Nephroprotective Effect of Ethanolic Extract of Tabernaemontana Coronaria in Mercuric Chloride Induced Renal Damage in Wistar Albino Rats.

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***Abstract*—Plants and plant-derived products are part of the healthcare system since ancient human civilization. Herbal medicines have been used for many years. The present study was carried out to evaluate the effect of ethanolic extract of *Tabernaemontana coronaria* in renal oxidative damage induced by mercuric chloride (HgCl2) in rats. HgCl2 (3 mg/kg) was administered intraperitoneally to the rats and the extract was fed orally for 21days. Results indicated that the levels of protein content, enzymic & non – enzymic antioxidants and membrane bound ATPases were decreased in the Group II rats and reverted back to the normal level in the treatment groups. In contrast, the serum levels of cholesterol, urea, uric acid, creatinine and liver marker enzymes were increased in the Group II rats and reverted back to normal level in the treatment groups. Our findings showed that treatment with ethanolic extract of *Tabernaemontana coronaria* offers imperative protection from HgCl2-induced nephrotoxicity. The deterioration of antioxidant enzymes and histological damage caused by HgCl2 are markedly improved by treatment the treatment with the ethanolic extract of *Tabernaemontana coronaria*. These observations may be attributed partially to the antioxidant effect of ethanolic extract of *Tabernaemontana coronaria* and suggest that it may be a clinically valuable agent in the prevention of acute renal failure caused by inorganic mercury intoxication.**

***Index Terms*—Tabernaemontana, coronaria, nephrotoxicity, mercuric chloride.**

I. Introduction

Mercury is one of the most common heavy metals, used for more than 3000 years in medicines (as disinfectants, vaccines), industries (fluorescent lamps, batteries, thermostats, thermometers), gold mining and therapeutically as a cathartic, diuretic, anti-inflammatory and in dental amalgams [1]. The general populations are exposed to methyl mercury through the diet: the main source is fish consumption [2]. Organic mercury has a lesser insult on kidneys than inorganic mercury. It is a transition metal and promotes the formation of reactive oxygen species. Mercuric chloride ($HgCl_2$) is inorganic mercury compound with ionic mercury. Short term exposure to mercuric chloride may cause tubular necrosis, interstitial pneumonitis and gastro intestinal ulcerations. By contrast, long term exposure can cause membranous nephropathy and central neurotoxicity [3].

Manuscript received July 20, 2012; revised August 28, 2012.

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Aerobic organs employ a battery of defense mechanisms such as antioxidant enzymes (SOD, Catalase, and glutathione peroxidase) to mitigate or prevent oxidative tissue damage. The diet derived antioxidants like ascorbic acid, Vitamin E, carotenoids, polyphenols and α-lipoic acid also served as primary lines of defense in destroying free radicals [4]. The general drugs given for nephrotoxicity include diuretics (like suresomide and torsemide) and steroids (like prednizole and β-metasone). Since these medicines have certain serious side effects, there is an urgent need to systematically evaluate plants for their activities. Such research could also lead to new discovery or advance the use of indigenous herbal medicines for orthodox treatment. *Tabernaemontana coronaria* R.Br. (syn. *Ervatamia coronaria*) is a glabrous, evergreen, dichotomously branched shrub, belonging to the family Apocynaceae. This species has been extensively investigated and a number of chemical constituents such as alkaloids, triterpenoids, steroids, flavanoids, phenyl propanoids and phenolic acids were isolated from leaves, roots and stems of the plant [5]. In this communication, the present study was investigated to study the nephroprotective effect of ethanolic extract of *Tabernaemontana coronaria* in mercuric chloride induced renal damage in Wistar albino rats.

II. Materials and Methods

Adult male albino rats weighing about 150-200 g were used for the study.

Group I	Control animals.
Group II	Mercuric chloride induced rats.
Group III	Nephrotoxic rats treated with plant extract (200mg/kg bwt).
Group IV	Nephrotoxic rats treated with plant extract at (400mg/kg bwt).
Group V	Control animals treated with plant extract only (200mg/kg bwt).
Group VI	Control animals treated with plant extract only (400mg/kg bwt).

The animals were weighed and dosed through oral intragastric tube everyday. The test drug was fed orally for 21days. A single intraperitoneal injection of freshly prepared Mercuric chloride (3 mg/kg body weight) was

given on the 18th day. The experiment was terminated in overnight fasted rats at the end of 21days.The rats were sacrificed by cervical dislocation after giving mild anesthesia using Chloroform. Blood was collected and serum was separated which was used for various parameters (Urea, uric acid, creatinine, cholesterol and protein). Kidneys were immediately dissected out, washed and stored in 0.9% ice cold saline for various biochemical evaluations (Enzymic – SOD, Catalase, GST, GPx & non-enzymic antioxidants - Vit C & GSH), LPO, membrane bound ATPases and also in 10% formalin for histopathological studies.

