



Original Research Article (Experimental)

Effect of ethanolic extract of *Rosa centifolia* against doxorubicin induced nephrotoxicity in albino rats

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ABSTRACT

Background: Efficacy of Anthracycline derivative Doxorubicin (Dox) has been proven in several malignancies such as breast cancer, Hodgkin and non-Hodgkin lymphoma, acute leukemia, lung, thyroid and ovarian cancer. However its clinical usefulness is restricted due to its cardiotoxicity and nephrotoxicity. *Rosa centifolia* belongs to family Rosaceae and in Ayurveda it is claimed for use in renal disorders. The main phyto-constituents of the plant are terpenoids, glycosides, flavonoids, tannins, phenolic compounds, pro-antrocyanides, pectin and riboflavin.

Objective: To investigate the ameliorative role of ethanolic extract of petals of *R. centifolia* in doxorubicin induced nephrotoxicity in rats.

Materials and methods: Nephrotoxicity was produced by administration of doxorubicin (2.5 mg/kg b.w., i.p. alternate day) in six equal injections for two weeks to achieve a cumulative concentration of 15 mg/kg. Low (LERC - 100 mg/kg p.o.) and high (HERC - 200 mg/kg p.o.) doses of ethanolic extract of petals of *R. centifolia* was administered as a pretreatment prior to doxorubicin administration. The general parameters such as body weight, food and water intake were measured throughout the study period. Serum biomarkers such as blood urea nitrogen (BUN), serum creatinine and albumin were measured before treatment and at the end of the experiments. Anti-oxidant enzymes such as glutathione (GSH), melonldehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were monitored after the last dose. Nephrotoxicity was assessed through histopathological analysis.

Results: The repeated administration of doxorubicin produces several morphological changes including reduction in the body weight as well as decreased food and water consumption. Serum biomarkers such as BUN, serum creatinine were increased and albumin concentration was decreased. The GSH, SOD and CAT concentrations were decreased, whereas MDA concentration was increased. Deteriorating changes in the histological architecture of kidney tissue were observed. In the LERC and HERC pretreated groups following changes were observed in dose dependent manner: increase in body weight, food and water intake ($p < 0.05$ and $p < 0.01$), decrease in the BUN ($p < 0.05$ and $p < 0.01$) and serum creatinine ($p < 0.05$ and $p < 0.05$) concentrations respectively. The significant increase in the albumin ($p < 0.01$) concentration was observed only in HERC. The pretreatment with LERC and HERC increased the antioxidant enzymes concentrations i.e. GSH ($p < 0.01$ and $p < 0.01$), SOD ($p < 0.01$ and $p < 0.01$), CAT ($p < 0.05$ and $p < 0.01$) and decreased the MDA concentration ($p < 0.05$ and $p < 0.01$) respectively. Histopathological studies showed that the pretreatment with low and high doses of ethanolic extract of petals of *Rosa centifolia* LERC and HERC groups minimized the tubular damage and reduced the inflammation as compared to doxorubicin treated group.

Conclusion: The biochemical and histopathological data from the present study clearly support the nephroprotective effect of ethanolic extract of petals of *R. centifolia*, which might be credited to its anti-oxidant property.

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1. Introduction

The anthracycline derivative doxorubicin (Dox) was introduced in 1969 for the treatment of various cancer tumours such as breast cancer, Hodgkin and non-Hodgkin lymphoma, acute leukemias, lung, thyroid and ovarian cancer [1]. However, its use is restricted greatly due to multiple adverse effects leading to cardiac, kidney, blood and testicular toxicity [2,3]. In kidney, doxorubicin increases permeability in glomerular capillary and also produces glomerular atrophy [4]. Although the exact mechanism of nephrotoxicity induced by doxorubicin has not been completely understood, it has been hypothesized that it may cause free radical production and lipid peroxidation [5] and subsequently results in the formation of reactive oxygen species (ROS) like superoxide anion, OH⁻ radicals and H₂O₂ molecules. Organs with less developed anti-oxidants protective mechanisms have been reported as vulnerable in doxorubicin induced nephrotoxicity in rats [6]. Doxorubicin produces impairment in mitochondrial membrane binding creatinine kinase enzymatic activity and assembling. It also causes suppression of DNA, RNA and protein synthesis [7]. Doxorubicin stimulates the formation of free radicals as well as reactive oxygen and nitrogen species by two pathways. First is enzymatic pathway utilizing cellular oxidoreductases and second is non-enzymatic pathway utilizing complexation with iron (Fe³⁺) [8].

