

Phytoremedial effect of *Lens culinaris* against doxorubicin-induced nephrotoxicity in male Wistar rats

Sreedevi Adikay, K. Saisruthi

Division of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India

Abstract

Objectives: The main objective of current study was to determine the phytoremedial effect of ethanol extract of seeds of *Lens culinaris* against doxorubicin-induced nephrotoxicity using biochemical and histopathological approaches. **Materials and Methods:** Ethanol extract of seeds of *L. culinaris* was prepared by hot extraction method, and preliminary phytochemical studies had been carried out. Nephrotoxicity was induced in male Wistar rats by single intraperitoneal administration of doxorubicin (15 mg/kg b.wt.). Nephroprotector effect of the extract was screened at two different dose levels, i.e. 200 and 400 mg/kg b.wt. by oral administration for 8 days. Nephrotoxicity was assessed by determining blood urea nitrogen (BUN), serum creatinine (SC), serum total protein (S_{TP}), urinary total protein (U_{TP}), and urinary creatinine (U_{Cr}). Renal oxidative stress markers superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO), and glutathione reduced (GSH) were also measured in kidney tissue. **Results:** The treatment with ethanol extract significantly decreased the levels of BUN, SC, S_{TP} , U_{TP} and LPO which were elevated by doxorubicin induction. In addition, extract also ameliorated the levels of U_{Cr} , SOD, CAT and GSH in dose-dependent manner. Histopathological studies had also substantiated the biochemical parameters. **Conclusion:** The results suggest that ethanol extract of seeds of *L. culinaris* possess potent phytoremedial effect against doxorubicin-induced nephrotoxicity.

Key words: Doxorubicin, *Lens culinaris*, lipid peroxidation, nephrotoxicity

INTRODUCTION

Plants have been used for the treatment of various ailments for several thousands of years. Worldwide about 35,000-70,000 species have, at one time or another, been used in some cultures for medicinal purposes.^[1] In developing countries, the majority of population still relies on herbal medicines to meet their health needs due to their low cost, fewer side effects, and easy availability. Scientific evaluation of functionality of medicinal plants is essential to develop them as effective phytochemicals.^[2] In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions of herbs or to confirm the claims made about them in various traditional systems.^[3]

Lens culinaris is an important medicinal plant belonging to the family Fabaceae is commonly called masoor dal in Hindi and lentils in English. Seeds are good sources of essential minerals such as calcium, vitamin B, and constitute an important

source of food.^[4] Polyphenols, tannins, and tannin-related compounds are principal components in lentils. The seed coat is rich in flavonol glycosides and proanthocyanidins.^[5] Lentils are being considered as one of the most beneficial legumes for health.^[6] Earlier reports suggested lentils possess anti-diabetic, anti-cancer, anti-hyperlipidemic, and excellent free radical scavenging activity.^[7] They are used in traditional medicine as blood purifier, diuretic, and antifungal. In folklore medicine, lentils are used to treat various kidney and gastric ailments.^[8]

Nephrotoxicity commonly known as renal disease or dysfunction may result as a side effect of many drugs

Address for correspondence:

Sreedevi Adikay, Division of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.
E-mail: sreedevi@spmrv.ac.in

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including doxorubicin. Doxorubicin is an anthracycline antibiotic with high anti-tumor efficacy. It was found to be highly effective against hematological malignancies and a wide variety of solid tumors.^[9] Its clinical use is limited due to diverse toxicities including cardiac, renal, hepatic, and testicular toxicity. Doxorubicin-induced nephrotoxicity believed through free radical formation, iron-dependent oxidative damage of biological macromolecules, and membrane lipid peroxidation (LPO).^[10] Several medicinal plants extract rich in antioxidants found to be effective against doxorubicin-induced nephrotoxicity. Although antioxidant property of seeds of *L. culinaris* is well known and it is used by folklore for the treatment of strangury and as a diuretic, their renoprotective effect is not yet studied.^[11] Therefore, the current study is aimed to study the phytoremedial effect of ethanol extract of seeds of *L. culinaris* against doxorubicin-induced nephrotoxicity.

MATERIALS AND METHODS

Plant Material

Seeds of *L. culinaris* were purchased from the local market, authenticated by Botanist Dr. Madhavachetty, Herbarium Keeper, Department of Botany, Sri Venkateswara University, Tirupati, India, and a specimen has been deposited in Department of Botany, Sri Venkateswara University, Tirupati, India. Seeds were powdered in Wiley mill.

