Original Article

Nephroprotective Effect Of Smilax China Against Mercuric Chloride Intoxication In Albino Rats- Histological Study

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Abstract

BACK GROUND : There is a growing problem of worldwide contamination of the environment with mercury. The fate and behaviour of mercury in the environment depends on its chemical form. Inorganic mercury compounds enter water bodies by different ways and undergo a process of methylation.

Mercury poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood stream. High exposures to inorganic mercury may result in damage to the gastrointestinal tract, the nervous system, and the kidneys. mercury combines with proteins in the plasma or enters the red blood cells but does not readily pass into the brain or fetus and instead, may enter other body organs.

Studies have revealed that mercuric chloride caused histopathological and ultra structural lesions in the kidney. Plants are considered to be a promising source of medicine in the traditional health care system. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are responsible for the physiological functions of living flora and hence they are believed to have better compatibility with thehuman body.

Keeping therefore in view, the growing interest of the use of herbal drugs the present study was undertaken with a view to evaluate the Protective effect of root tuber of Smilax china (Linn) against mercuric chloride intoxication in kidney.

METHODS: The animals were divided in to 5 groups for phase I of Study.
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 of kidney were done.
Phase II was Carried out for 15 days.
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 VI - Post treatment with Smilax china 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.
Group VII - Post treatment with Smilax china 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.
Group VIII - Post treatment with Smilax china 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days. Group IX - Post treatment with Smilax china 400mg/kg/BW orally(15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.

RESULTS: Animals treated orally with Mercury chloride shows tubular Necrosis in lumen, sloughing of epithelium in the lumen , and Haemorrghaic changes in the glomeruli. They also exhibited high levels of Urea and creatinine in the blood. Animals treated Prophylatically with Smilax china shows decreased tubular necrosis with few normal tubules, occasional haemorrghic changes and there is no detachment of the epithelial cells, showing the Protective effect of Smilax china on Merucric chloride intoxication on Renal Tissue .
CONCLUSION: Hence Smilax china can be used as a drug of choice to protect the kidney from Mercuric chloride intoxication when affect with low dose concentration as prophylatixc and as Treatment. The study supports the ancient traditional review that Smilax china can be used in condition hyperuricemia and chronic renal failure.

Key Words: Smilax china, Mercuric chloride, Nephroprotective Abbrevations: HD- High Dose, LD–Low Dose, SC-Smilax china, NR-Natural Recoery, PT-Post Treatment

INRODUCTION

Kidneys accumulate highest levels of mercury compared to brain and liver [1]. Renal toxicity of mercuric chloride is well documented in literature. Nuclear factor B (NF- B) is a thiol dependent transcriptional factor that promotes cell survival and protects cells from apop- totic stimuli. Mercuric ion Hg (2+) is, one of the strongest thiol-binding agents known, impairs NF- B activation and DNA binding at low M concentrations in kidney epithelial cells leading to apoptosis [2]. Renal function and immunologic markers among chloralkali workers with long-term low exposure to mercury vapor when examined indicated an effect of exposure on the kidney proximal tubule cells [3].

Renal dysfunction increases plasma Creatinine level upon methyl mercury intoxication for 5 ppm mercury for 2 years [4]. Decrease in protein (brain and liver) acid and alkaline phosphatase and glutathione S transferase was observed upon 0.5 mol/ml mercury for five consecutive days, while thiobarbituric acid reactive substances (TBARS) was found to be significantly increased in brain and liver indicating free radical stress [5] .

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 [6]. 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 [7] and it exhibits anti- inflammatory, diuretic, anti-diabetic, anti-psoriatic and digestive properties [8]. Steroidal saponins have been isolated from Smilax riparia and Smilax china L. and the anti-inflammatory activities of the isolated fractions have been investigated. Steroidal saponins, isolated from Smilax china L. have been reported to possess anti-inflammatory activity. Therefore the present studies to confirm the protective activity of smilax chinensis L. on kidney 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 Material

The plant selected for present work was Smilax china (Family: Liliaceae). The root tuber was collected from Tirunelveli district, Tamilnadu which was identified and certified from the Bharathira University, Department of Botany, Coimbatore.

