

Evaluation of nephroprotective activity of *Ichnocarpus frutescens*

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ABSTRACT

Nephrotoxicity is one of the most common kidney problems and occurs when exposed to antitumor drug Cisplatin. The aim of this study was to evaluate the nephroprotective activity of ethanolic extract of *Ichnocarpus frutescens* against cisplatin induced nephrotoxicity. Nephrotoxicity was induced in rats by administrating a single dose of cisplatin 6mg/kg i.p. and was manifested by high levels of BUN, serum creatinine, serum total proteins, and urinarytotal proteins and decreased creatinine clearance. The alcoholic extract of *Ichnocarpus frutescens* was administrated in two different doses from day 6-day 15th in curative regimen and the higher dose was administered for ten days in the prophylactic regimen. Experimental results suggest that the supplementation of extract reduced the elevated serum creatinine, blood urea nitrogen levels, lipid peroxidation level and improved the creatinine clearance. The dose dependent protective effect of plant *Ichnocarpus frutescens* may be due to free radical scavenging property.

Key words: Nephrotoxicity; *Ichnocarpus frutescens*; Cisplatin; Lipid peroxidation.

INTRODUCTION

Nephrotoxicity-defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines, industrial or environmental chemicals. The periodical management of present day kidney associated diseases in allopathy is expensive and has severe side effects. Hence the research for nephroprotective drugs from natural sources has greater importance.

Ichnocarpus frutescens L. (Family-Apocynaceae) is a woody black creeper with slender branches found almost throughout India up to an altitude of 4000ft. Previous studies on chemical constituents of the plant revealed the presence of phenyl propanoids, sterols, flavanoids, coumarins, phenolic acids (Khanet al.,1997; Lakshmi et al.,1985). A review of previous reported medicinal properties of *Ichnocarpus frutescens* include antidiabetic (Rakeshet al., 2008), antipyretic (Pandurangan et al., 2009), antitumor (Chidambaram et al., 2007), hepatoprotective activity (Chidambaram et al., 2011) antioxidant activity (Faheem et al., 2014), analgesic and anti-inflammatory activity (Nitinet al., 2010).

Many Indian medicinal plants are reviewed as nephroprotective with fewer side effects and are also inexpensive. The Indian traditional medicine suggests that the plant *I. frutescens* as an alternative plant for Indian Sarassaparilla (*Hemidde musindicus*) and was used by tribals of Idukki, Kerala for urinary diseases (Kumaret al., 2012). The root powder of the plant was used with milk for diabetes, kidney stones and fever. But the scientific data supporting the nephroprotective property of this plant is lacking. Hence the present study was undertaken for evaluating the nephroprotective activity of *Ichnocarpus frutescens* since, no reports exists on this plant till today.

MATERIALS AND METHODS

Plant Material and Chemicals: *Ichnocarpus frutescens* was collected from Idukki, Kerala, India and authenticated by department of botany, Nagarjuna ayurveda centre. A specimen voucher number was deposited in college (Visveswarapura Institute of Pharmaceutical Sciences) herbarium for future references. The chemicals were purchased from Merck India and Cisplatin was a gift sample from Caplin Labartories, Pondicherry.

Preparation of the extract: The plant *Ichnocarpus frutescens* was collected, shade dried and coarsely powdered to reflux with alcohol for 3 hours maintaining the temperature between 60-70°C and collected the filtrate after filtration. The residue was further refluxed two times and collected the filtrate. The resulting three filtrates were collected and distilled to collect to get pure extract of 140g and were suspended in sterile water for experimental studies.

Pharmacological studies

Acute toxicity studies: Acute toxicity studies were performed on albino mice of either sex weighed (25-30g). The acute oral toxicity study was carried out as per guide lines set by Organization for Economic Corporation and Development (OECD) received for the purpose of control and supervision of experiments on animals. One tenth of median lethal dose (LD₅₀) was considered as effective dose (Ghosh et al., 1984).