TABLE I: THE CONCENTRATION OF UREA, URIC ACID, CREATININE, CHOLESTEROL AND PROTEIN IN SERUM OF CONTROL AND EXPERIMENTAL GROUPS

Particulars	Control (Group I)	$HgCl_2$ control (Group II)	$HgCl_2$+ plant extract treated (200mg/kg) (Group III)	$HgCl_2$+plant extract treated (400mg/kg) (Group IV)	Plant extract alone treated (200mg/kg) (Group V)	Plant extract alone treated (400mg/kg) (Group VI)
Urea (mg/dl)	25.13±0.040[a]	46.11±0.281[b]	38.90±0.353[c]	34.04±0.120[d]	25.08±0.076[a]	25.09±0.071[a]
Creatinine (mg/dl)	0.932±0.033[a]	2.58±0.049[b]	1.94±0.073[c]	1.13±0.111[d]	0.91±0.012[a]	0.93±0.0068[a]
Uric acid (mg/dl)	4.1±0.089[a]	5.9±0.268[b]	5.13±0.225[c]	4.61±0.201[d]	4.0±0.089[a]	4.03±0.136[a]
Cholesterol (mg/dl)	119.95±0.448[a]	225.27±0.462[b]	199.15±0.393[c]	174.24±0.469[d]	119.81±0.482[a]	119.40±0.377[a]
Protein(g/dl)	7.28±0.053[a]	5.08±0.026[b]	6.14±0.022[c]	6.86±0.027[d]	7.27±0.058[a]	7.26±0.058[a]

Values are expressed as mean ± S.D. Values are taken as a mean of six individual experiments.Values not sharing a common superscript letter differ significantly(DMRT)

TABLE II: THE CONCENTRATION OF SGOT, SGPT AND ALP IN SERUM OF CONTROL AND EXPERIMENTAL GROUPS

Particulars	Control (Group I)	$HgCl_2$ control (Group II)	$HgCl_2$+ plant extract treated (200mg/kg) (Group III)	$HgCl_2$+plant extract treated (400mg/kg) (Group IV)	Plant extract alone treated (200mg/kg) (Group V)	Plant extract alone treated (400mg/kg) (Group VI)
SGOT (IU/L)	72.56±0.498[a]	157.53±0.450[b]	105.2±0.325[c]	92.96±0.403[d]	72.17±0.306[a]	72.28±0.159[a]
SGPT (IU/L)	35.64±0.064[a]	55.15±0.159[b]	40.36±0.478[c]	38.32±0.317[d]	35.46±0.300[a]	35.64±0.078[a]
ALP(IU/L)	260.29±0.457[a]	374.06±0.494[b]	319.48±0.448[c]	305.11±0.359[d]	260.03±0.064[a]	260.10±0.098[a]

Values are expressed as mean ± S.D. Values are taken as a mean of six individual experiments. Values not sharing a common superscript letter differ significantly (DMRT)

TABLE II: THE ACTIVITIES OF ANTIOXIDANT ENZYMES OF GPX, SUPEROXIDE DISMUTASE, GST, CATALASE AND CONCENTRATION OF LIPID PEROXIDATION IN KIDNEY OF CONTROL AND EXPERIMENTAL GROUPS

Particulars	Control (Group I)	$HgCl_2$ control (Group II)	$HgCl_2$+ plant extract treated (200mg/kg) (Group III)	$HgCl_2$+ plant extract treated (400mg/kg) (Group IV)	Plant extract alone treated (200mg/kg) (Group V)	Plant extract alone treated (400mg/kg) (Group VI)
GPx(µg of GSH/mg of protein)	39.33±0.335[a]	20.18±0.187[b]	32.62±0.364[c]	34.51 ±0.447[d]	37.60 ±0.437[e]	38.78 ±0.420[a]
Superoxide dismutase (Units/g tissue)	1.69±0.314[a]	1.13 ±0.166[b]	1.281±0.253[a]	1.45±0.279[a]	1.588±0.272[a]	1.656±0.368[a]
Catalase (µ moles of H_2O_2 utilized /min/mg/protein)	23.19±0.161[a]	10.22 ±0.251[b]	17.52 ±0.273[c]	20.25±0.291[d]	20.55±0.454[d]	22.38±0.314[e]
GST(µmoles of CDNB Conjugate formed/mg of protein)	8.51±0.228[a]	4.31±0.389[b]	8.063±0.113[a]	8.35±0.174[a]	8.506±0.254[a]	8.51±0.362[a]
Lipid peroxidation (n moles/gm of tissue)	14.68±0.317[a]	29.49±0.403[b]	19.59±0.413[c]	16.25±0.410[d]	14.53±0.450[a]	14.42±0.323[a]