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/kg/Bw [9].

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:

Animals were divided into 5 groups as follows containing 8 animals in each for phase of Study Group (Phase I Study)
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 from both control and experimental groups were dissected under ether anesthesia, and the abdominal wall was exposed and Kidney 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.

Phase II:

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 - Post treatment with Smilax china 400mg/kg/BW orally (15 days) 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 1mg/kg/BW orally for 30days.
Group 9 - Post treatment with Smilax china 400mg/kg/BW orally (15 days) after receiving Mercuric chloride 1mg/kg/BW orally for 30days.

HISTOPATHOLOGICAL STUDIES

Kidney removed from the control and experimental rats, were washed thoroughly in physiological saline, cut into pieces of desired size and fixed in buffered **formalin.** 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 thickness sections 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.

BIOCHEMICAL ANALYSIS: Urea and Creatinine were analyzed to support the Histopathological changes of the kidney. Periorbital Blood was collected from the animals and analysed in Autoanalyzer. Theanalysis are done under regular intervals. 2nd (48 Hours), 7th , 15th and 30th day (phase 1) and 45thday (phase 2) were analysed.

STATISTICAL ANALYSIS

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

RESULTS

The effect of Smilax china on Renal tissue was exhibited on this study by Histopathological Study and Biochemical Analysis.

HISTOPATHOLOGICAL CHANGES IN KIDNEY

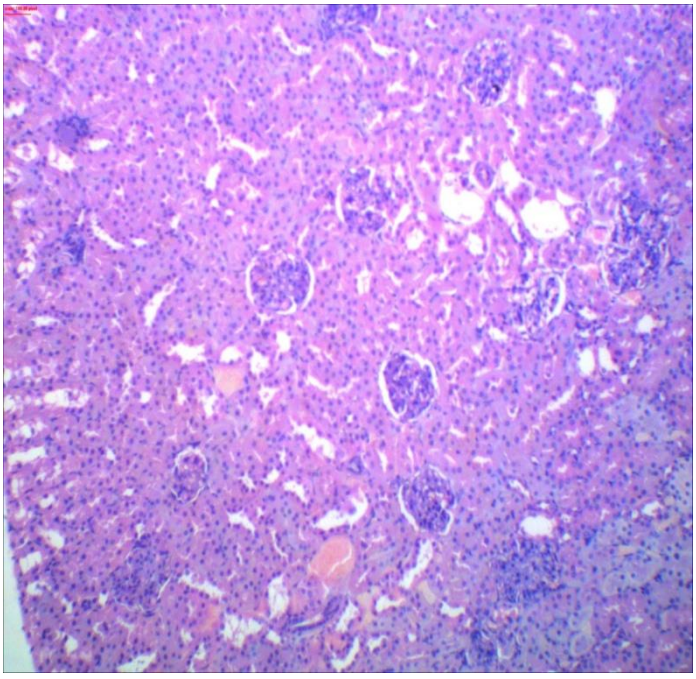

Fig.No.1 – Group I –Normal Kidney – Control – H & E (10x)