Animal selection: Wister rats weighed (200-250g) were selected and acclimatized to standard laboratory conditions (25±0.2°C) and maintained for 12hour day and night cycle. They were provided with regular rat chow and drinking water. The animals were maintained in these conditions for one week before the experimental session. The studies were carried out according to the guidelines of CPCSEA (Reg NO.152/99/ CPCSEA dt23-09-10).

Experimental Model for Nehroprotector Activity: Wistar rats were weighed (200-250g) and divided into 6 groups of six animals each. Group-I, received (1% tween 80) for 15 days and Group-II, (curative control) received Cisplatin (6 mg/Kg, i.p; single dose) on the first day and vehicle (1% twin 80) from 6th-day15th. Group-III, received Cisplatin on first day and plant extract (250mg) from 6th-day15th. Group-IV, treated with Cisplatin on first day and plant extract (500 mg) from 6-day15th. Group-V, acts as a prophylactic control in which the animals were given with vehicle from 1-day10th and Cisplatin on 11th day. Group-VI, was treated with plant extract (500mg) from 1-day10th and cisplatin on 11th day. On 16th day blood and urine were collected from all six groups for biochemical estimation. For lipid peroxidation studies in kidney, on 16th day, the rats from each group have to be anaesthetized using sodium phenobarbitone (60mg/Kg) and kidneys were isolated. The blood was collected on 15th day to analyze Serum Creatinine, (Jaffes Alkaline Picrate method, (Philip, 1994) Serum Total Protiens, (Biuret method) (Godkar, 1994) and Blood Urea Nitrogen (BUN); (DAM method), (Wybengo 1971). Urine was collected to analyze Urine

Creatinine (Alkaline Picrate method) (Godkar, 1994) and Urine Total Proteins (Turbidimetry method) (Godkar, 1994). Formalin preserved samples of kidneys from various groups used for histopathological studies and centrifugated, homogenate was used for lipid peroxidation studies (Heath and Backer method, 1968).

Serum Analysis: The blood was collected on 16th day and serum was separated by centrifugation at 10000rpm for 10 min. and analyzed for creatinine, blood urea nitrogen and serum proteins (Caraway et al., 1963).

Collection and Urine Analysis: All animals were kept in individual metabolic cages and had free access to drinking water during the urine collection period. The urine was collected for 24h on 15th day. A drop of concentrated hydrochloric acid was added to the urine to analyze urine creatinine and urinary protein (Caraway et al., 1963)

In vivo Lipid peroxidation: The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation. Stock solution of 15% w/v trichloro acetic acid (TCA) 0.375% w/v of thiobarbituric acid (TBA) and 0.25 N HCl. The solution was mildly heated to assist the dissolution of TCA. Further combine 1ml of biological sample (1-2mg of membrane protein or 0.1-0.2μmole of lipid phosphate) with 2ml of TCA-TBA HCl and mixed thoroughly. Then the solution was heated for in a boiling water bath and allowed to cool. After cooling, the flocculent precipitate was removed by centrifugation at 2500rpm for 2min and at 535nm against the blank. The concentration of malonodi aldehyde of the sample was calculated by following formula using an extinction coefficient $1.56 \times 10^5 M^{-1} cm^{-1}$ % inhibition.

$$\text{Inhibition(\%)} = (\text{ControlOD} - \text{TestOD}) \times 100 / \text{ControlOD}$$

Histopathological studies: The kidneys from all groups were isolated and preserved in 10% neutral buffered formalin solution. Sections were stained with haematoxylin and eosin and observed under standard microscope. The centrifuge homogenate was used for lipid peroxidation studies.

Statistical analysis: The results were expressed as Mean±SEM and all data were subjected to ANOVA followed by Dunnett's *t* test. The *P* value of <0.05 was considered statistically significant.

