ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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# Reno-Protective and Membrane stabilizing the effect of *Premna tomentosa* in Carbon tetrachloride induced toxicity in rats

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*Article History:*

Received on: 13.01.2018
Revised on: 23.06.2018
Accepted on: 26.06.2018

*Keywords:*

$CCl_4$,
Membrane stabilizing effect,
*Premna tomentosa*,
Reno-protection

ABSTRACT

The ethanolic extract of *Premna tomentosa* was evaluated for its reno-protective and membrane stabilizing effect against $CCl_4$ induced toxicity in Rats. Animals were divided into four groups. The first group was considered normal. The second group received CCl4.Third and fourth groups were received $CCl_4$ and different doses of extract. The treatment was continued for 21 days. On the 22nd day, animals were sacrificed and analyzed various biochemical parameters include $Na^+$ $K^+$ ATP ase, $Ca^{2+}$ ATP ase, $Mg^{2+}$ ATP ase, aspartate transaminase, alanine transaminase, alkaline phosphatase, acid phosphatase, catalase, glutathione –s-transferase, reduced glutathione and malondialdehyde. Alkaline phosphatase, acid phosphatase, aspartate transaminase and alanine transaminase were decreased significantly while antioxidants like catalase, glutathione-s-transferase and reduced glutathione remained the other way. Membrane stabilizing enzymes like $Na^+$, $K^+$ ATPase, $Ca^{2+}$ ATPase, $Mg^{2+}$ ATPase were increased significantly in treated animals. These observations suggest that ethanolic extract of *Premna tomentosa* at a dose of 250 mg/kg, b.wt as an effective dose for treating $CCl_4$ intoxicated rats.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1620

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## INTRODUCTION

The kidney performs excretory homeostatic and endocrine functions. Kidney, skin, lung, gastrointestinal tract, salivary glands and liver are the major organs through which excretion takes place. Among the channels of excretion, kidneys are considered to be the chief. The kidney serves as the primary vehicle for excreting nitrogenous waste and other unnecessary substances from the body. Every day the kidneys filter several litres of fluid from the bloodstream, allowing toxins, metabolic wastes, and excess ions to leave the body through urine while returning only essentially needful substances to the blood.

Failure of a kidney to excrete waste products leads to accumulation of these products particularly nitrogenous substances. When kidney function is impaired, loss of control of homeostasis mechanism occurs. It is causing ill health. This is called renal failure. There are two types of renal failure viz, acute and chronic.

An estimated 3.83 percent of adults aged 20 or more (7.7 million adults) have physiological evidence of chronic kidney disease having a moderately or severely reduced glomerular filtration rate (Coresh *et al*., 2005)

Some chemicals including various environmental toxicants and clinically useful drugs cause severe cellular changes in different organs of our body when exposed to a condition of more than optimum. Chronic exposure of $CCl_4$ at lower dose cause damage to the liver and necrosis of the renal tubular epithelium (Gosselin *et al*., 1984).

$CCl_4$ is converted to trichloromethyl Gusselinradical by cytochrome $P_{450}$ (Noguchi *et al*., 1982). These free radicals initiate the peroxidation of membrane leading to the generation of polyunsaturated fatty acids, which in turn, covalently binds to microsomal lipids and proteins (Tom *et al*., 1984).

This phenomenon results in the generation of reactive oxygen species like superoxide anion $^{\cdot}O_2$, $H_2O_2$ and the hydroxyl radical. Evidence suggests that the cell to scope has developed various enzymatic and non-enzymatic systems with the reactive oxygen species and other free radicals. However, when a condition of oxidative stress establishes, the defence capacities against reactive oxygen species becomes insufficient (Halliwell *et al*., 2000)

Thus, the administration of $CCl_4$ results in oxidative damage in the kidney (Ozturk *et al*., 2003). The damages in kidney include glomerular necrosis and alteration in proximal and distal tubules progressing ultimately to detachment of the epithelial cells and tubular necrosis (Doik *et al*., 1991)

Majority of the world population in developing countries rely on herbal medicine. Currently, 80 % of the world population depends on the plant-derived medicine for the first line of primary health care because of its lack of side effects (Farnsworth and Bingal, 1977) *Premna tomentosa*, a moderate sized, deciduous tree, distributed in Madhya Pradesh and the rest of the country. Bark, root, leaves and wood of this tree are commonly used as medicine.