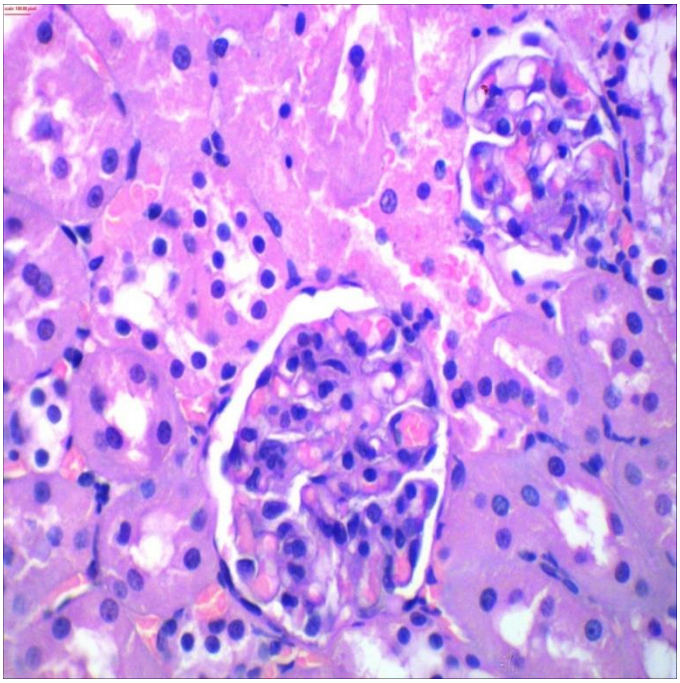

Fig.No. 2 –Group II – High Dose HgCl2 Treated – H & E (40x)
,Showing Tubular Necrosis , Sloughing of epithelium in lumen , Haemorrhagic changes in Glomeruli Seen.

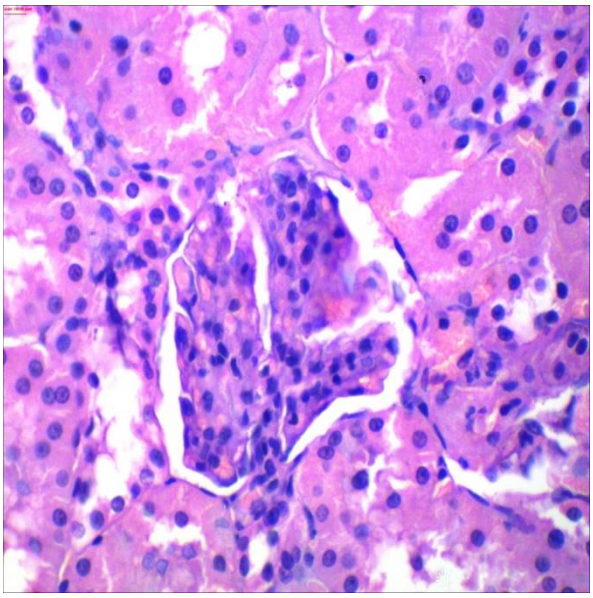

Fig.No.3- Group III-Low dose HgCl2 Treated Animals – H&E(40x)- Showing Mild Tubular necrosis , Sloughing of epithelium in the lumen.

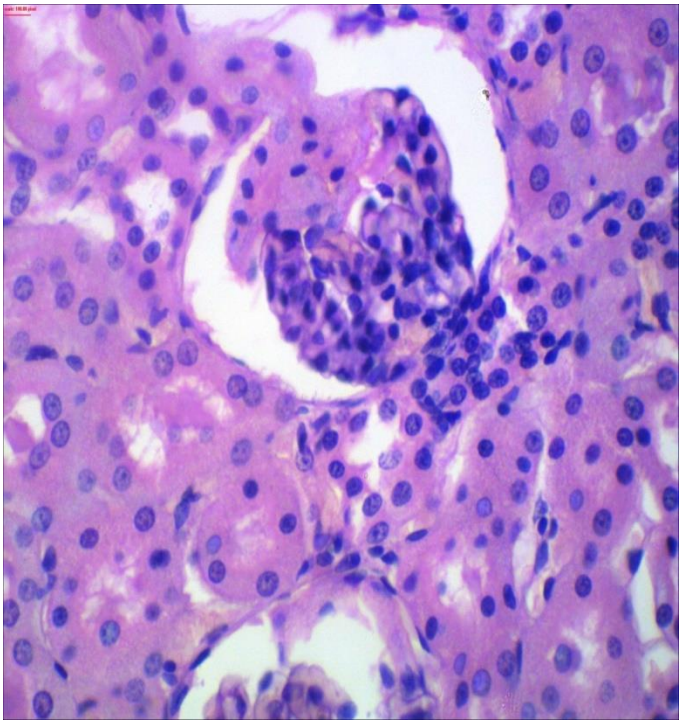

Fig.No.4- Group IV- High dose of HgCl2 + Smilax China Treated Animals – H&E(40x)- Showing Few Tubules are normal and Few Tubules showing necrosis

