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EFFECT OF *OCIMUM BASILICUM* ON CISPLATIN MODELS OF ACUTE RENAL FAILURE

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ABSTRACT:

The hydroalcoholic extract of the entire plant of *Ocimum basilicum* was studied for its nephroprotective activity in cisplatin-induced acute renal injury in albino rats of either sex. In the curative regimen, the extract at dose levels of 100, 300 and 500 mg/kg showed dose-dependent reduction in the elevated blood urea and serum creatinine and normalized the histopathological changes in the curative regimen. The findings suggested that the ethanol extract of *Ocimum basilicum* possesses marked nephroprotective activity with minimal toxicity and could offer a promising role in the treatment of acute renal injury caused by nephrotoxins like cisplatin.

KEYWORDS: *Ocimum basilicum*, Cisplatin, Nephrotoxicity, Nephroprotection, Acute renal failure.

INTRODUCTION

Ancient literature has prescribed various herbs for the cure of kidney disease. The term "Pasanabheda" has been cited in the literature to identify the group of plants which have been extensively used in the indigenous system of medicine to dissolve urinary calculi and stones[1] like *Coleus aromaticus, Aerva lanata, Aerva javanica, Rotula aquatica, Kalancho pinnata, Ocimum basilicum. Ocimum basilicum* is commonly known as “Damaro” in Gujarati. It is an ancient herb. *Ocimum basilicum* is an erect branching herb, is native to tropical Asia. It is also cultivated commercially in southern Europe, Egypt, Morocco, Indonesia and California. The plant is indigenous to the lower hills of Punjab and cultivated through India. *Ocimum basilicum* is used as the principle source of “Pashanabeda” a Sanskrit term cited in literature[2] to identify a group of plants extensively used in the Ayurvedic system of medicine to dissolve urinary calculi and stones. Folkloric medicine of Rayalaseema region, Andhra Pradesh reports the use of *Ocimum basilicum* as a nephroprotector in the treatment of various kidney ailments[3]. The plant is also used by the Yanadi tribal’s of the Chittor district as a diuretic and for the treatment of nephrocarcinosis and urethral stones[4]. The plant has been documented earlier for its therapeutic effects in renal diseases by some Unani physicians[5]. In the dry zones of Sri Lanka, *Ocimum basilicum* has been identified for its usefulness in controlling kidney disorders[6]. The seeds of this plant was widely used in nephrosis and used to treat kidney ailments. The seeds are used in curing urinary problems. A literature survey revealed that *Ocimum basilicum* was endowed with various chemical components such as flavonoids, steroids, polysaccharides, tannins, saponins, etc[7], which possibly contribute to its diverse uses in folklore medicine. Acute renal failure refers to the sudden and usually reversible loss of renal function, which develops over a period of days or weeks. Among the causes of acute renal failure, acute tubular necrosis, which occurs due to ischemia or nephrotoxins like cisplatin is most common, accounting for 85% of the incidence. There is a continuous search for agents which provide nephroprotection against the renal impairment caused by drugs like cisplatin for which allopathy offers no remedial measures. Thus, it was imperative that mankind turns towards alternative systems of medicine for solace. Hence, the present study was an attempt to screen the hydroalcoholic extract of aerial parts of *Ocimum basilicum* for its nephroprotective activity.

MATERIALS AND METHODS

Collection of the Herb:

Fresh herb of *Ocimum basilicum* was collected from Ayurvedic garden Gandhinagar, Gujarat, India. It was dried and reduced into coarse powder. Its botanical identity was

authenticated by Dr. S.K. Patel, Department of Botany, Government Science College, Gandhinagar, Gujarat, India. A voucher herbarium specimen number PH/509/0013 has been deposited in the Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, India**.**

Preparation of Hydroalcoholic Extract:

The aerial parts of the plant were dried under shade and powdered to 60# separately and stored in air tight container. The shade dried powdered plant was extracted with 95% ethanol and water (75:25). This mixture was taken in conical flask, reflux on water bath for 4 hrs with gradual shaking. Then it was filtered rapidly taking precautions against loss of the solvent. The filtrate of total hydroalcoholic extract was then concentrated in vacuum to dryness in a tared flat-bottomed petridish, dried at 105^0 C and weighed (yield 16.67% w/w).

