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Protective Effect of *Cissampelos pareira* Linn. on Paracetamol Induced Nephrotoxicity in Male Albino Rats.

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ABSTRACT

Cissampelos pareira Linn. (Menispermaceae) widely distributed all over India and it is widely used for various kidney disorders. According to the Ayurveda, the kidneys are made up of the "Rakta" and "Meda" dhatus. Treating these two dhatus is also an effective way to treat the kidneys. The plant *Cissampelos pareira* Linn. is used to treat these two dhatus. This promotes us to undertake a study to examine the possible antioxidant and nephroprotective activity of the whole plant extract in paracetamol induced nephrotoxic rats. The degree of protection was determined by estimating urinary creatinine, urinary glucose, urinary sodium, urinary potasium blood urea, serum creatinine levels and body weight of the animals. The *In-vivo* antioxidant activity was determined by estimating the tissue levels of GSH, SOD, catalase and lipid peroxidation. The treatment with 70% hydroalcoholic extract of *Cissampelos pareira* Linn. whole plant (HACP) (200 and 400 mg/kg body weight, p.o) markedly reduced paracetamol induced elevation of urinary sodium, potassium electrolytes, urinary glucose, blood urea and creatinine levels. It also increased the body weights and urinary creatinine. In addition treatment with HACP significantly restored the tissue SOD, catalase, GSH and reduced the LP. In conclusion, these results suggested that HACP of the whole plant possess nephroprotective activity against paracetamol induced kidney damage and significant antioxidant activity.

Keywords: Nephrotoxicity, *Cissampelos pareira* Linn, Paracetamol, Antioxidant.

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INTRODUCTION

Nephrotoxicity is important concern during drug development when selecting new drug. At present the world population is having lots of kidney disorders, which has several reasons to be explained for the causes of disorders. Kidneys are the major organs which are responsible for various functions in our body like regulate blood volume and composition, help to regulate blood pressure, synthesize glucose, release erythropoietin and excrete wastes in the urine [1]. Liver and kidney are major organ systems physiologically involved in the metabolism and excretion of various xenobiotics, consequently they are exposed to oxidative stress and free radicals. This results in the tissue necrosis and damage of these organ systems. Therefore, several attempts are been made to protect these organs from the free radical challenges. Reactive oxygen species developed form the chemicals or drugs that are exposed to the renal cells will lead to the renal necrosis.

In our body free radicals are generated through several processes that might be exposure to ultraviolet radiation in sun light, exposure to X- rays, and during normal metabolism of the cells. Chemical substances like carbon tetrachloride and various drugs which we are using for various diseases also produce free radicals when they are metabolised in the body [1]. There are several types of reactive oxygen species which include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. These reactive species will react with membrane lipids, proteins, nucleic acids, enzymes and other small molecules it may lead to the cellular damage. The cellular damage which is caused by these free radicals is the main reason for many degenerative diseases of ageing such as cardiovascular diseases, brain dysfunction, cancer, cataracts, and immune system decline etc. Free radicals are charged molecules which are having unpaired electrons, these charged molecules will try to get neutralised by capturing electrons from other substances. During neutralization process of these free radicals they will generate new free radicals, so it is a chain reaction which will generate thousands of free radicals within seconds. Antioxidants are able to stabilising or deactivating those free radicals before they attack cells. So, antioxidants are essential for maintaining optimal cellular and systemic health. To protect from these free radicals human have evolved highly sophisticated antioxidant protection system. The protection system involves both exogenous and endogenous in origin. The inbuilt antioxidant systems like glutathione reductase, superoxide dismutase, catalase etc., will protect the cells in our body from free radicals. But, sometimes the generation of free radicals are so high such that they may over power the inbuilt antioxidant systems and damage the cells. So we need extra supplements of antioxidants to protect from these free radicals. By using vitamins and phytonutrients we can protect our body cells from these free radicals. As we know many plants contain phytochemicals constituents that possess medicinal properties, among them majority of plants shows antioxidant activity. Hence, the present study was to detect the nephroprotective activity of (70%v/v) hydroalcoholic extract of *Cissampelos pareira* Linn. The nephroprotective activity was screened against paracetamol induced nephrotoxiciy in rats. In addition the antioxidant activity was found out by using *in-vivo* methods like tissue lipid peroxidation (LP), superoxide dismutase

(SOD), catalase and glutathione (GSH). Paracetamol used as antipyretic and analgesic drug. It will cause hepatic and renal damage but hepatic damage is more common than renal damage in paracetamol over dose. In the case of paracetamol over dose acute renal failure will occur even in the absence of liver injury [2]. So, the study was under taken to examine the preventive effect of HACP on paracetamol induced nephrotoxicity in albino rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *Cissampelos pareira* Linn. used for the present study was collected from Chittoor district of Andhra Pradesh. The whole plant was identified, confirmed and authenticated by comparing with voucher specimen available at survey of Medicinal plants and collection unit, Department of Botany, by field Botanist Dr. K. Madava Chetty, Assistant Professor, Sri Venkateswara University, Tirupathi. The whole plant material was cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

Extraction

The powder was extracted directly with 70% v/v ethanol, which was used for biological investigations and *in-vitro* antioxidant studies, after subjecting it to preliminary qualitative phytochemical studies [3]. The extract was concentrated under reduced pressure and stored in vacuum desiccators.