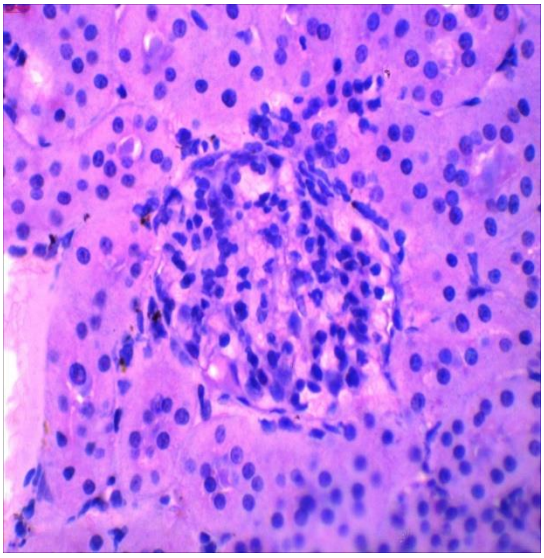

Fig.No.5- Group V- Low dose of HgCl2 + Smilax China Treated Animals – H&E(40x)- Showing Regenerative Tubular Epithelial Cells.

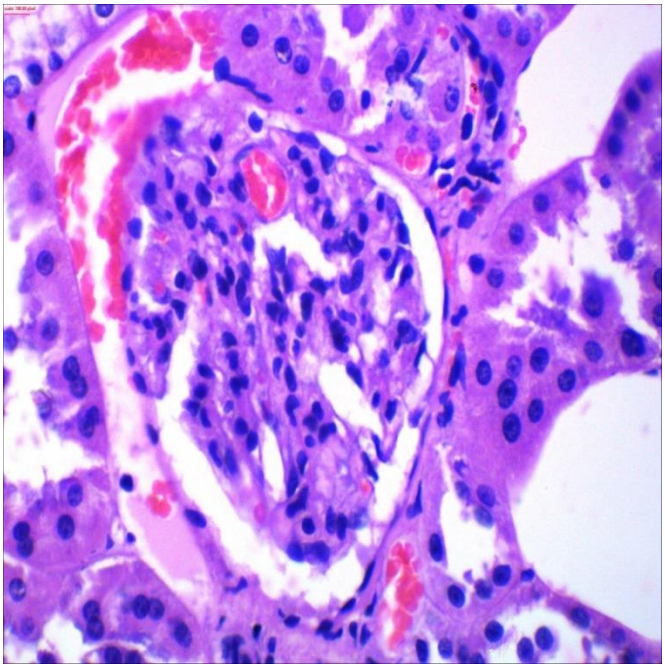

Fig.No.6- Group VI- High dose of HgCl2 , Post treatment with Smilax China Treated Animals – H&E(40x)- Showing Premodimante normal Tubules & Few showing Tubular necrosis.