Values are expressed as mean ± S.D. Values are taken as a mean of six individual experiments. Values not sharing a common superscript letter differ significantly (DMRT)

TABLE IV: THE CONCENTRATION OF VITAMIN C AND REDUCED GLUTATHIONE IN KIDNEY OF CONTROL AND EXPERIMENTAL GROUPS

Particulars	Control (Group I)	$HgCl_2$ control (Group II)	$HgCl_2$+ plant extract treated (200mg/kg) (Group III)	$HgCl_2$+ plant extract treated (400mg/kg) (Group IV)	Plant extract alone treated (200mg/kg) (Group V)	Plant extract alone treated (400mg/kg) (Group VI)
Vitamin C (mg/g of protein)	1.64±0.273[a]	0.72±0.022[b]	1.30±0.032[a]	1.44±0.121[a]	1.63±0.273[a]	1.65±0.269[a]
Glutathione (µg/mg protein)	48.23±0.393[a]	22.40±0.327[b]	40.30±0.345[c]	44.38±0.335[d]	48.02±0.153[a]	48.15±0.072[a]

Values are expressed as mean ± S.D. Values are taken as a mean of six individual experiments. Values not sharing a common superscript letter differ significantly (DMRT)

TABLE V: MEMBRANE BOUND ATPASE ASSAYS DONE IN KIDNEY OF CONTROL AND EXPERIMENTAL GROUPS

Particulars	Control (Group I)	$HgCl_2$ control (Group II)	$HgCl_2$+ plant extract treated (200mg/kg) (Group III)	$HgCl_2$+plant extract treated (400mg/kg) (Group IV)	Plant extract alone treated (200mg/kg) (Group V)	Plant extract alone treated (400mg/kg) (Group VI)
Na^+-K^+ATPase (µmoles of phosphorous liberated/hr/mg of protein)	3.91±.018[a]	2.94±0.075[b]	3.11±0.017[bc]	3.24±0.250[c]	3.56±0.037[d]	3.9±0.023[a]
Ca^{2+}ATPase (µmoles of phosphorous liberated/hr/mg of protein)	2.31±0.017[a]	1.28±0.027[b]	2.09±0.062[c]	2.12±0.017[ac]	2.21±0.139[ac]	2.30±0.174[a]
Mg^{2+}ATPase (µmoles of phosphorous liberated/hr/mg of protein)	1.49±0.179[a]	0.93±0.035[b]	1.33±0.268[a]	1.36±0.063[a]	1.40±0.049[a]	1.47±0.116[a]

Values are expressed as mean ± S.D. Values are taken as a mean of six individual experiments. Values not sharing a common superscript letter differ significantly (DMRT)

TABLE VI: LEVEL OF PROTEIN (IN MG/G OF TISSUE) IN DIFFERENT ORGANS OF RAT AT THE END OF 21 DAYS STUDY

Particulars	Control (Group I)	$HgCl_2$ control (Group II)	$HgCl_2$+ plant extract treated (200mg/kg) (Group III)	$HgCl_2$+plant extract treated (400mg/kg) (Group IV)	Plant extract alone treated (200mg/kg) (Group V)	Plant extract alone treated (400mg/kg) (Group VI)
Kidney	71.35±0.027[a]	42.34±0.10[b]	57.43±0.037[c]	65.72±0.052[d]	71.36±0.226[a]	71.37±0.013[a]

Values are expressed as mean ± S.D. Values are taken as a mean of six individual experiments. Values not sharing a common superscript letter differ significantly (DMRT)

III. RESULTS AND DISCUSSION

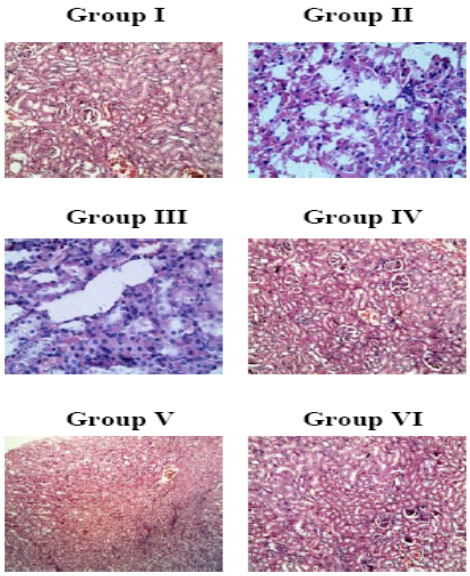
A. Variation in Urea, Creatinine and Uric Acid Levels in Rat Serum

As shown in the Table I, urea, creatinine and uric acid levels were elevated in *$HgCl_2$* induced renal toxicity when compared to group Ị normal rats. Oral administration of ethanolic extract of *Tabernaemontana* coronaria significantly reduced urea, creatinine and uric acid levels in rats affected by nephropathy. There was no significant change in the above parameters in the rats treated with plant extract alone and is also similar to control group. However maximum protection (activity) was offered by the 400mg/kg of *T. coronaria* pretreatment. The results of the present study are in corroboration with earlier reports [6].

