

## Nephroprotective Effect of *Jatropha curcas* Fruit Extracts Against Carbon Tetrachloride Induced Nephrotoxicity in Rats

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### ABSTRACT

The present study was undertaken to explore the nephroprotective potential of *Jatropha curcas* fruit extracts against carbon tetrachloride (CCl<sub>4</sub>) induced nephrotoxicity in rats. Nephrotoxicity was induced by CCl<sub>4</sub> (3ml/kg body weight) in animals. Blood biochemical parameters, urine analysis and histopathological studies were carried out to assess the nephroprotective effect. CCl<sub>4</sub> administration induced significant nephrotoxicity in rats, which was evident from enhanced levels of albumin, potassium. From the obtained results it may be concluded that pretreatment of silymarin (50mg/kg dose orally) significantly reversed carbon tetrachloride induced nephrotoxicity where as *Jatropha curcas* methanol extract (250mg/kg body weight) showed significant effect against CCl<sub>4</sub> induced nephrotoxicity in rats than *Jatropha curcas* aqueous extract (p<0.001) for most of the blood biochemical parameters, hematological parameters as well in attenuation of pathological changes in kidney tissues.

**Keywords:** Nephrotoxicity, carbon tetrachloride, silymarin, flavonoids, *Jatropha curcas*, *Curcaspurgans*.

### INTRODUCTION

Kidney failure is nowadays increasing at an alarming rate. Acute renal failure (ARF), is characterized by sudden loss of the ability of the kidneys to excrete wastes, concentrate urine, conserve electrolytes, and maintains fluid balance. The mortality rate of patients with ARF has remained 25–70% despite the use of various pharmacologic agents. Nephrotoxicity is mostly related to oxidative stress and nowadays much attention has been made towards the possible nephroprotective properties of medicinal plants<sup>1</sup>. Carbon tetrachloride (CCl<sub>4</sub>) is a toxic chemical, widely used in the dry cleaning industry, in filling fire extinguishers, in the fumigation of grains, and as an insecticide. Recent studies have shown that CCl<sub>4</sub> is associated with advanced production of free radicals leading to dysfunction of several organs. Chronic CCl<sub>4</sub> treatment is a common practice to induce hepatic fibrosis, renal, pulmonary and testicular injuries, and cardiac tissue damage in rats as an experimental model. Tissue damage by CCl<sub>4</sub> depends on the amount of dosage and duration of exposure of the experimental animals to this toxicant. Its action is based on membrane lipid peroxidation and induction of trichloromethyl radical (.CCl<sub>3</sub>), resulting in severe cell damage. It is evidenced that metabolic activation of CCl<sub>4</sub> by cytochrome P450 resulted in the production of trichloromethyl radical (.CCl<sub>3</sub>) and peroxytrichloromethyl radical (.OOCCl<sub>3</sub>) that, in turn, initiate subsequent lipid peroxidation, responsible for injuries in various organs such as liver and kidney. Therefore, it can be stated that CCl<sub>4</sub> is the best-characterized tool for the study of oxidative stress trials as it consistently generates free radicals with the implication

of pathological environment. These free radicals damage the integrity of liver cell membranes by releasing the cytosolic enzymes such as alanine transaminase, aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase into the blood stream and elevating thiobarbituric acid reactive substances (TBARS) level with subsequent necrosis and inflammatory cell infiltration; affect physical parameters of kidney such as urinary and serum profile; increase lysosomal enzymes activities of testis and kidney<sup>2,3,4</sup>.

Despite advances in western system of medicine and medical technology world over, its increasingly being realized that if we have to support the healthcare requirements of our ever increasing population, we will have resort to economical, yet effective alternatives and there cannot be a better alternative than the herbal drugs which have had a long history of safe usage in different parts of the world, including India.

The attention of the world is now being drawn more and more to herbs and herbal medicines as the synthetic drugs seem to have come up against a wall in the treatment of illness which is described as life style diseases<sup>5</sup>.

Silymarin is obtained from the *Silybum marianum* (milk thistle) an edible plant that has been used medicinally for the centuries as a herbal medicine. It is a mixture of mainly three flavonolignans, silybin, silidianin, and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, because of its antioxidant activity and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration<sup>6,7</sup>.