Extract of *Premna tomentosa* is immunomodulatory and cytoprotective (Sairam *et al*., 2003) and hypolipidemic (Devi *et al*., 2004). It increases the total ATP ase, $Ca^{2+}$ ATP ase and $Mg^{2+}$ ATP ase in acetaminophen intoxicated rats (Pandima Devi *et al*., 2004). Effect of *Premna tomentosa* in $CCl_4$ induced toxicity has not yet been studied. Renoprotective and membrane stabilizing the effect of *Premna tomentosa* in $CCl_4$ induced toxicity have been evaluated here.

## MATERIALS AND METHODS

### Preparation of *Premna tomentosa* extract

The roots of *Premna tomentosa* were collected from different areas of Tamilnadu. They were authenticated at Rabinot herbarium, Trichy and Botanical Survey of India, Coimbatore, and Tamilnadu. They were shade dried and coarsely powdered. The extract of the same was made with ethanol using soxhlet apparatus (Mohanasundaram *et al*., 2016). The extract was concentrated *invaccuo*. The brown coloured semisolid extract was used for the following study.

### Experimental animals

Healthy Wistar albino rats weighing 180 – 200 g were obtained from the SASTRA animal house, Tanjore, Tamilnadu. They were maintained in the controlled temperature (23 ±. 3° C) and humidity 60-65 % with 12 hr dark and light cycle at CARISM Animal house, SASTRA Tanjore. The animals were fed with commercial diet (Tetragon Chemie Pvt. Ltd., Doddaballapur, Bangalore) the Institutional Animal Ethical Committee permitted the study with Reg No. 817/04/ac/cpcsea

### Treatment of animals

Animals were randomly divided into 4 groups with 8 animals in each. Group 1 Normal distilled water, 0.3 ml, p.o. Group 2 (Control) received 30% $CCl_4$ in liquid paraffin (1 ml/kg body weight, i.p) Group 3 and 4 received 30 % $CCl_4$ in liquid paraffin (1 ml/kg body weight, i.p) and *Premna tomentosa extract* at the dose of 150 and 250 mg/kg, p.o, respectively. Treatment duration was 21 days and the dose of $CCl_4$ was administered after every 72- hr. (Manoj, B and K. Aqueed, 2003) The overnight fasted animals were sacrificed 24 h after the last injection of $CCl_4$ and blood was collected in tubes containing 10 % EDTA as an anticoagulant. The organs were excised and they were washed in ice-cold saline and then, homogenized. 10 % kidney homogenate was prepared in 0.1 M Tris HCl buffer pH 7.4.

In homogenate and plasma, various biochemical parameters like Aspartate transaminase, alanine transaminase (Reitman and Frankel, 1957), alkaline phosphatase acid phosphatase (King and Armstrong, 1934) were analyzed. Erythrocyte was isolated by Quist (1980) method and various membrane stabilizing enzymes like $Na^+ K^+$ ATPase (Bonting *et al*., 1970), $Ca^{2+}$ ATP ase, $Mg^{2+}$ ATP ase (Hjerten and Pan, 1983) were analyzed, Enzymatic antioxidants like catalase (Aebi *et al*., 1983), in organs and glutathione-s-transferase (Habig *et al*., 1974) in plasma and organs were estimated. A nonenzymatic antioxidant like reduced glutathione (Beutlar, 1967) in plasma and various other organs was estimated. Lipid peroxidation was evaluated by estimating thiobarbituric acid reactive substances (Ohkawa *et al*., 1979) in plasma and various other organs.

### Statistics

Values are Mean ± SE of 6 animals and the significant difference was calculated using One Way ANOVA using SPSS software version 11.0.