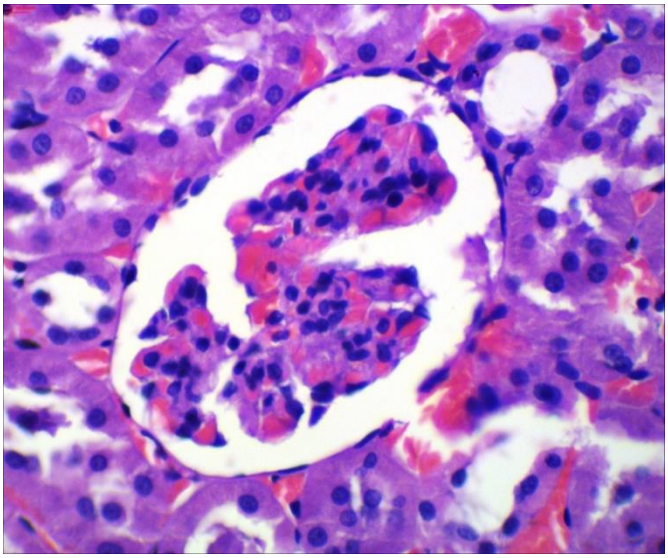

Fig.No.7- Group VII- High dose of HgCl2 treated Animals on Natural Recovery – H&E(40x)- Showing sloughing Tubular epithelium,Few mild tubular Necrosis & other tubules are normal

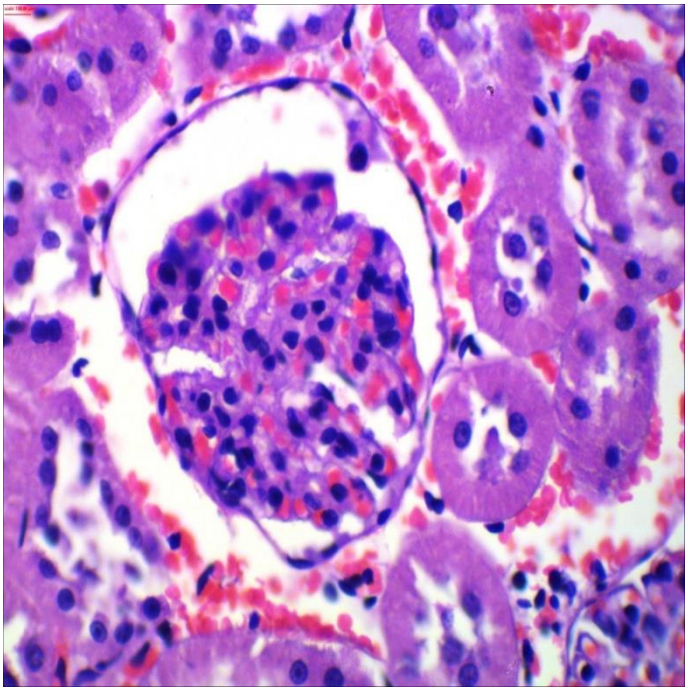

Fig.No.8-Group-VIII- Low dose of HgCl2 , Post treatment with Smilax China Treated Animals – H&E(40x)- Showing Premodimante normal Tubules & Few showing Tubular necrosis.

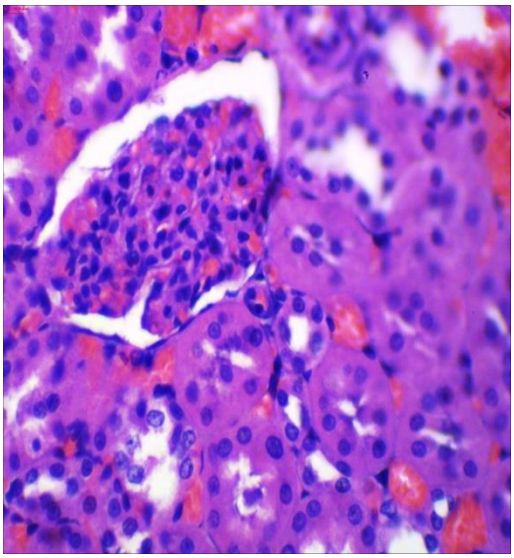

Fig.No.9.Group IX - Low dose of HgCl2 treated Animals on Natural Recovery – H&E(40x)- Showing sloughing Tubular epithelium