Although there are various therapeutic strategies available to prevent nephrotoxicity induced by doxorubicin, herbal preparations are preferred due to being economical, effective, easily available and safe [9]. The WHO estimates that 80% of world's population from developing countries use traditional medicines derived from plants [10]. Some plant extracts like *Lepidium sativum* [11], *Solanum torvum* [12] and proanthocyanidins [13] are scientifically proven as nephroprotective against doxorubicin induced nephrotoxicity.

Rosa centifolia Mill Rose flower belongs to family Rosaceae, found across India and has been traditionally used in culinary practices. Traditionally in Ayurveda, it has been used for the various disorders including cardiac and renal disorders [9,10]. Therapeutic uses of Rose petals include the heart diseases, wound healing, eye problems. Rose oil is useful in aromatherapy in insomnia and blood pressure [14]. *R. centifolia* is scientifically reported to have analgesic [15], anti-inflammatory [15], antibacterial [16], antioxidant [17], anti-tussive [18] and anti-depressant activities [19].

Literature review of the plant reveals the presence of following phytoconstituents terpenoids, glycosides, flavonoids, tannins, phenolic compounds, oligomeric compounds, proanthocyanidins, saccharine matter, mineral salts, salt of mallic acid and tartaric acid, pectin and riboflavin [20]. Earlier literatures reported that phytoconstituents like flavonoids [21] and triterpenoids [22] present in other plants are responsible for antioxidant activity and scientifically proven as nephroprotective. *R. centifolia* plant contains these types of antioxidant phytoconstituents. Therefore the present study was designed to evaluate the role of *R. centifolia* in doxorubicin induced nephrotoxicity in rats.

2. Materials and methods

2.1. Plant material

The authenticated petals of *R. centifolia* were procured in the month of April from Department of Botany, Sri Venkateshwar University, Tirupati, India. The petals were washed thoroughly with water, rinsed with distilled water to remove soil and foreign materials. The petals were shade dried grinded and sieved to obtain uniform powder of 40 mesh size. The petal powder was subjected to organoleptic evaluation like colour, odour and taste.

2.2. Ethanolic extract of petals of *Rosa centifolia* preparation and phytochemical investigation

Accurately weighed 25 g of petal powder and 250 mL of ethanol were placed in iodine flask and were kept for 1 h with occasional shaking. A reflux condenser was attached to the flask and the contents were allowed to boil for an hour. Later the ethanolic extract of petals of *Rosa centifolia* was cooled and concentrated using rotary flash evaporator [23]. The dried ethanolic extract was subjected to qualitative phytochemical investigation using various tests such as Molish's, Shinoda, Liebermann Burchard, Ferric chloride etc [24].

2.3. Chemicals

Doxorubicin was a gift sample from Get Well Pharmaceuticals, India. Other analytical grade chemicals and enzyme assay kits were procured from Sigma Aldrich and ERBA Mannheim, India respectively.

2.4. Animals

Total 30 normal Wistar rats (12 females) of either sex weighing 150–200 g and six female mice of 18–25 g were used, after securing the ethical approval from Institutional Animal Ethical Committee (Ref. No. KLEU's-08-IAEC.HBL-31/Aug2013). All the animals were housed in a group of six under environmentally controlled room with 12 h light/dark cycle in polypropylene cages and maintained at controlled room temperature (22 ± 2 °C) and relative humidity of 40–60% with free access to standard laboratory chow (Gold Mohur Lipton India Ltd.) and water *ad libitum* was provided. Before the initiation of experiment, rats were acclimatized for seven days to laboratory environment.