## RESULTS AND DISCUSSION

Our research was focused on the membrane stabilizing the effect of *Premna tomentosa*, which was

**Table 1: Effect of *Premna tomentosa* extract in plasma marker enzymes of $CCl_4$ intoxicated rats**

| Treatment | (IU/L) | GPT (IU/L) | ALP (IU/L) | ACP (IU/L) | Total protein (g/dl) |
|---|---|---|---|---|---|
| Normal | 21.8 ± 2.04 | 9.4 ± 0.1 | 396.0 ± 10.8 | 99.0 ± 2.9 | 4.6 ± 0.1 |
| Control ($CCl_4$) | 58.8 ± 0.85 * | 17.7 ± 2.17 * | 1094.0 ± 11.7 * | 512.0 ± 9.0 * | 2.8 ± 0.3 * |
| PT 150mg/ kg | 39.5 ± 3.8 | 13.4 ± 1.1 | 717.3 ± 12.2 | 277.4 ± 1.06 | 4.2 ± 0.6 |
| PT 250mg/ kg | 23.8 ± 2.6 * | 10.3 ± 1.3 * | 425.0 ± 4.95 * | 121.0 ± 5.8 * | 5.6 ± 0.6 * |

Note - Values are mean ± SE. * - Values are differing significantly at p<0.05

**Table 2: Effect of *Premna tomentosa* extract in plasma antioxidant levels of $CCl_4$ intoxicated rats**

| Treatment | TBARS (nanomoles of MDA /mg of protein) | GSH (µg of GSH/mg of protein) | GST (nanomoles of CDNB-GSH conjugate formed/min/mg of protein) |
|---|---|---|---|
| Normal | 0.22 ± 0.01 | 2.13 ± 0.02 | 2.15 ± 0.02 |
| Control ($CCl_4$) | 1.3 ± 0.02 * | 1.38 ± 0.01 * | 0.07 v 0.001 * |
| PT 150mg/ kg | 0.39 ± 0.08 * | 1.79 ± 0.02 | 1.98 ± 0.01 * |
| PT 250mg/ kg | 0.21 ± 0.02 * | 2.18 ± 0.02 * | 2.00 ± 0.01 * |

Note - Values are mean ± SE. * - Values are differ significantly at p<0.05

**Table 3: Effect of *Premna tomentosa* extract in marker enzymes levels of $CCl_4$ intoxicated rats**

| Treatment | GOT (IU/L) | GPT (IU/L) | ALP (IU/L) | ACP (IU/L) | Total protein (g/dl) |
|---|---|---|---|---|---|
| Normal | 21.8 ± 2.0 | 19.3 ± 0.1 | 196.0 ± 11.9 | 2476.0 ± 74.9 | 448.3 ± 17.9 |
| Control ($CCl_4$) | 54.8 ± 2.1 * | 47.6 ± 1.6 | 2035.0 ± 18.3 * | 7232.0 ± 33.0 | 302.2 ± 14.4 * |
| PT 150mg/ kg | 40.3 ± 0.9 | 30.3 ± 2.6 | 1048.2 ± 12.5 | 5436.0 ± 48.6 | 453.2 ± 36.8 |
| PT 250mg/ kg | 25.4 ± 1.0 * | 20.1 ± 0.5 | 546.0 ± 18.2 * | 2989.5 ± 30.7 | 612.2 ± 25.7 * |

Note - Values are mean ± SE. * - Values are differ significantly at p<0.05

**Table 4: Effect of *Premna tomentosa* extract in kidney antioxidant levels of $CCl_4$ intoxicated rats**

| Treatment | TBARS (nanomoles of MDA /mg of protein) | GSH (µg of GSH/mg of protein) | GST (nanomoles of CDNB-GSH conjugate formed/min/mg of protein) | Catalase (µmoles of $H_2O_2$ used /min/mg of protein) |
|---|---|---|---|---|
| Normal | 0.37 ± 0.02 | 4.43 ± 0.19 | 6.21 ± 0.02 | 196.0 ± 12.5 |
| Control ($CCl_4$) | 1.5 ± 0.1 * | 2.07 ± 0.09 * | 3.65 ± 0.19 * | 45.6 ± 2.5 * |
| PT 150mg/ kg | 0.9 ± 0.03 | 4.18 ± 0.17 * | 5.67 ± 0.42 | 80.2 ± 4..39 |
| PT 250mg/ kg | 0.4 ± 0.02 * | 5.15 ± 0.29 * | 6.33 ± 0.4 * | 142.3 ± 14.4 * |