RESULTS

Effect of *Ichnocarpus frutescens* serum parameters: The effect of *I. frutescens* on serum parameters are shown in **Table 1**. In Cisplatin induced group, the levels of serum creatinine, serum protein and Blood Urea Nitrogen (BUN) were significantly increased (*P*<0.05) when compared with the positive control. Upon supplementation of *Ichnocarpus frutescens* with (250 and 500mg) in Group III and IV, there was a significant decline (*P*<0.05) in serum marker levels. In prophylactic regimen, the treatment with the extract of (500mg/Kg) for ten days showed a significant reduction in serum marker levels in Group VI when compared to positive control group.

Table-1: Effect of *Ichnocarpus frutescens* extract on serum parameters.

Groups	B UN (mg/dl)	Serum creatinine (mg/dl)	Serum totalprotein (g/dl)	Lipid peroxidation (% inhibition)
I	39.92 ± 1.45	0.56 ± 0.11	5.53 ± 1.1	-
II	56.82 ± 0.95	2.19 ± 0.8	9.1 ± 0.4	-
III	49.15 ± 1.5 ^a	1.93 ± 0.6 ^a	8.2 ± 0.2 ^a	14*
IV	38.2 ± 2.18 ^b	0.72 ± 0.5 ^b	6.5 ± 0.7 ^b	35*
V	58.92 ± 1.7	2.98 ± 0.5	7.1 ± 0.5	-
VI	40.85 ± 0.97 ^c	1.16 ± 0.8 ^c	6.1 ± 0.1 ^c	9*

- Mean SD, n=6, *Significant pharmacological activity, **P*<0.05; ^a*P*<0.05 compared with curative control. ^b*P*<0.05 compared with curative control. ^c*P*<0.05 compared with prophylactic control.

Effect of *Ichnocarpus frutescens* urine parameters: The deterioration of the renal functions induced by the cisplatin shown in **Table 2**. The administration of Cisplatin caused significant reduction ($P<0.05$) in creatinine clearance and increased the excretion of urinary protein in curative control Group II and preventive control in Group V when compared to the normal control group. In curative regimen, the treatment with extracts in Group III and IV animals increased creatinine clearance and reduced the elevated levels of total protein excretion caused by cisplatin compared to cisplatin control Group II. Animals in the preventive regimen (Group VI) also showed significant protection against cisplatin induced effects.

Table-2: Effect of extract of *Ichnocarpus frutescens* on renal parameters.

Groups	Urine total protein (mg/day)	Creatinine clearance (ml/h/100gBd.Wt)	Body weight (g)
I	5.9 ± 0.8	19.8 ± 0.5	215.0 ± 5.0
II	14.5 ± 1.8	9.5 ± 0.1	180.8 ± 5.5
III	12.1 ± 0.3*a	12.5 ± 0.8*a	189 ± 3.4*a
IV	7.8 ± 0.5*b	16.0 ± 0.28*b	209.3 ± 3.0*b
V	15.2 ± 1.5	7.2 ± 0.5	182.5 ± 2.8
VI	13.5 ± 0.6*c	10.6 ± 1.1*c	192 ± 5.1*c

- Foot notes are same as mention in table-1.

Lipid peroxidation: Administration of Cisplatin caused significant increase in the levels of Malondialdehyde (MDA) in curative control (Group II) and preventive control (Group V) when compared to normal control group ($P<0.05$). This indicated that increased lipid peroxidation in Group II and V animals. A significant reduction in MDA level was observed in animals treated with plant extracts (Groups III and Group IV) animals when compared to curative control group. The higher dose was very effective in prevention of oxidative damage induced by cisplatin. The prophylactic treatment group which is treated with extract for ten days and cisplatin on 11th day also decreased the MDA levels compared to prophylactic control group ($P<0.05$).

Histopathological studies: Studies showed degeneration of glomeruli due to Cisplatin administration. The pathological conditions were reduced and almost reversed when administered with 500mg/kg of alcoholic extract. The prophylactic extract also sustained with mild degenerative changes and cast cells.