Animals:

Healthy adult male albino rats (200–250 g) of Wistar strain were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (12-h light: 12-h dark cycle; 25±30C; 35–60% humidity). The animals had free access to standard lab chow (Hindustan Lever Ltd., Mumbai, India) and tap water. Study was conducted after obtaining institutional animal ethical committee clearance (KBIPER/10/163).

Drugs and chemicals:

All different organic solvents were used for extraction under study were obtained from Finar chemicals ltd., Ahmedabad, India and Cisplatin injection (Cadila healthcare limited, Ahmedabad,India), Urea estimation kit (Span Diagnostics Ltd, Surat, India), Creatinine estimation kit (Span Diagnostics Ltd, Surat, India) were obtained.

Acute toxicity studies:

The rats were fed with hydroalcoholic extract of *Ocimum basilicum* suspended in carboxy methyl cellulose (CMC) 1% w/v in increasing dose levels of 10, 20, 100, 200, 300, 500, 1000 and 3000 mg/kg body weight[8]. The animals were observed continuously 2 hr for the gross behavioural changes and then intermittently every 2 h for a period of 24 h and finally at the end of 72 h to note for any signs of toxicity including death.

Induction of nephrotoxicity and drug feeding schedule:

Cisplatin-induced renal injury*:*

Five groups of six rats each were used in this model. Group 1 was administered with vehicle 1 % Carboxy Methyl Cellulose solution (2 ml) for 15 days. Group 2 was rats were treated with cisplatin 5 mg/kg body weight, single dose, intraperitoneally on 1^{st} day, and blood was withdrawn through retro-orbital vein from animals on 6^{th} day to assess for renal

function tests. Kidney was isolated for histopathology.

***Curative group**:*

The rats of Groups 3 and 4, were after, initial administration of a single dose of cisplatin 5 mg/kg body weight treated orally with hydroalcoholic extract of *Ocimum basilicum* at doses of 300 and 500 mg/kg body weight from the 6th day onwards for 10 days. Blood was withdrawn on the 16th day to estimate blood urea and serum creatinine for their curative effect.

***Prophylactic group**:*

Animals of Group 5 were treated orally with 300 mg/kg body weight of the extract for 10 days. On the 11th day single intra-peritoneal dose of cisplatin was administered. The blood was withdrawn on the 16th day to assess blood urea and serum creatinine levels.

Assessment of renal function

Body weight:

The weights (g) of the animals were noted on the first and last day of treatment and the percentage change in body weight was calculated.

Blood urea:

Urea concentration in the blood was estimated by enzymatic method using Urease enzyme kit by modified Berthelot method[9]. Absorbance was measured using UV-240 Vis spectrophotometer (Shimadzu Corporation, Japan).

Serum creatinine:

Creatinine level in serum was estimated by the alkaline picrate method using creatinine kit method[9]. Absorbance was measured from UV-240 Vis spectrophotometer (Shimadzu Corporation, Japan).

Histopathological examination:

Two animals from each group were sacrificed on the day of blood withdrawal and kidneys were isolated. Tissue samples were immersed in 10% formalin for histopathological studies. Samples were embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Statistical analysis:

Results are given as mean ±S.E.M. Data were analyzed using one-way ANOVA followed by post hoc Sheffe's test using SPSS computer software version 7.5. The statistical significance of difference was taken as $P < 0.05$.

In vitro antioxidant study

In vitro free radical scavenging activity was determined using the DPPH method[10].