Preparation of Extract

Seed powder was defatted with petroleum ether (60-80°C). The defatted marc was air-dried and macerated with ethanol for 12 h. Macerated material was allowed for triple solvent extraction with ethanol for 3 h. The extract was concentrated under reduced pressure and obtained semisolid mass was kept at 4°C until use.

Preliminary Phytochemical Studies

Preliminary phytochemical studies had been carried out using standard procedures to identify the presence of alkaloids, glycosides, flavonoids, phenolic compounds, tannins, etc.^[12]

Experimental Animals

Albino-Wistar rats weighing 150-180 g were selected for the study. The animals were maintained in polypropylene cages. They had free access to standard pellets as basal diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee and carried out according to the guidelines of CPCSEA.

Acute Toxicity Studies

Acute toxicity studies were carried out according to the Organization for Economic Cooperation and Development 423 guidelines.^[13]

Experimental Design

After acclimatization, rats were randomly assigned into six groups of six each.

- Group I: Normal control (only vehicle from day 1 to 8)
- Group II: Vehicle day 1 to day 8 + doxorubicin (15 mg/kg) on day 4
- Group III: Lower dose of ethanol extract from day 1 to 8 + doxorubicin (15 mg/kg) on day 4
- Group IV: Higher dose of ethanol extract from day 1 to 8 + doxorubicin (15 mg/kg) on day 4
- Group V: Cystone (5 ml/kg) from day 1 to 8 + doxorubicin (15 mg/kg) on day 4
- Group VI: Only higher dose of ethanol extract from day 1 to 8.

On day 8, urine was collected with the help of metabolic cages and urine samples were subjected for estimation of urinary functional parameters. On day 9, animals were sacrificed by cervical decapitation and blood samples were collected by cardiac puncture and were used for estimation of serum markers. Kidneys were isolated and left kidney was used for assessing antioxidant parameters and right kidney for histopathological studies.

Assessment of Renal Function

Serum and urinary markers

Nephroprotector activity was evaluated in terms of biochemical parameters such as blood urea nitrogen (BUN) (diacetylmonoxime method), serum creatinine (SC) (Jaffe's alkaline picrate method), serum total protein (S_{Tp}), urinary total protein (U_{Tp}) (turbidity method), and urinary creatinine (U_{Cr}) (alkaline picrate method) were estimated using commercial kits in respective days as mentioned in the treatment protocol.^[14]

Anti-oxidant studies

Weighed portions of the kidneys were homogenized in ice cold 0.05 M phosphate buffer pH 7.8 to obtain a 20% (w/v) homogenate. The homogenates were centrifuged at 10,000 rpm for 15 min and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes. Antioxidant studies were carried out by the estimation of levels of renal oxidative stress markers namely catalase (CAT),^[15] superoxide dismutase,^[16] reduced glutathione (GSH),^[17] and LPO.^[18]

Histopathological studies

The isolated right kidney was fixed with 10% buffered formalin and processed to paraffin wax. Sections (5 μ) were

stained with hematoxylin and eosin and were examined under light microscope.

Statistical Analysis

The data were expressed as mean \pm standard deviation. Mean values between the groups were considered statistically significant $P < 0.05$ after analyzing by one-way ANOVA and was compared using Tukey-Kramer multiple comparison tests.

RESULTS

Preliminary Phytochemical Studies

Phytochemical screening of the ethanol extract of seeds of *L. culinaris* revealed the presence of alkaloids, saponins, flavonoids, glycosides, tannins, and terpenoids.

Acute Toxicity Studies

Animals which received extract at 2000 mg/kg b.wt. observed for 14 days had not shown any clinical signs of toxicity and mortality.

Nephroprotector Activity

Administration of high dose of ethanol extract for 8 days does not show any deteriorative effects on the kidney.

Serum and Urinary Markers

Animals treated with doxorubicin (15 mg/kg b.wt.) significantly increased the levels of SC, BUN, S_{TP} , U_{TP} and decreased the levels of U_{Cr} . Animals treated with ethanol extract at 200 and 400 mg/kg b.wt. reversed the effects induced by the doxorubicin in dose-dependent manner. Animals which received standard (cystone) also reversed all the effects induced by the doxorubicin [Table 1].