*Jatrophacurcas* Linn. is a bush or small tree and belongs to Euphorbiaceae family. It is widely distributed in Mexico and Central America. The other name of the plant is *Curcaspurgans*. Pharmacological reports revealed that it is having anti microbial<sup>8,9,10</sup>, anti-inflammatory<sup>11</sup>, antimetastatic<sup>12</sup>, antitumor<sup>12</sup>, coagulant and anti-coagulant (dose dependent)<sup>13</sup>, disinfectant<sup>14</sup>, antiparasitic<sup>14</sup>.

## MATERIALS AND METHODS

### Animals

Wistar rats of either sex, weighing around 200- 250 g. were employed in the present study. They were obtained from in house breed animals of Chalapathi Institute of Pharmaceutical Sciences, Guntur. The rats were provided standard laboratory feed and water ad libitum. They were exposed to an alternate light and dark cycle of 12 h and had free access to food and water. The animals were acclimatized to the laboratory conditions for at least 5 days before the nephrotoxicity test. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh (Approval No: 09/IAEC/CIPS/2016-17; dt 05/04/2016) and care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests, Environment and Climate Change, Government of India.

### Plant material

The plant material consists of dried powdered fruit of *Jatrophacurcas* Linn. belonging to the family Euphorbiaceae. Fresh fruits of *Jatrophacurcas* Linn. was collected from medicinal plant garden of Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, India. The plant material was authenticated by a botanist and the specimen sample is deposited in the Pharmacognosy Division, Chalapathi Institute of Pharmaceutical Sciences, Guntur.

### Preparation of plant extract

The shade dried and coarsely powdered fruits were extracted with solvents like methanol and water by hot percolation extraction (soxhlation method) then the extracted solvents was filtered and filtrate was concentrated using a rotary evaporator. The concentrated plant extracts was used for the pharmacological activities.

### Drugs and reagents

Silymarin was purchased from Sigma Aldrich, Bangalore, India. Carbon tetrachloride and formalin was obtained from Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences. CCl<sub>4</sub> was administered intraperitoneally (i.p.) in rats.

### Experimental groups

The efficacy of *Jatrophacurcas* aqueous extract (JCAE) was compared *Jatrophacurcas* methanol extract (JCAE) by evaluating *in vivo* nephroprotective activity in rats against CCl<sub>4</sub> induced nephrotoxicity.

Four groups, each comprising of five wistar rats, were employed in the study.

Group I (Control group): Rats were administered 0.9% w/v normal saline, orally for 14 days.

Group II (CCl<sub>4</sub> - treated control group): Rats were administered CCl<sub>4</sub> (3 ml/kg, i.p.) on the day 14.

Group III (Silymarin + CCl<sub>4</sub>- treated group): Rats were treated with silymarin (50 mg/kg, orally) for 14 days. On the 14<sup>th</sup> day silymarin was administered 60 min prior the administration of CCl<sub>4</sub>.

Group IV (JCAE + CCl<sub>4</sub> -treated group): Rats were treated with JCAE (250mg/kg body weight) for 14 days. On 14<sup>th</sup> day JCAE was administered 60 min before the administration of CCl<sub>4</sub>.

Group V (JCME + CCl<sub>4</sub> -treated group): Rats were treated with JCME (250mg/kg body weight) for 14 days. On 14<sup>th</sup> day JCME was administered 60 min before the administration of CCl<sub>4</sub>.

### Parameters evaluated

Blood biochemical parameters: creatinine (mg/dl), total calcium (mg/dl), blood urea (mg/dl), albumin (g/dl), sodium (mmol/l), potassium (mmol/l), total protein (g/dl) Urine analysis: color, appearance, pH albumin, sugar, microscopic examination of urinary sedimentation.

Histopathological studies for kidney tissues.

### Statistical analysis

The results are expressed as mean ± standard error of means (S.E.M.). The data of nephrotoxicity results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test using graph pad prism version 6.0. A p-value <0.05 was considered to be statistically significant.

## RESULTS

Various pharmacological interventions employed in the present study did not show any significant mortality. Further, no significant difference was observed between the results obtained from rats of either sex.