RESULTS

The in vitro antioxidant studies of hydroalcoholic extract of *Ocimum basilicum* exhibited DPPH scavenging properties in a dose dependent manner. Acute toxicity studies

carried out showed the extract to be safe up to a dose of 3000 mg/kg. The toxicity group 2 showed definite signs of nephrotoxicity, as compared to the control groups, evidenced by the loss of body weight and elevation of the biochemical parameters, viz. blood urea and serum creatinine as shown in table 1 and 2.

Table 1: Effect of hydroalcoholic extract of aerial parts of the *Ocimum basilicum* on Blood Urea

Groups	1st day mean ± S.E.M	6th day mean ± S.E.M	16thday mean ± S.E.M
Control	50.95±0.76	50.76±0.72	52.018±0.765
Cisplatin-induced	50.74±0.41	387.44±6.89	392.085±2.19
Ocimum basilicum (300mg/kg)	51.37±0.29	324.21±30.68*	75.57±3.21*
Ocimum basilicum (500mg/kg)	51.45±0.31	321.22±25.43*	92.26±2.6

*Significant difference from control group, at P<0.05

Results represent mean ± S.E.M., n=6, Statistical analysis was done by one-way ANOVA followed by post Dunnett test. All groups were compared with control group.

Table 2: Effect of hydroalcoholic extract of aerial parts of the *Ocimum basilicum* on serum creatinine level

Groups	1st day mean ± S.E.M	6th day mean ± S.E.M	16thday mean ± S.E.M
Control	0.883±0.025	0.867±0.027	0.817±0.044
Cisplatin-induced	0.885±0.009	3.188±0.827	4.745±0.44
Ocimum basilicum (300mg/kg)	0.852±0.023	4.098±0.443*	1.268±0.202*
Ocimum basilicum (500mg/kg)	0.868±0.023	4.263±0.571*	1.042±0.057*

*Significant difference from control group, at P<0.05

Results represent mean ± S.E.M., n=6, Statistical analysis was done by one-way ANOVA followed by post Dunnett test. All groups were compared with control group.

The presence of peritubular and glomerular congestion, tubular casts, epithelial degeneration, interstitial edema, blood vessel congestion and infiltration by inflammatory cells, which are features of acute tubular necrosis, were also observed in the histopathological sections of the kidneys in these groups, indicative of the extent of damage done at the tissue level. The curative regimen, i.e. Groups 3 and 4, in this model showed a dose-dependent normalization of the toxicity induced by cisplatin with the groups treated with 300 mg/kg body weight of the extract showing significant activity. The rats of Group 5 in this model, i.e. prophylactic and the preventive regimen, showed significant reversal of the raised blood urea and serum creatinine levels compared to Group 3 but it was accompanied by a loss of body weight. However, the histopathological sections (Fig. 1) continued to showed light epithelial

degeneration in the prophylactic group of the cisplatin model.

DISCUSSION

While investigating into the possible protective effect of *Ocimum basilicum,* it was observed that 10 days administration of the extract (300 mg/kg body weight) prior to cisplatin administration (5 mg/kg, single dose) in the prophylactic regimen, provided marked protection against cisplatin-induced renal injury. Suggested, that it has nephroprotective activity in cisplatin model of renal injury. Numerous studies have shown that cisplatin-induces renal damage by free radical generation[11,12]. Hence, natural and synthetic antioxidants and free radical scavengers are claimed to provide nephroprotection in cisplatin renal injury. Vitamin C, Vitamin E, selenium, etc. among the natural free radical scavengers and glycine, probucol, etc[13] amongst the synthetic molecules, have been shown to possess partial protection against cisplatin-induced oxidative damages. Till date only natural antioxidants isolated from Panax ginseng have been reported to have action in nephrotoxic models. Flavonoids such as kaempferol 3-rhamnoside and kaempferol 3-rhamnogalactoside have been reported to be present[7]. Flavonoids are well known potent antioxidant and free radical scavengers. The DPPH scavenging model of *Ocimum basilicum,* showed significant free radical