Anti-oxidant Levels

Administration of doxorubicin decreased the levels of CAT, super oxide dismutase (SOD), GSH and increased LPO levels in kidney tissue. Treatment with ethanol extract of *L. culinaris* increased CAT, SOD, GSH and decreased LPO levels significantly in dose-dependent manner [Table 2].

Histopathology

In histopathological examination, normal architecture was observed in normal animals whereas renal lesions including marked glomerular congestion, tubular necrosis, and inflammatory changes were observed in doxorubicin-induced animals. The renal lesions were reduced in animals received ethanol extract [Figures 1-6].

Table 1: Effect of ethanol extract of *L. culinaris* on renal functional parameters

Group	BUN (mg/dl)	SC (mg/dl)	S_{TP} (g/dl)	U_{TP} (mg/24 hrs)	U_{Cr} (mg/24 hrs)
I	14.50 \pm 0.78	1.06 \pm 0.05	5.75 \pm 0.08	1.62 \pm 0.15	17.63 \pm 0.53
II	35.28 \pm 1.34 ^a	2.53 \pm 0.18 ^a	6.88 \pm 0.10 ^a	4.54 \pm 0.22 ^a	1.67 \pm 0.22 ^a
III	20.77 \pm 0.49 ^b	1.63 \pm 0.13 ^b	6.33 \pm 0.05 ^b	3.18 \pm 0.12 ^b	3.65 \pm 0.26 ^{**b}
IV	19.94 \pm 0.79 ^b	1.40 \pm 0.06 ^b	6.20 \pm 0.04 ^b	2.45 \pm 0.12 ^b	7.08 \pm 0.11 ^b
V	24.68 \pm 0.95 ^b	1.63 \pm 0.03 ^{ns,b}	6.10 \pm 0.12 ^{ns,b}	2.30 \pm 0.15 ^{*b}	17.42 \pm 0.52 ^{ns,b}
VI	11.96 \pm 0.66 ^{ns,a}	0.91 \pm 0.04 ^{ns,a}	5.85 \pm 0.07 ^{ns,a}	1.09 \pm 0.07 ^{ns,a}	18.83 \pm 0.40 ^{ns,a}

Each value represents the Mean \pm S.E.M from 6 animals in each group. ^a $p < 0.05$ when compared with Group-I; ^b $p < 0.05$ when compared with Group-II; ns: not significant.

Table 2: Effect of ethanol extract of *L. culinaris* on anti-oxidant levels against doxorubicin induced nephrotoxicity

Group	LPO nmol/g of wet tissue	SOD U/mg of wet tissue	CAT U/mg of protein	GSH μ mol/g of wet tissue
I	1.15 \pm 0.26	42.63 \pm 0.60	42.37 \pm 0.74	80.68 \pm 0.59
II	7.73 \pm 0.39 ^a	16.80 \pm 0.35 ^a	11.88 \pm 0.42 ^a	34.91 \pm 0.32 ^a
III	5.03 \pm 0.34 ^b	27.37 \pm 0.59 ^b	30.92 \pm 0.22 ^b	46.38 \pm 0.47 ^b
IV	3.16 \pm 0.29 ^b	32.45 \pm 0.88 ^b	33.82 \pm 0.72 ^b	64.54 \pm 0.55 ^b
V	2.81 \pm 0.19 ^b	35.23 \pm 0.83 ^b	33.45 \pm 0.87 ^b	64.54 \pm 0.54 ^b
VI	1.27 \pm 0.16 ^{ns,a}	44.27 \pm 0.82 ^{ns,a}	42.87 \pm 0.65 ^{ns,a}	74.69 \pm 0.92 ^{ns,a}

Each value represents the Mean \pm S.E.M from 6 animals in each group. ^a $p < 0.05$ when compared with Group-I; ^b $p < 0.05$ when compared with Group-II; ns: not significant