### Effect on blood biochemical and urinalysis parameters

CCl<sub>4</sub> (3ml/kg i.p.) significantly decreased the levels of blood biochemical parameters except albumin and potassium when compared to control group.

Silymarin (50 mg/kg, orally) pre-treated animals showed significant increased levels of blood biochemical parameters except creatinine, albumin, total protein which is evident from Table 1.

JCAE (250mg/kg body weight) pre-treated animals showed significant decreased levels of blood biochemical parameters except blood urea, sodium.

JCME (250mg/kg body weight) pre-treated animals showed significant increased levels of blood biochemical parameters except blood urea, sodium, total protein which is evident from Table 1.

The urinalysis of various treatment groups did not show much difference except the microscopic examination of urinary sediment which is evident from Table 2.

Histopathological studies of CCl<sub>4</sub> induced nephrotoxicity group showed significant damage in kidney tissue when compared with control group. The CCl<sub>4</sub> treated group showed interstitial inflammation where as in control group glomerular and tubules with normal structure in kidney tissue. The histopathological slides of silymarin pre-treatment group showed significantly attenuated congested

Table 1: Blood biochemical parameters of various treatment groups.

S.No.	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
1	Creatinine (mg/dl)	1.32±0.08	1.12±0.08	1±0.07	0.74±0.05	1.11±0.07
2	Calcium (mg/dl)	12.2±0.31	12.1±0.56	13.78±0.18	8.61±0.19	11.2±0.49
3	BloodUrea (mg/dl)	69.4±2.83	34±3.24	35.8±2.49	38.4±2.31	38.2±2.08
4	Albumin(g/dl)	4.74±0.15	5.4±0.57	5.29±0.07	4.08±0.26	4.5±0.24
5	Sodium (mmol/l)	135.4±1.5	116.6±5.44	134.8±1.88	138.8±2.35	135.6±1.312
6	Potassium (mmol/l)	7.24±0.2	9.8±0.09	10±0	8.68±0.18	10±0
7	Total Protein(g/dl)	9.22±0.36	8.94±0.62	6.9±0.14	5.54±0.37	5.5±0.14

Table 2: Urine analysis parameters of various treatment groups.

S.No.	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
1	Color	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
2	Appearance	Cloudiness	Cloudiness	Cloudiness	Cloudiness	Cloudiness
3	pH	7	7	7	7	7
4	Albumin	***	***	****	****	***
5	Sugar	Nil	Nil	Nil	Nil	Nil
6	Microscopic examination of urinary sediment	Normal	Plenty of uric acid crystals and bacteria	Normal	Plenty of uric acid crystals	Normal