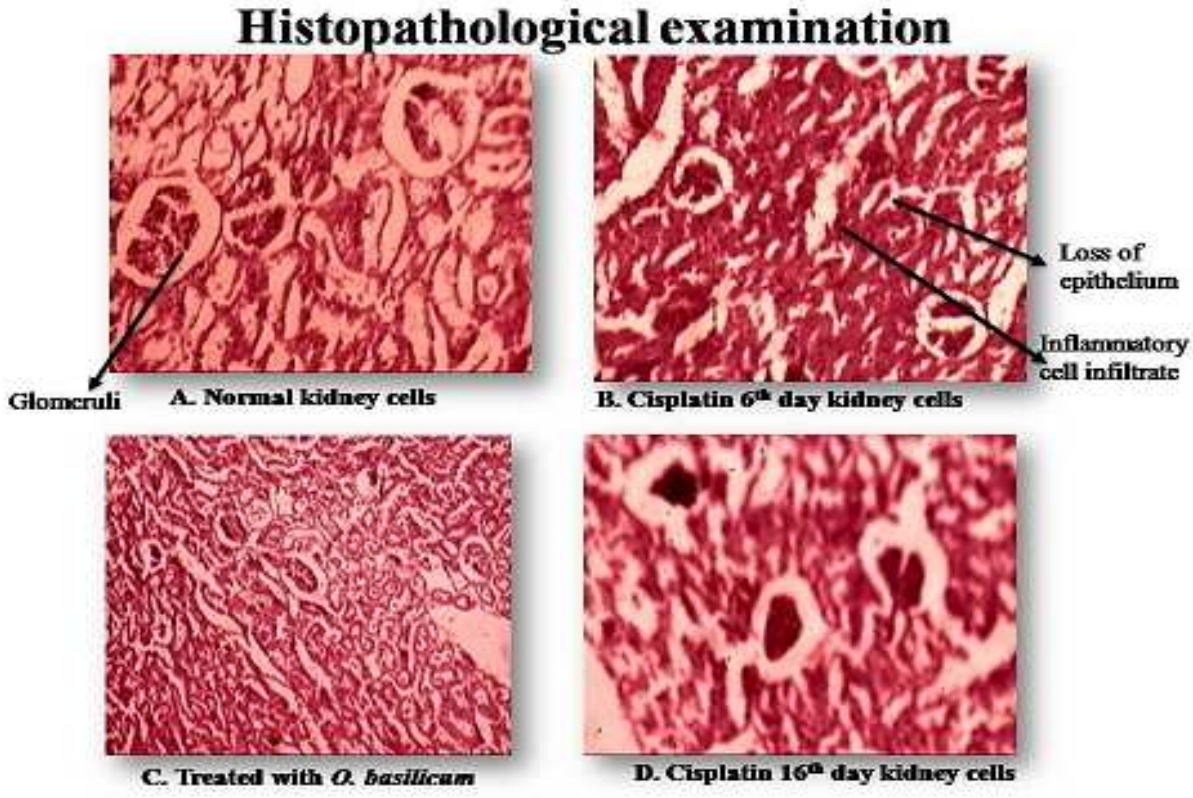

Fig 1: Histopathological section

scavenging activity. Hence, the probable mechanism of nephroprotection by *Ocimum basilicum*, may be attributed to its antioxidant and free radical scavenging property. To conclude, our studies have shown that the entire plant of *Ocimum basilicum*, possesses marked nephroprotective activity with minimal toxicity and thus has a promising role in the treatment of acute renal injury induced by nephrotoxins, especially cisplatin and gentamicin. Further isolation of active components and its nephroprotective activity. The results showed that the hydroalcoholic extract of the aerial parts of this plant possesses nephroprotective and in-vitro antioxidant activity. *Ocimum basilicum* contains various phenolic compounds, tannins, various phytosterols and fatty acids. The main constituents were linalool, limonene, methyl eugenol, beta-caryophyllene and farnesene. Mainly phenolic compounds were present in the plant extract and they were responsible for the nephroprotective activity of plant. *Ocimum basilicum* plant was reported to posses medicinal properties like anti-bacterial, antifungal, anti-inflammatory, antiplatelet, antiulcer, antiviral, anticancer and also used to treat kidney troubles and renal diseases[14,15,16].