Note - Values are mean ± SE. * - Values are differ significantly at p<0.05

**Table 5: Effect of *Premna tomentosa* extract in renal ATP ase enzyme levels of $CCl_4$ intoxicated rats**

| Treatment | $Na^+K^+$ATP ase (nanomoles of P liberated/min/mg of protein) | $Mg^{2+}$ ATP ase (nanomoles of P liberated/min/mg of protein) | $Ca^{2+}$ ATP ase (nanomoles of P liberated/min/mg of protein) |
|---|---|---|---|
| Normal | 10.6 ± 0.2 | 10.6 ± 0.04 | 7.2 ± 0.1 |
| Control ($CCl_4$) | 5.6 ± 0.4* | 6.9 ± 0.4* | 3.5 ± 002* |
| PT 150mg/ kg | 9.0 ± 0.2* | 8.5 ± 0.1 | 5.3 ± 0.07 |
| PT 250mg/ kg | 10.2 ± 0.2* | 10.4 ± 0.5* | 7.0 ± 0.4* |

Note - Values are mean ± SE. * - Values are differ significantly at p<0.05

evaluated by assessing the biochemical parameters such as aspartate transaminase, alanine transaminase, alkaline phosphatease, acid phosphatase, $Na^+K^+$ATPase, $Ca^{2+}$ ATP ase, $Mg^{2+}$ ATP ase, catalase, glutathione-s-transferase, reduced glutathione and thiobarbituric acid reactive substances in experimentally $CCl_4$ intoxicated rats.

Basu *et al*., (2003) have explained that increased free radical production and lipid peroxidation have been proposed as a major cellular mechanism involved in $CCl_4$ in toxicity. Slater *et al*., (1971) have shown that free radical or reactive oxygen species

such as hydroxyl radical, peroxy radical and hydrogen peroxide are produced during lipid peroxidation.

The level of thiobarbituric acid reactive substances was significantly ($p<0.05$, Table 2, 4) elevated in $CCl_4$ intoxicated group. On treating $CCl_4$ intoxicated animals with different doses of extract, Malondialdehyde level was decreased significantly ($p<0.05$, Table 2,4). This may be due to the anti-lipid peroxidation activity of the extract. Antilipid peroxidative Effect of *Premna tomentosa* at a dose of 250 mg/kg b.wt was also observed in kidney tissue The effect on the kidney is mentioned in Table 2,4.

Catalase is one of the important enzymes in the supportive team of defence against reactive oxygen species, which is present in most cells and catalyses the decomposition of $H_2O_2$ to $H_2O$ and $O_2$. (Tolbert *et al*., (1981). Catalase plays a vital role in scavenging of radicals. The inhibition of catalase activity may be due to the enhanced production of $O_2$ and peroxyl radical during the chronic administration of $CCl_4$ (Yan 1992).

Our present study revealed that the activity of catalase in renal tissue was significantly ($p<0.05$, Table 2) lowered in $CCl_4$ intoxicated rats. Decreased activity of catalase in $CCl_4$ treated rats may increase their susceptibility to oxidative injury.

Administration of *Premna tomentosa* root extract increases the activity of catalase ($p<0.05$, Table 2). This may be due to the increased synthesis of catalase by the extract. This increased catalase may scavenge the produced peroxy radical.

Reduced glutathione is a tripeptide. It contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione, an antioxidant, protects cells from toxins such as free radicals (Strużńka *et al.,* 2005).

Glutathione constitutes the first line of defence against free radical. It performs many functions. Reduced glutathione can react with singlet oxygen, superoxide and hydroxyl radical and therefore function directly as a free radical scavenger. Reduced glutathione may stabilize membrane structure by removing acyl peroxide formed by lipid peroxidation reaction (Prince *et al*., 1990).

In $CCl_4$ intoxicated rats glutathione level was decreased significantly ($p<0.05$, Table 2, 4) in both plasma and renal tissue. This may be due to the utilization of reduced glutathione to scavenge the produced free radicals. On treating animals with *Premna tomentosa,* reduced glutathione level was increased significantly ($p<0.05$, Table 2, 4) against disease control animals. These results also reveal that *Premna tomentosa* extract can increase the synthesis of reduced glutathione.