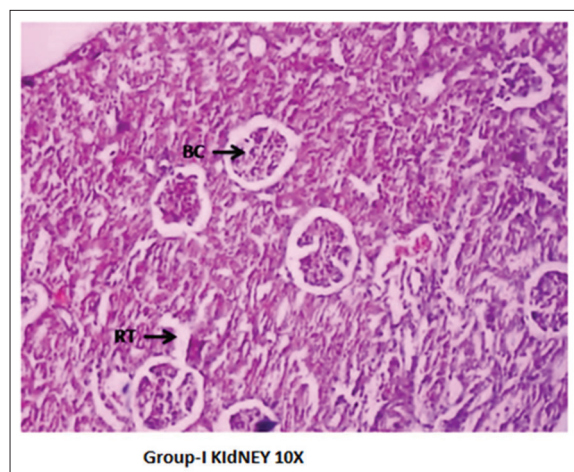


Figure 1: Section of rat kidney showing normal organization. BC: Bowman's capsule, RT: Renal tubule

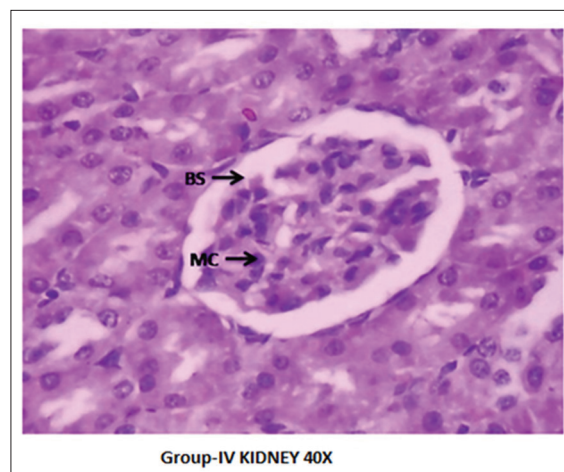


Figure 4: Section of rat kidney treated with ethanolic extract (400 mg/kg bd. Wt.) showing regenerative changes. BS: Bowman's space, MC: Messangial cells

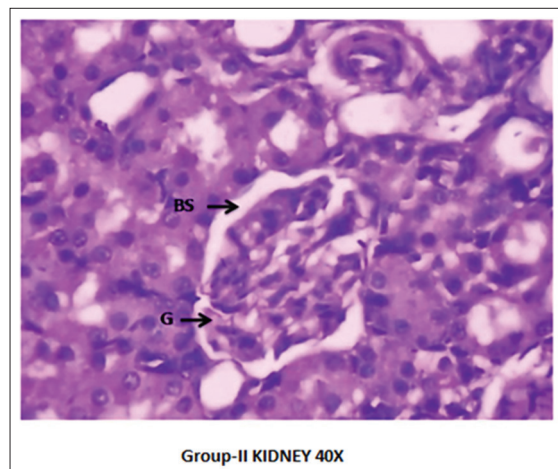


Figure 2: Section of rat kidney showing doxorubicin induced necrotic changes and congestion. BS: Bowman's space, G: Glomeruli

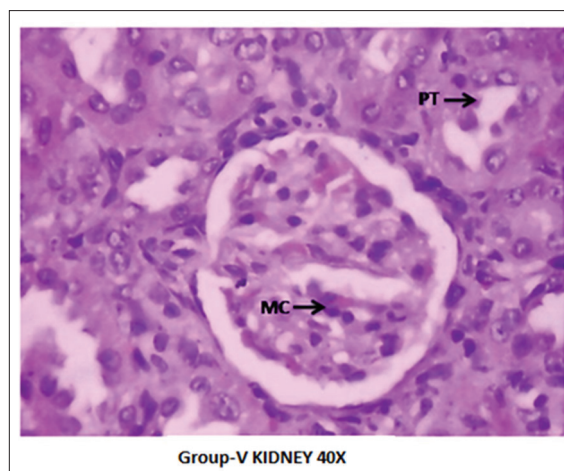


Figure 5: Section of rat kidney treated with standard (cystone) showing almost normal organization. PT: Proximal tubule; MC: Messangial cells