Animals

Albino wistar rats weighing 150-250g were procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy. Animals were maintained under controlled condition of temperature at $27^{o} \pm 2^{o}$ C and 12-h light-dark cycles. They were housed in polypropylene cages and had a free access to standard pellets (Amruth) and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (GCP-IAEC/021/12/2010) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

Evaluation of Nephroprotective Activity in Paracetamol Induced Nephrotoxicity[4]

The rats were randomly assigned into 4 groups of 6 animals each.

Group I: Animals of this group served as untreated control and is fed p.o with normal saline 5 ml/kg body weight daily for 14 days.

Group II: The animals of this group were treated as similarly as first group, on 14th day paracetamol suspension was given by p.o, in a dose of 750 mg/kg.

Group III: The animals of this group were given with 200 mg/kg, o.p HACP for 13 days, on 14th day paracetamol suspension was given by p.o, at a dose of 750 mg/kg, after one hour the HACP was given to the animals.

Group IV: The animals of this group were given with 400 mg/kg, o.p HACP for 13 days, on 14th day paracetamol suspension was given by p.o, in a dose of 750 mg/kg, after one hour the HACP was given to the animals.

After 24 hours all group of animals were kept in metabolic cages for urine collection, the urine was collected for next 24 hours and sacrificed with mild ether anaesthesia and the kidney tissues, urine and blood samples were collected and assessed.

Physical and biochemical parameters:

The percentage change in body weight was calculated, biochemical parameters such as Na^+ [5-8]′ K^+ [5-8]′ Glucose[9,10]′ Creatinine[11,12] in urine and Creatinine[11,12] Urea in blood were estimated.

Glutathione Estimation[13]

Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in a tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure (Aykae, et.al.,) [14] Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. % increase in OD is directly proportional to the increase in the levels of Glutathione. Hence, % increase in OD was calculated.

Lipid Peroxidation[13]

Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid. Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 μmol of lipid phosphate) with 2.0 ml of TCA-TBA-HCl and mix thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the sample was determined at 535 nm against a blank that contains all the reagents minus the lipid. % decrease in OD was directly proportional to the decrease in the levels of lipid peroxidation. Hence, % decrease in OD was calculated.

Superoxide Dismutase Levels[15,16]

2.8 ml of sodium carbonate buffer (0.05 mM) and 0.1 ml of tissue homogenate or sucrose (Blank) was incubated at 30^0C for 45 minutes. Then, the absorbance was adjusted to 0 to sample. Thereafter, the reaction was initiated by adding 10µl of adrenaline solution (9mM). The change in absorbance was recorded at 480nm for 8-12 minutes. Throughout the assay, the temperature was maintained at 30^0C. Similarly, SOD calibration curve was prepared by taking 10 units/ ml as standard solution. 1 units of SOD produce approximately 50% of inhibition of auto-oxidation of adrenaline. The results are expressed as unit (U) of SOD activity per mg of tissue.

Calculation:

$$SOD = \frac{C \times \text{Total Volume} \times 1000}{50 \text{ X Sample Volume X mg protein per ml}}$$

Unit: Units/ mg Protein.

Catalase[17]**:**

Catalase measurement was done based on its ability to decompose hydrogen peroxide (H_2O_2). Briefly, to 0.95 ml of 10 mM H_2O_2 in 60 mM phosphate buffer (pH = 7.0), 50 µl of the tissue supernatant was added and the rate of degradation of H_2O_2 was followed at 240 nm per min. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2 using the formula k = 2.303/ Δt x log (A1/A2) s-1 (A unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH = 7.0 at 25^o C).

Statistical Analysis

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Effect on Change in Body Weights

There was slight decrease of body weights in paracetamol treated group (II) when compared with control group (I) but not significant. However, there was slight dose dependent increase of body weights but not significantly in animals treated with HACP 200 mg/kg, p.o and 400 mg/kg, p.o (III, IV).(Table No 1)

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Table No 1: Effect on Change in Body Weights

Group	Treatment	Dose	Change in body weights (g) Mean ± SEM
I	Normal control	Vehicle	1.500 ± 0.182**
II	Toxicant control	Paracetamol 750 mg/kg p.o	-0.333 ± 0.333
III	HACP	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HACP	0.083 ± 0.416^{ns}
IV	HACP	Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HACP	1.167 ± 0.459*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control.HACP: Hydro alcoholic extract of *Cissampelos pareira* Linn.