Hence, here an attempt has been made to evaluate a nephroprotective activity of hydroalcoholic extract of *Ocimum basilicum* on cisplatin-induced nephrotoxicity in rats

From this study, it was observed that cisplatin-induced renal toxicity was evidenced by the elevated biochemical markers such as serum urea, serum creatinine, total protein, and serum albumin and by the histopathological features of acute tubular necrosis. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in renal tubules. The hydroalcoholic extract of *Ocimum basilicum* at 300 mg/kg the dose levels was found to decrease the changed serum urea, serum creatinine, total protein and serum albumin level and sets it near to the normal value and bring about a marked recovery in kidneys as evidenced microscopically.

In the present study, it was observed that 10 days administration of hydroalcoholic extract of aerial parts *Ocimum basilicum* 300 mg/kg and 500 mg/kg body weight prior to cisplatin administration (5 mg/kg, single dose) effectively prevented the cisplatin induced renal injury as evidenced by decreased serum urea and serum creatinine levels and by increased total protein and albumin levels and normalized the histopathological features. In cisplatin-induced group there was acute tubular necrosis and glomerular congestion observed because of toxicity. Administration of hydroalcoholic extract of *Ocimum basilicum* for ten days recovered the damage produce in kidney cells. There was less glomerular congestion observed in treated groups.

Both of this dose level recovered the damage produced in cisplatin induced renal injury but at the dose level of 300 mg/kg recovery was more as compared with 500 mg/kg. Serum urea and serum creatinine level was found to decrease more at the dose level of 300 mg/kg bodyweight. So, it showed that significant nephroprotective action in 300 mg/kg as compared to 500 mg/kg dose of hydroalcoholic extract of *Ocimum basilicum.* Therefore 300 mg/kg dose was considered as potent dose for further study.

Numerous in-vivo and in-vitro studies have demonstrated, that reactive oxygen species viz. Free radical species are involved in various tissue injury process and various oxygen free radicals have been implicated in several biological processes potentially important in glomerular diseases. Previous reports suggest that cisplatin induced nephrotoxicity is by inhibition of lipid peroxidation and depletion of cellular thiols. Cisplatin inhibits the activity of antioxidant enzymes (Glutathion and lipid peroxidase) in rat kidneys suggesting that cisplatin nephrotoxicity results from generation of reactive oxygen species. A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental animal models[17]. One mechanism proposed is that cisplatin induces renal damage by free radical generation, by altering arginine metabolism and by increasing the activity of calcium dependant nitric oxide synthase[17,18].

Acute toxicity study was performed to check the safety profile of plant extract and observed that any death or hazardous effects has been observed in rats or not.

Antioxidant activity of plant extract was one of the supportive parameter in the nephroprotective activity of plant extract. The hydroalcoholic extract showed significant free radical scavenging activity in various models like ABTS scavenging, lipid peroxidation, total antioxidant and O-phenanthroline activity. Hence, the probable mechanism of nephroprotection by *Ocimum basilicum* may be attributed to its antioxidant and free radical scavenging property. In this plant extract various phenolics and tannins were present. They were mainly responsible for the nephroprotective activity of plant extract.

CONCLUSION

It can be concluded that our studies have shown that hydroalcoholic extract of *Ocimum basilicum* possess marked nephroprotective activity which, one is already reported in ancient literature under the heading of “Pashanbheda”. Further, isolation of active components and its nephroprotective activity in chronic renal failure model have to be evaluated.

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