Glutathione-s-transferase acts like peroxidase and removes the stable peroxide from the system, resulting in the reduction of peroxide-induced damage (Jagetia *et al*., 2004). Significantly decreased ($p<0.05$, Table 2, 4) activity of GST in both plasma and renal tissue was seen in $CCl_4$ intoxicated arts. However, the reconstitution of the level of GST activity was seen in rats treated with *Premna tomentosa* extract. These results also revealed the antioxidant activity of *Premna tomentosa.*

Necrosis in the kidney by $CCl_4$ usually associated with elevated level of plasma enzymes, the indicator of cellular leakage and loss of functional integrity of the cell membrane in the kidney (Drotman *et al*., 1978). Aspartate transaminase, alanine transaminase and alkaline phosphatase are ubiquitously distributed in the body tissue including the heart, liver, kidney and muscle.

From the present study it was observed that aspartate and alanine transaminase and alkaline phosphatase activity was increased significantly ($p<0.05$, Table 1) in plasma and decreased significantly in renal tissue ($p<0.05$, Table 1, 3) in $CCl_4$ intoxicated rats as reported by González *et al*., 2006). This may be due to the membrane damage caused by trichloro peroxy radical, a free radical formed from $CCl_4$.

On treating animals with different doses of extract, release of enzymes decreased dose-dependently and significant difference in both plasma and tissue ($p<0.05$, Table 1, 3) was observed at a dose of 250 mg/kg b.wt such protective effect of *Premna tomentosa* confirms it's antioxidant activity and thereby, protection of membrane.

Acid phosphatase is frequently employed as a marker enzyme to assess the lysosomal changes invivo because it is a localized almost exclusively in the particulars and its release parallels that of lysosomal hydrolysis (Tanaka *et al*., 1968).

A significant increase ($p<0.05$, Table 1) in plasma and decrease ($p<0.05$, Table 1) in kidney acid phosphatase was observed in $CCl_4$ intoxicated rats. This may be due to the damage of the lysosomal membrane caused by $CCl_4$. However, on treating with *Premna tomentosa* extract at a dose of 250 mg/kg b.wt, a significant decrease ($p<0.05$, Table 1, 3) in plasma and increase ($p<0.05$, Table 1) of acid phosphatase in kidney tissue was observed.

ATP ases are lipid-dependent as well as thiol-dependent membrane-bound enzymes. Enhanced susceptibility to lipid peroxidation of the membrane can lead to thiol formation. Thereby, there is

a change in membrane function. The ionic concentration of hemolymph in crustaceans is maintained by active absorption of sodium chloride from the surrounding medium

Enzymes like $Na^+ K^+$ ATP ase, $Mg^{2+}$ ATP ase and $Ca^{2+}$ ATP ase are responsible for the ionic regulation of hemolymph (Proverbio *et al*., 1990). Membrane-bound $Na^+ K^+$ ATPase is an important enzyme utilizing the energy of ATP hydrolysis for transport of several cations. The inhibition of this enzyme produces an increase in intracellular calcium and a decrease in intracellular magnesium (Kramer *et al*., 1991).

The plasma membrane $Na^+ K^+$ ATPase is concerned with the maintenance of low concentration of $Na^+$ and consequently of cellular water content. Decreased activity of $Na^+ K^+$ ATPase can lead to a decrease in sodium efflux thereby alter the membrane permeability (Kako *et al*., 1988)

$Ca^{2+}$ATP ase regulates the calcium pump activity. Decrease $Ca^{2+}$ ATP ase activity has been reported during oxidative stress due to hydroperoxides (Alkon *et al*., 1988). $Mg^{2+}$ ATP ase is the major ATP ase for the plasma membrane for the amino phospholipids translocase activity of the plasma membrane (Vajreswari *et al*., 1992).

## CONCLUSION

The root extract of *Premna tomentosa* was found to exhibit antioxidant activity at a dose of 250 mg/kg b.wt. Moreover, it prevents the renal damage caused by $CCl_4$. Membrane stabilizing the effect of *Premna tomentosa* at a dose of 250 mg/kg b.wt was also evaluated. The mechanism of action of *Premna tomentosa* in membrane stabilizing effect and the phytoconstituents responsible for this protection should be evaluated.

## ACKNOWLEDGEMENT

The authors are grateful to Prof. R. Sethuraman, Vice-Chancellor, SASTRA and his able administrative colleagues for allowing us to complete this work. The CARISM, SASTRA are also gratefully acknowledged.

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