0.5/mg/bw shown marked changes histopathologically. It showed oblong tubules, the seminiferous tubules exhibited big vacuole in their lumen due to toxic load. In some of the tubules the epithelial lining were detached. The necrotic changes are noted in the lumen, primary spermatocytes showing intracytoplasmic vaculoation (fig.no.2) where as secondary spermatocytes showed intranuclear vaculoation (fig.no.3). The spermatids became hypertrophied and sertolicells were damaged. Degenerative changes were also seen in the Leydig cells and connective tissue.

Mercury and Smilax china Treated Group: Animals treated with Merucry chloride on both doses were administered with Smilax china 400mg/kg/bw prophylactically for 30days, shows marked decreased in the vaculoation on the spermatocytes, increased mitotic activity.(fig.No.4 & 5)

Mercury affected and Post treatment of Smilax china Group: Animals affected with Merucry chloride both high and low dose (30days) toxic load was treated with smilax china for 15days, which marked histological changes in the seminiferous tubules. In high dose post treated group the lumen is normal and sloughing of the spermatocytes in the lumen of the seminiferous tubules is seen (fig.No.6), where as in low dose post treated group the spermatid are normal , the sloughing is not seen, no dyscohesive seen betweem the cells are seen(fig.No.8)

Mercury affected and Natural recovery group: Animals affected with both dose of mercuric chloride after 30days are allowed for natural recovery. Showing Persistence of the intranuclear and intracytoplasmic vaculoation on high dose group, which new spermatogenic cells are recovering. Hypertrophied serotli and spermatid cells are seen (fig.No.7), where in low dose mercury chloride treated animals,dyschohesive of primary and secondary spermatocytes are seen with decreased necrosis in the lumen(fig.No.9).

DISCUSSION

Exposure of albinorats to mercurichloride signigicantly affects the spermatogenesis. Administration of heavy metals causes degenerative changes in testicular tissue and accessory reproductive organs[15]. they revealed that inhibition of steroidogenesis and level of testosterone was decline. Similarly testicular generation and cellular degeneration in the seminiferous tubules and leydig cells due to administration of Merucric chloride (0.5mg/kg) to rats are reported[16], and our observation agree with the results of it. Mercuric chloride at the dose of 05mg/kg/BW and 1mg/kg/BW for a period of 30days decreases the spermatogenesis and decreases the testosterone levels,[17] which is well exhibited on our study . In the present investigation, the animals were treated simultaneously with mercury and Smilax china 400mg/kg/BW showed protection against mercury induced cytotoxicity alterations. The seminiferus tubules showed less shrinkage and reorganized and malformation of different spermatogenic cells were reduced. The sdtudy of Wan.M. Hilmi et.al., Smilax myosotiflora can be given as aphrodisiac[18] and this is the first study reporting Smilax china against mercuric chloride intoxiciation. It also show when the animals were treated after mercuric chloride intoxication for a period of 15days showed marked changes in the histoarchitecture of the seminiferous tubules showing its positive affinity through the steriodgenesis action. On the basis of this study it is concluded that mercury causes severe toxic tissuse damage. This damage may be caused by the reactive oxygen species produced by mercury within the animals body. Smilax china interact with mercury ions, neutralize them or bind with transition metals and prevent the ROS mediated oxidative damage in testis and protect the tissue from intoxication and enhances spermatogenesis.

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