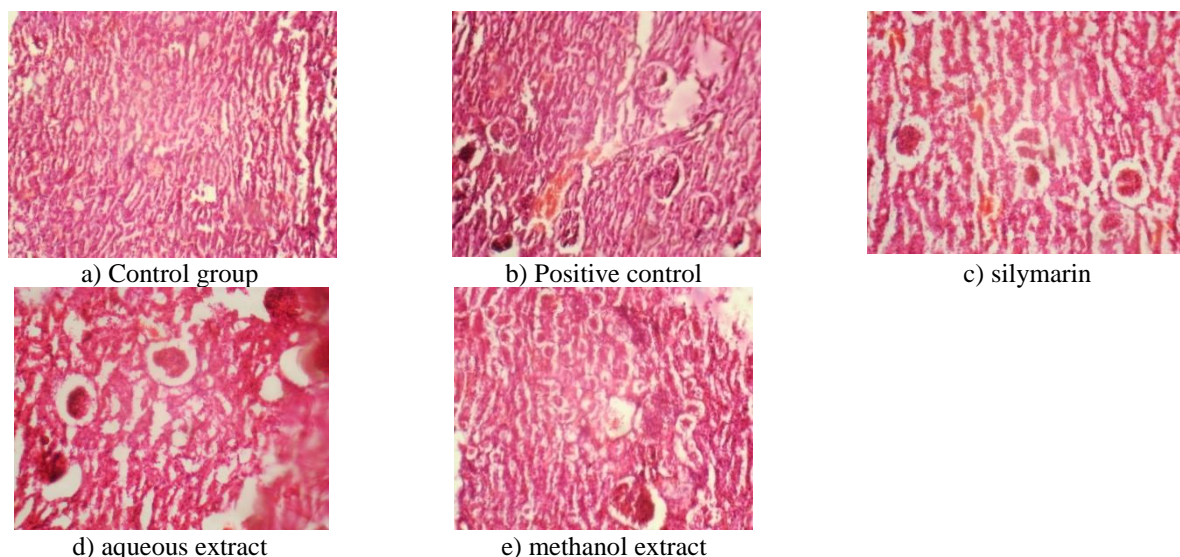


Figure1: Histology slides of the isolated kidney of various treatment groups showing haematoxylin and eosin stained cells.

vessels and interstitial inflammation when compared with CCl<sub>4</sub> group. The histopathological slides of JCAE treated group showed mild interstitial inflammation where as JCME group showed significant recovery of kidney tissue after CCl<sub>4</sub> challenge similar to silymarin treatment group as shown in [Figure 1].

**DISCUSSION**

Carbon tetrachloride induced nephrotoxicity employed in the present study is one of the most widely accepted models to evaluate nephroprotective activity in laboratory animals. In the present study<sup>15,16,17</sup>. CCl<sub>4</sub> treatment group showed a significant decrease in creatinine (p<0.87), calcium (p<0.13), blood urea (p<0.0001), sodium (p<0.001), total proteins (p<0.2) and increase in albumin (p<0.4), potassium (p<0.0001), when compared with control group.

Pretreatment with silymarin attenuated CCl<sub>4</sub> induced nephrotoxicity when compared with positive control group which is evident form significant increase in observed blood biochemical parameters except creatinine (p<0.87), albumin (p<0.4), total proteins (p<0.05). Silymarin treatment group showed decrease in creatinine (p<0.87), blood urea (p<0.0001), sodium (p<0.25), total proteins (p<0.2) and increase in calcium (p<0.13), albumin (p<0.4), potassium levels (p<0.0001) when compared with control group.

Pretreatment with JCAE showed decrease in observed blood biochemical parameters except blood urea (p<0.92), sodium (p<0.25) when compared with silymarin group. JCAE also showed decrease in observed blood biochemical parameters except blood urea (p<0.92), sodium (p<0.0001) when compared with CCl<sub>4</sub> group. Furthermore JCAE treatment group showed decrease in

observed blood biochemical parameters except sodium ( $p < 0.25$ ), potassium ( $p < 0.0001$ ) when compared with control group.

Pretreatment with JCME showed increase in observed blood biochemical parameters except blood urea ( $p < 0.92$ ), sodium ( $p < 0.25$ ) when compared with JCAE group. Total protein was found to be similar with JCAE group. JCME also showed decrease in calcium ( $p < 0.001$ ), albumin ( $p < 0.4$ ), total proteins ( $p < 0.2$ ) and increase in creatinine ( $p < 0.87$ ), blood urea ( $p < 0.92$ ), sodium ( $p < 0.25$ ) when compared with Silymarin group. Potassium was found to be similar with Silymarin group. Likewise JCME treatment group showed decrease in creatinine ( $p < 0.87$ ), calcium ( $p < 0.13$ ), albumin ( $p < 0.4$ ), total proteins ( $p < 0.0001$ ), and increase in blood urea ( $p < 0.92$ ), sodium ( $p < 0.001$ ), potassium ( $p < 0.3$ ) when compared with  $\text{CCl}_4$  group. Furthermore JCME treatment group showed decrease in observed blood biochemical parameters except potassium ( $p < 0.0001$ ) when compared with control group. Sodium levels were found to be similar with control group. The histopathological slides of JCAE treated group showed interstitial inflammation whereas JCME group showed significant recovery of kidney tissue after  $\text{CCl}_4$  challenge similar to silymarin treatment group.

## CONCLUSION

From the obtained results it may be concluded that *Jatropha curcas* methanolic extract showed significant nephroprotective effect against  $\text{CCl}_4$  induced nephrotoxicity in rats than *Jatropha curcas* aqueous extract for most of the blood biochemical parameters, urine analysis ( $p < 0.001$ ) as well in attenuation of pathological changes in kidney tissue.

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