Table.No.1 COMPARISON OF LEVELS OF UREA AND CREATININE IN DIFFERENT INTERVALS OF TIME –PHASE I OF STUDY

Parameter	Time interval	Group1**	Group 2**	Group 3**	Group 4*	Group 5*
Creatinine (mg/dl)	48hrs	0.55±0.05[a]	1.3962±0.01[a]	1.2225±0.01[a]	1.17±0.02[a]	1.0675±0.01[a]
	7th Day	0.65±0.05[b]	1.415±0.01[b]	1.2625±0.01[b]	1.1125±0.01[b]	1.0475±0.01[b]
	15th Day	0.7375±0.05[c]	1.4425±0.01[c]	1.2937±0.01[c]	1.0725±0.02[c]	1.0262±0.01[c]
	30th Day	0.8125±0.06[d]	1.4625±0.01[d]	1.32±0.01[d]	0.96±0.09[d]	0.925±0.08[d]
Urea (mgs/dl)	Duration	**Group1****	**Group 2***	**Group 3****	**Group 4***	**Group 5****
	48hrs	19.87±0.42[a]	27.77±0.23[a]	25.22±0.23[a]	26.7±0.26[a]	23.85±0.13[a]
	7th Day	20.83±0.79[b]	28.25±0.47[b]	25.73±0.21[b]	26.2±0.16[b]	23.41±0.25[b]
	15th Day	20.81±0.84[b]	28.76±0.77[c]	26.51±0.37[c]	25.8±0.53[c]	22.93±0.59[c]
	30th Day	20.83±0.79[b]	29.2±1.11[d]	27.1±0.56[d]	25.4±0.88[d]	21.75±0.95[d]

Values are expressed as Mean±SD. Values not sharing a common superscript letter differ significantly at 5% level of significance. **indicates RANOVA significance with P value <0.0001, *indicates RANOVA significance with P value <0.05, #indicates no significant difference

TABLE.NO.2 COMPARISON OF LEVELS OF UREA & CREATININE ON 30th & 45th DAY

Parameter	Group	30th Day	45th Day	Significance
UREA (mgs/dl)	Group 6	29.225±1.18	22.875±0.85	Significant*
	Group 7	29.175±1.22	26.25±0.64	Significant**
	Group 8	27.4±0.69	16.025±0.80	Significant*
	Group 9	26.8±0.11	23.125±0.85	Significant**
CREATININE (mg/dl)	Group 6	1.4675±0.01	0.97±0.05	Significant*
	Group 7	1.4575±0.02	1.33±0.02	Significant**
	Group 8	1.32±0.01	0.775±0.09	Significant**
	Group 9	1.32±0.01	1.28±0.01	Significant**

The 30th day and 45th day values are compared using paired t test, Values are represented as Mean ± SD,*significant at p<0.0001, **significant at p<0.05

TABLE.NO. 3 COMPARISON OF UREA AND CREATININE LEVELS BETWEEN HD HgCl2 + SC, HD HgCL2 PT & NR

PARAMETER	CREATININE (mg/dl)	UREA(mgs/dl)
G1	0.8125±0.06[a]	20.837±0.79[a]
G4	0.96±0.09[b]	25.425±0.88[b]
G6	0.97±0.05[b]	22.875±0.85[c]
G7	1.33±0.02[c]	26.25±0.64[b]

Oneway ANOVA test was used (Note: In group 1 and 4, only 30th day values are taken. group 6 and 7 are the values taken at 45th day from group 2.) Values are expressed as Mean ± SD. Values not sharing a common superscript letter differ significantly at 5% level of significance (Least Significant Difference test (LSD).

TABLE.NO. 4 COMPARISON OF UREA AND CREATININE LEVELS BETWEEN LD HgCl2 + SC, LD HgCL2 PT & NR

PARAMETER	CREATININE (mg/dl)	UREA(mgs/dl)
G1	0.8125 ± 0.06^{a}	20.837 ± 0.79^{a}
G5	0.925 ± 0.08^{b}	21.75 ± 0.95^{a}
G8	0.775 ± 0.09^{a}	16.025 ± 0.80^{b}
G9	1.28 ± 0.01^{c}	23.125 ± 0.85^{c}

Oneway ANOVA test was used (Note: In group 1 and 5, only 30th day values are taken. group 8 and 9 are the values taken at 45th day from group 2.) Values are expressed as Mean ± SD. Values not sharing a common superscript letter differ significantly at 5% level of significance (Least Significant Difference test (LSD).