Effect on Urinary Sodium, Potassium, Glucose and Creatinine

There was significant (p<0.05) increase of sodium levels in paracetamol treated group (II) when compared to control (I). However 200 mg/kg, p.o (III) HACP slightly increased the levels of sodium but not significant and in the case of 400 mg/kg, p.o (IV) HACP decreased the levels of sodium significantly (p<0.05) in urine samples were observed

Potassium levels in paracetamol treated group (II) were increased significant (p<0.05) when compared to control group (I). However 200 mg/kg, p.o (III) HACP slightly decreased the levels of potassium but not significant and in the case of 400 mg/kg, p.o (IV) HACP decreased the levels of sodium significantly (p<0.05) in urine samples were observed.

Glucose levels in paracetamol treated group (II) were increased significantly (p<0.001) when compared to control group (I). However 200 mg/kg, p.o (III) HACP slightly decreased the levels of glucose but not significant and 400 mg/kg, p.o (IV) HACP reduced the glucose levels significantly (p<0.01) in the urine samples.

Urinary creatinine levels in paracetamol group (II) were decreased significantly (p<0.01) when compared to control group (I). However 200 mg/kg, p.o (III) HACP increased the levels of creatinine but not significant and in the case of 400 mg/kg, p.o (IV) HACP increased the levels of creatinine significantly (p<0.05) in urine samples were observed. (Table No 2)

Effect on Blood Urea and Blood Creatinine

Blood urea level increased significantly (p<0.001) in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg, p.o (III) and 400 mg/kg, p.o (IV) HACP decreased urea levels significantly (p<0.001) in the blood samples. Blood creatinine level increased significantly (p<0.01) in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg, p.o (III) HACP decrease the blood creatinine significantly (p<0.05) and 400 mg/kg, p.o (IV) HACP decreased creatinine levels significantly (p<0.01) in the blood samples. (Table No 3)

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Table No 2: Effect on Urinary Sodium, Potassium, Glucose and Creatinine

Group	Treatment	Dose	Urinary sodium levels (meq/l) Mean ± SEM	Urinary potassium levels (meq/l) Mean ± SEM	Urinary glucose levels (mg/dl) Mean ± SEM	Urinary creatinine levels (mg/dl) Mean ± SEM
I	Normal control	Vehicle	149.9 ± 3.280*	1.333 ± 0.235*	3.593 ± 1.922***	3.038 ± 0.216**
II	Toxicant control	Paracetamol 750 mg/kg p.o	171.8 ± 6.331	2.542 ± 0.419	25.16 ± 6.106	2.012 ± 0.233
III	HACP	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HACP	172.9 ± 5.324^{ns}	1.740 ± 0.228^{ns}	16.02 ± 2.204^{ns}	1.900 ± 0.163^{ns}
IV	HACP	Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HACP	151.7 ± 4.345*	1.274 ± 0.208*	7.953 ± 1.943**	2.762 ± 0.184*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control.HACP: Hydro alcoholic extract of *Cissampelos pareira* Linn.

Table No 3: Effect on Blood Urea and Blood Creatinine

Group	Treatment	Dose	Blood urea (mg/dl) Mean ± SEM	Blood creatinine (mg/dl) Mean ± SEM
I	Normal control	Vehicle	18.63 ± 1.565***	1.498 ± 0.217**
II	Toxicant control	Paracetamol 750 mg/kg p.o	66.03 ± 4.624	2.244 ± 0.098
III	HACP	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HACP	43.64 ± 1.111***	1.613 ± 0.122*
IV	HACP	Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HACP	33.67 ± 2.342***	1.523 ± 0.128**

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01 and *P<0.05. All values are compared with Toxicant control.
HACP: Hydro alcoholic extract of *Cissampelos pareira* Linn.

Effect of HACP on Tissue Lipid Peroxidation (LP) and Glutathione (GSH)

There was dose dependent inhibition of *in-vivo* LP significantly (p< 0.001) by both the doses of HACP. 200 mg/kg, p.o HACP showed 44.20% inhibition, whereas 400 mg/kg, p.o HACP showed 64.49% inhibition (Table No 4).There was a marked depletion of GSH level in paracetamol treated groups. HACP showed a dose dependent increase in the level of GSH.

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However, at 200 mg/kg, p.o HACP showed 44.65% increased in GSH level and 400 mg/kg, p.o HACP showed 65.39% increased in GSH levels. (Table No 5)

Table No 4: Effect of HACP on Tissue LP in Paracetamol Induced Nephrotoxicity Model.