B. Changes in Cholesterol and Protein

The change in the level of cholesterol and protein in both the control and experimental animals was shown in Table I. The levels of serum cholesterol and serum protein were increased and decreased respectively in *$HgCl_2$* induced nephrotoxicity as compared with normal rats. Treatment with ethanol extract of *Tabernaemontana coronaria* at the doses of 200mg/kg and 400mg/kg reduced and increased the levels of cholesterol and protein respectively, which is comparable to normal rats. This result is in consistent with earlier reports by Hetta and Yassin [7]. where different

fractions of Hyphaene thebaica fruit were used.



Group I: Kidney of control animal showing normal histology.

Group II: Kidney of Mercuric chloride intoxicated rats showing focal renal tubular atrophy.

Group III: Kidney of nephrotoxic animal treated with ethanolic extract of *T. coronaria* (200mg/kg) showing normal histology.

Group IV: Kidney of nephrotoxic animal treated with ethanolic extract of *T. coronaria* (400mg/kg) showing normal histology.

Group V: Kidney of normal animal treated with plant extract *T. coronaria* (200mg/kg) showing normal histological structure.

Group VI: Kidney of normal animal treated with plant extract *T. coronaria* (400mg/kg) showing normal histological structure.

Fig. 1. Histopathology of kidney

C. Level of SGOT, SGPT and ALP

The enzymes SGOT, SGPT and ALP were increased significantly in $HgCl_2$ induced rats as shown in Table II. The *Tabernaemontana coronaria* treatment significantly reversed the levels of above enzymes when compared to $HgCl_2$ induced renal toxicity. The *Tabernaemontana coronaria* treatment alone did not have significant effect on the levels of the above mentioned enzymes. These results are supported by the report of Kamis *et al* where ethanolic pulp extract was used [8].

D. Concentration of Lipid Peroxidation

The effect of the ethanolic extract of *Tabernaemontana coronaria* in lipid peroxidation is given in Table III. The elevated levels of lipid peroxidation in $HgCl_2$ induced nephrotoxicants were reduced significantly to near normal levels upon treatment with *Tabernaemontana coronaria* extract. The plant extract alone treated rats did not show any significant change. Similar result was observed by Sivaprasad *et al* [9].

E. Activities of Antioxidant Enzymes Glutathione pEroxidase, SOD, GST and Catalase.

Administration of $HgCl_2$ alone in group II animals caused a significant decrease in the levels of antioxidant enzymes *GPx*, SOD, GST and catalase when compared with group 1 animals (Table III). Group V and group VI animals which received Tabernaemontana coronaria alone (200mg & 400mg/kg respectively) did not show any significant variation in the above parameters when compared with group I control animals. Rats given concomitant doses of $HgCl_2$ and *Tabernaemontana coronaria* showed a marked rise in the levels of above mentioned enzymes compared to group II animals. Our results were in agreement with the findings of Samipillai and Jagadeesan [10].

F. Activities of Non Enzymic Antioxidants—Vitamin C and Glutathione

Table IV represents the level of Vitamin C and Glutathione in control and experimental rats. It was observed that the level of vitamin C and glutathione were reduced in $HgCl_2$ induced nephrotoxic rats (group II) when compared with control animals (group I). By the administration of *Tabernaemontana coronaria* the elevated levels of Vitamin C and glutathione were restored to near normal values in group III and group IV animals. No significant changes were observed in *Tabernaemontana coronaria* alone treated animals in group V and group VI when compared with control animals. These results are supported by the results given by Aqil *et.al* [11].

G. Alterations in Na+ K+, Ca2+ and Mg2+ ATPases

The activities of Na^+K^+ ATPase, Ca^{2+}ATPase and Mg^{2+} ATPase were significantly diminished in $HgCl_2$ induced rats when compared with control rats (Table V). The activities of these enzymes were reverted to near normal values in Tabernaemontana *coronaria* administered rats. No significant change was observed in *Tabernaemontana coronaria* treated drug control rats as compared to normal control groups. Our results were supported by Soundararajan *et al* [12]. and Srinivasan *et al* [13].

IV. Conclusion

In conclusion, the findings reported in the study indicate that the oral administration of *Tabernaemontana coronaria* to mercuric chloride intoxicated rats exhibited significant nephroprotective effect. Although promising results have been obtained, more concerted efforts are still needed for the isolation, characterization and biological evaluation for the active principles of the extract.

Acknowledgement

We, the authors are thankful to our Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

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