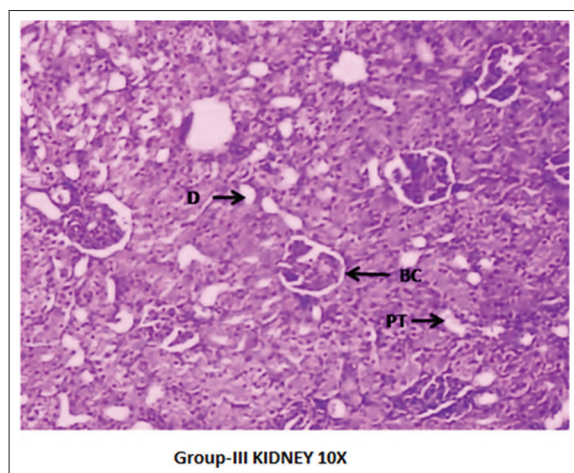


Figure 3: Section of rat kidney treated with ethanol extract (200 mg/kg bd.wt.) showing mild regeneration. D: Distal tubule, BC: Bowman's capsule, PT: Proximal tubule

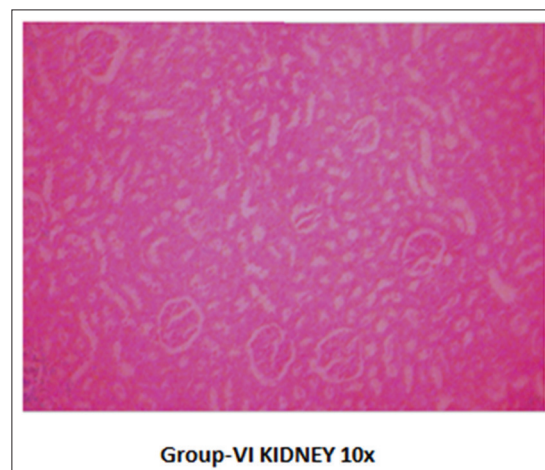


Figure 6: Section of rat kidney administered only with higher dose of extract showing normal architecture

DISCUSSION

Doxorubicin-induced nephrotoxicity is very well documented phenomenon in both humans and animal models.^[19,20] The proposed mechanism of doxorubicin-induced nephrotoxicity is through the formation of its free radical semiquinone form.^[21] The formed semiquinones are unstable in aerobic conditions and thereby generate superoxide anion radicals by interacting with molecular oxygen.^[22] The increase in initial free radicals leads to locally infiltrated neutrophils and activated glomerular mesangial cells to continue free radical production causing renal tissue damage which may impair the normal filtration process by doxorubicin.^[23,24] Administration of doxorubicin in rats significantly increased BUN, SC and S_{TP} which are in good agreement with those previous reported.^[25-27] Like *Solanum torvum*, *Zingiber officinale*, and *Olea europaea*, administration of ethanol extract of seeds of *L. culinaris* (200 and 400 mg/kg b.wt.) has resulted in significant decrease in BUN, SC and S_{TP} in doxorubicin-induced animals in dose-dependent manner thus offering considerable protection against doxorubicin-induced nephrotoxicity.^[27-29] In the current study ethanol extract of *L. culinaris* reduced the serum marker levels and reversal of the effects on urinary functional parameters may be due to amelioration of glomerular filtration rate. The nephroprotective mechanism also appears by modulation of various antioxidant parameters thereby ameliorating the antioxidant defense of renal tissue. Equilibrium between antioxidant enzymes is an important process for effective removal of oxygen stress in intracellular organelles. In the present study, administration of doxorubicin decreased the SOD, CAT, and GSH levels and increased LPO significantly which are in good agreement with previous reports.^[25-27] The ethanol extract at both doses significantly improved the antioxidant status in doxorubicin-treated animals in dose-dependent manner. The enhanced antioxidant activity of extract might be involved in the scavenging of free radicals generated from doxorubicin.^[30] The antioxidant activity may be attributed by the presence of phenolic compounds and flavonoids in seeds of *L. culinaris* which was confirmed by preliminary phytochemical studies.^[31] Further, results of histopathological studies revealed that extract has ameliorated the renal tissue damage induced by doxorubicin. Administration of high dose of ethanol extract alone for 8 days does not show any deteriorative effects on kidney revealing that the extract is safe. Thus the biochemical, antioxidant and histopathological results substantiated the nephroprotector activity of ethanol extract of seeds of *L. culinaris*.

CONCLUSION

The present study suggests that ethanol extract of seeds of *L. culinaris* possess potent phytoremedial effect against doxorubicin-induced nephrotoxicity. Furthermore, it provides corroborative scientific evidence for folklore use of seeds of the plant for the treatment of various renal ailments.

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