Group	Treatment	Dose	**Absorbance Mean ± SEM** (LP)	**% Inhibition** (LP)
I	Nornmal control	Vehicle	0.114 ± 0.001***	-
II	Toxicant control	Paracetamol 750 mg/kg, p.o	0.276 ± 0.001	-
III	HACP	Paracetamol 750 mg/kg, p.o + 200 mg/kg, p.o HACP	0.154 ± 0.001***	44.20
IV	HACP	Paracetamol 750 mg/kg, p.o + 400 mg/kg, p.o HACP	0.098 ± 0.002***	64.49

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with Toxicant control. HACP: Hydro alcoholic extract of *Cissampelos pareira* Linn.

Table No 5: Effect of HACP on Tissue GSH Levels in Paracetamol Induced Nephrotoxicity Model.

Group	Treatment	Dose	**Absorbance Mean ± SEM** (GSH)	**% Increase** (GSH)
I	Nornmal control	Vehicle	1.766 ± 0.003***	-
II	Toxicant control	Paracetamol 750 mg/kg, p.o	0.916 ± 0.002	-
III	HACP	Paracetamol 750 mg/kg, p.o + 200 mg/kg, p.o HACP	1.325 ± 0.003***	44.65
IV	HACP	Paracetamol 750 mg/kg, p.o + 400 mg/kg, p.o HACP	1.515 ± 0.002***	65.39

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with Toxicant control. HACP: Hydro alcoholic extract of *Cissampelos pareira* Linn.

Effect of HACP on Superoxide Dismutase (SOD) and Catalase

The rats which were treated with paracetamol exhibited significant lowered SOD (8.650 ± 0.520) as compared to those of control rats (17.430 ± 0.520), treatment with HACP significantly (p<0.001) elevated the reduced SOD levels when compared to the toxicant control.

The rats which were treated with paracetamol exhibited significant lowered catalase (7.773 ± 0.649) as compared to those of control rats (13.970 ± 0.435), treatment with HACP significantly (p<0.001) elevated the reduced catalase levels when compared to the toxicant control. (Table No 6)

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Table No 6: Effect of HACP on Superoxide dismutase (SOD) and Catalase in Paracetamol Induced Nephrotoxic Rats.

Group	Treatment	Dose	SOD U/mg protein	Catalase U/mg Protein
I	Normal control	Vehicle	17.430 ± 0.520***	13.970 ± 0.435***
II	Toxicant control	Paracetamol 750 mg/kg p.o	8.430 ± 0.246	7.773 ± 0.649
III	HACP	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HACP	15.790 ± 0.666***	12.130 ± 0.206***
IV	HACP	Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HACP	16.980 ± 0.550***	12.760 ± 0.487***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with Toxicant control. HACP: Hydro alcoholic extract of *Cissampelos pareira* Linn.

DISCUSSION

In clinical practice there are several categories of nephrotoxic drugs especially drugs used in the treatment of cancer and certain diseases like tuberculosis. Most of these nephrotixic drugs reported to produce renal toxicity due to generation of free radicals. Thus generated free radicals over powers the inbuilt protective mechanism, resulting in the nephrotic damage and necrosis[18].

Therefore it is thought to be beneficial to administer free radicals scavengers like antioxidants along with such nephrotoxic drugs so as to reduce their toxicity without affecting the therapeutic efficacy.

The availability of safer and synthetic antioxidants and free radical scavengers is very less. Therefore it was thought to search for the natural antioxidants and free radical scavengers, which are useful in reducing the drug induced nephrotoxicity. In one of our field survey we found that, the plant was abundantly available in all over India and a native practitioner has claimed that the plant is a very good nephroprotective agent. Keeping all these things in view the present study was planned to assess the anti-oxidant and nephroprotective properties.

The HACP significantly restored the tissue SOD, catalase, GSH and reduced the LP. These results are indicating HACP possess antioxidant property. The HACP also prevented the reduction in body weight, urinary creatinine and reduced the elevated urinary sodium, potassium, glucose, blood urea and serum creatinine, Therefore, the HACP has organ protective potential against xenobiotic induced nephrotoxicity in rats.

The HACP possess alkaloids, flavonoids, and these compounds are known to possess antioxidant activity and antioxidant activity may be involved in organ protective activity. Therefore, the antioxidant and organ protective property of HACP can be assigned to antioxidant principle of it. However, further studies are needed to identify, isolate, characterize and screen the organ protective activity.

CONCLUSION

The HACP demonstrated significant dose dependant increase in depleted tissue GSH, SOD, catalase levels and reduction in lipid peroxidation caused by paracetamol induced nephrotoxicity. The HACP prevented the reduction in body weight, urinary creatinine and reduced the elevated urinary sodium, potassium, glucose, and blood urea and serum creatinine in paracetamol induced nephrotoxicity. Hence, the HACP possess the antioxidant and nephroprotective activity.

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