DISCUSSION

Cisplatin, a widely accepted antineoplastic agent used in the treatment of solid tumors, head neck, ovarian and lung cancers. However the clinical usefulness of this drug is limited due to its dose related nephrotoxicity (Taguchi et al., 2005). The kidney accumulates cisplatin to a greater degree than other organs and is the major route of excretion. The concentrations of cisplatin in proximal tubule epithelial cells are about five times the serum concentration (Borch et al., 1987). Cisplatin is accumulated by peritubular uptake in both the proximal and distal nephrons. The S₃ segment of the proximal tubule accumulates the highest concentration of cisplatin (Baddreldin et al., 2006). This disproportionate accumulation of cisplatin in kidney tissue contributes to nephrotoxicity. This can lead to acute renal failure (Kawai et al., 2006). The mechanism for renal injury has been the focus of intense investigation and recent studies suggest that inflammation, oxidative stress injury, and apoptosis probably explain part of this injury (Hanigan et al., 2003; Ramesh et al., 1991). Several evidences reported that cisplatin induces renal damage by free radical generation (Kawai et al., 2006). Nephrotoxic effect of cisplatin ranges from mild sub lethal changes to inflammatory responses.

The result of our study for serum parameters revealed that cisplatin administration produced acute tubular damage which is evidenced from the elevated levels of serum creatinine, serum urea, serum proteins and decreased creatinine clearance. This is a clear indication of decrease in glomerular filtration rate. Treatment of animals with different doses of *Ichnocarpus frutescens* reduced the levels of raised serum urea, serum creatinine, and serum proteins and increased the creatinine clearance in a dose dependent manner. The extract also showed significant preventive effect due to the presence of sufficient amount of extract in animals to prevent cisplatin toxicity.

The renal damage is produced within one hour after administration. Hence the presence of protective agent in the renal tissues may have reduced the toxic effects of cisplatin. In the prophylactic regimen the treatment of animals with 500mg/Kg reduced the effects caused by cisplatin.

Previous report suggests that the plants possessing flavonoids exhibits antioxidant property and they can scavenge free radicals resulting in nephro protection (Wegner et al., 1999). Hence antioxidants and free radical scavengers of natural, synthetic and semi synthetic origin might provide nephro protection against cisplatin induced renal injury. *Ichnocarpus frutescens* is one such plant possessing antioxidant properties due to presence of flavonoids and can combat cisplatin nephrotoxicity (Khan et al., 1997). Several studies stated that the mechanism of nephrotoxicity is related to depletion of antioxidant mechanism and by initiation of lipid peroxidation (Safirstein et al., 1984). Similar effective nephroprotection was seen in ethanolic extract of *Graptophyllum pictum* (L) and glucosides of ascorbic acid and α -tocopherol (Dani et al., 2008). The potential of ethanolic extract of *Ichnocarpus frutescens* against cisplatin therapy may be attributed to the antioxidant mechanism.

In the present lipid peroxidation studies, animals pretreated with plant extract showed moderate protection against cisplatin induced elevated levels of MDA. In curative regimen, group IV received higher dose of *Ichnocarpus frutescens* showed better protection and decreased levels of MDA. The same was confirmed by histopathological studies (**Figure 1-6**). Hence the antioxidants and free radical scavengers present in the plant might have provided nephroprotection.

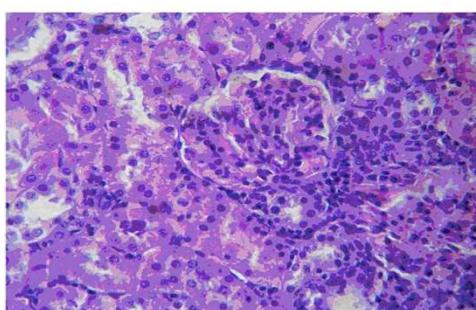


Fig-1: Section of rat kidney with normal glomerulai, proximal and distal tubules.