DISCUSSION:

Exposure to different concentrations of mercuric chloride causes renal damage. Inorganic mercury has been shown to acculumate in the renal cortex and affect the morphologyand function of the tubules [10]. The degree of necrosis in proximal tubules were higher in High dose administered Mercuric chloride group, where as animals treated with smilax china showed decreased histopathological changes which was correlated with the biochemical paramaters.

In the present study, the higher levels of urea and, in particular, creatinine clearly reflected progressing renal insufficiency in rats treated with mercuric chloride [11,12] reported higher urea and creatinine levels in rats administered mercury either in the form of inorganic salts or as a complex with metallothioneine. Such functional impairment probably resulted from both vasoconstriction and a direct cytotoxic effect of mercury [13,14]. Besides, the detrimental effect may be attributed mainly to the accumulation of this toxic metal in kidney. In the present work, renal concentration of mercury was highest among all tissues and organs tested, probably indicating the most serious organ affection in Group I and II of Animals. The Histoarchiture of these group of animals treated with two different doses of Mercuric chloride showed marked tubular Necrosis, sloughing of epithelium in the lumen and haemorraghic changes in the glomeruli. When Smilax china is given as prophylactic drug in Group 4 and 5 along with High and low dose of Mercuric chloride, only few tubules marked changes when compared with Group 1 and Group 2. Similarly Urea and Creatinine levels were also lowered in animals with prophylactic administration of Smilax china on High dose (Group 4) and low dose (Group 5) animals.

There is evidence that chronic exposure to low concentration of metals like mercury causes tissue or organ damage [15]. This study demonstrates that administration of Smilax china prior to HgCl2 treatment appears to moderate the renal damage caused by HgCl2. The administration of HgCl2 brings about an impairment of renal function which is evident by increase in urea and creatinine levels in the blood. Other manifestations of mercury toxicity include impairment of electrolyte, water and nonelectrolyte transport in variety of cells and tissues, the principal target organ being the kidneys [16].

The kidneys are paired bean-shaped organs located on either side of the spinal column. The kidneys perform a variety of functions for the body, the most important being removal of unwanted substances (waste and surplus) from the plasma, homeostasis of the body's water, electrolyte and acid/base status and participation in endocrine regulation. Human acute renal failure is often caused by ischemic and nephrotoxic insults commonly acting in combination [17]. There are very few agents that inhibit the acute renal failure in mercuric chloride induced model of nehrotoxicity.

One of them is Smilax China, which has been shown to afford protection to HgCl2 induced nephrotoxicity even if it is administered after HgCl2. In this study the renal damage was assessed by biochemical parameters

measured in the blood along with histologic damage. The relative magnitude and degree of necrosis in proximal tubules based on quantitative histopathological analysis showed that the degree of renal damage observed was higher in High dose of HgCl2 treated animals.

Our studies have shown that at low doses of mercuric chloride, a very mild level of cellular necrosis was detected while severe treated with HgCl2 there was a decrease cellular necrosis involving primarily the pars recta of proximal tubules was observed in rats treated with 0.5 and 1.0 mg of HgCl2/kg body weight (unpublished data). It has been reported that at small doses, HgCl2 affects the cortico-medullary area of the kidney.

As the dose of HgCl2 is increased the injury spreads to involve proximal convoluted tubules. The renal damage caused by HgCl2 was reversed by administration of Smilax china before HgCl2. The administration smilax china before HgCl2 restored the altered indices to near normal levels.

Conclusion:

This is the First study to report Smilax china on Mercuric chloride intoxication. Hence to concluded, Smilax china protects on the renal tubules of the kidney when affect with low dose concentration of HgCl2 in both Prophylatic and post treatment group when compared to High dose concentration of HgCl2 affects group.

CONFLICT OF INTEREST: NIL

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