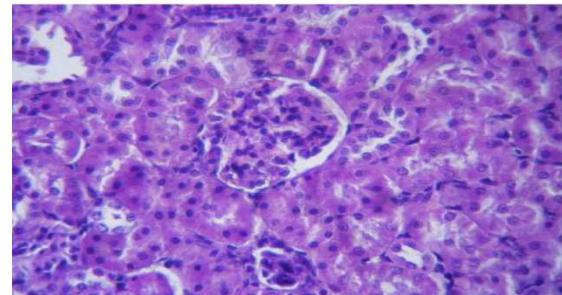


Fig-2: Section of rat kidney treated with cisplatin (curative control).

- Showed glomeruline unremarkable and most of the tubules show desquamation with epithelial casts. (Massive total necrosis).

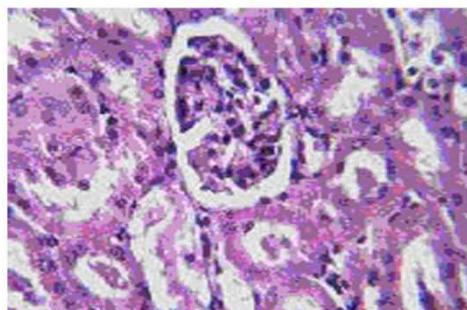


Fig-3: Section of rat kidney with extract of *I. frutescens* (200mg/kg).

- Showed congestion in glomerulai, proximal and distal tubules (Moderate interstitial congestion).

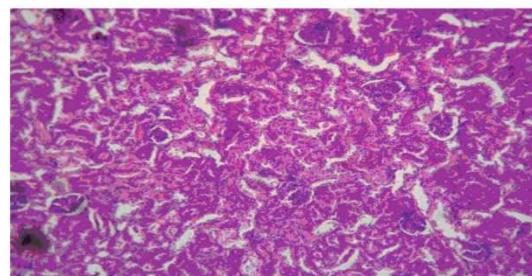


Fig-4: Section of rat kidney with extract of *I. frutescens* (400mg/kg) with maintained glomerulai, proximal and distal tubules.

- Recovery of interstitial congestion.

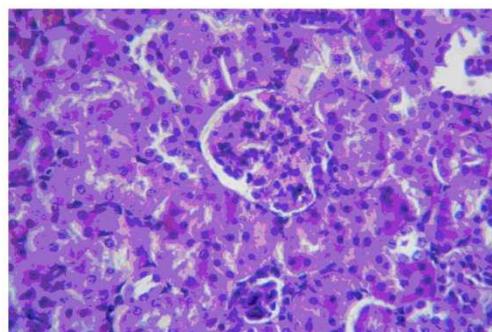


Fig-5: Section of rat kidney in prophylactic control.

- Showed glomerulaein remarkable, desquamation is seen with epithelial cells and lymphocytic infiltration (Severe interstitial congestion).

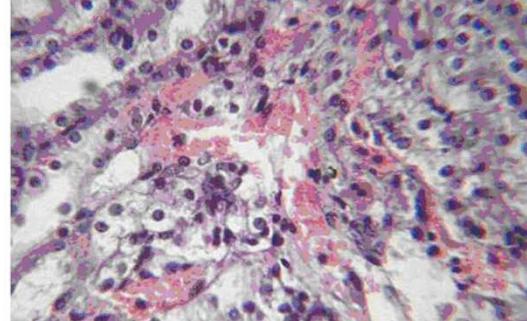


Fig-6: Section of rat kidney treated with extract of *I. frutescens* (Prophylactic control).

- Showed presence of cast cells and less interstitial congestion (Moderate recovery in interstitial congestion).

CONCLUSION

The experimental result proves the ethanolic extract of *Ichnocarpus frutescens* could prevent cisplatin nephrotoxicity. Supplementation of *Ichnocarpus frutescens* during cisplatin therapy reduced the risk of cisplatin induced nephrotoxicity in a dose dependent manner. The extract also showed significant preventive effect due to presence of sufficient amount of antioxidants to prevent the effects of cisplatin. Therefore the ethanolic extract of *Ichnocarpus frutescens* can be used as a natural source of nephroprotective agent against cisplatin induced nephrotoxicity.

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