2.5. Acute oral toxicity

The toxicity studies were carried out as per OECD guidelines, revised draft 423. The albino mice were chosen to carry out acute toxicity studies by up and down method. Ethanolic extract of petals of *R. centifolia* was administered at a dose of 2000 mg/kg body weight orally and food was withheld for up to 4 h after administration of the extract. The animals were observed for changes in general behavioral, weight, tremor, convulsion, salivation, sleep, skin, eye and death at 30 m, 1, 2, 3, 4, 24 h and once daily for remaining 14 days [25].

2.6. Experimental design

After the end of one week of acclimatization period, the rats were divided into five groups of six animals in each as follows [26].

Group I received vehicle 5 mL/kg (saline) body weight *p.o* (4 males and 2 females).

Group II were treated with doxorubicin 2.5 mg/kg body weight by *i.p.* in 6 equal injections on alternate days for 2 weeks (3 males and 3 females).

Group III (ERC) received only ethanolic extract of petals of *Rosa centifolia* (200 mg/kg body weight *p.o.*) daily for 2 weeks. For next two weeks the vehicle was administered on alternative day (4 males and 2 females).

Group IV (LERC) received low dose (100 mg/kg body weight *p.o.*) of ethanolic extract of petals of *Rosa centifolia* for 2 weeks as a pretreatment followed by doxorubicin as in group II (4 males and 2 females).

Group V (HERC) received high dose (200 mg/kg body weight *p.o.*) of ethanolic extract of petals of *Rosa centifolia* for 2 weeks as a

pretreatment followed by doxorubicin as in group II (3 males and 3 females).

2.7. Body weight, food and water

Body weight, food and water intake were regularly monitored throughout the study period, before and after the treatment with doxorubicin for all the animals.

2.8. Serum biomarkers

Blood samples were collected at the end of the study period by retro orbital route and plasma was separated by centrifuging and concentrations of blood urea nitrogen (BUN), serum creatinine (SCr) and albumin were assessed by commercially available kits using clinical chemistry analyzer (Chem7, Erbamannheim).

2.9. Enzyme assays in kidney tissue

The animals were anaesthetized by isoflurane and sacrificed by carotid bleeding followed by quick dissection of kidney tissue. The kidney tissue was washed by cold saline, dried using filter paper and weighed. Kidneys of all the animals were used to prepare 10% w/v homogenate in Tris–HCl buffer (pH 7.4). The homogenates were processed for the estimation of endogenous antioxidants in kidney tissue such as glutathione (GSH), malonaldehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The remaining portion was used for histopathological studies.

2.9.1. Estimation of reduced glutathione (GSH) in kidney tissue

The GSH was determined by Ellman method [27]. One mL of homogenate was added to 1 mL of 10% TCA, centrifuged and the supernatant was separated. One mL of supernatant was treated with 0.5 mL of reagent (Ellman), 3 mL of buffer was added and the absorbance was recorded immediately at 412 nm. The amount of glutathione is calculated using the absorption $13,600 \text{ M}^{-1} \text{ cm}^{-1}$.

2.9.2. Estimation of lipid peroxidation (MDA) in kidney tissue

It was assessed by TBARS (thiobarbituric acid reactive species) using MDA as standard by Buege and Aust method [28]. Homogenate (0.1 mL) was added to 2 mL of TCA- TBA- HCl reagent and then boiled for 15 min. After that centrifugation at 1000 rpm for 10 min, the absorbance of supernatant was measured at 535 nm and malondialdehyde concentration of the sample was calculated.

2.9.3. Estimation of superoxide dismutase (SOD) in kidney tissue

The estimation was determined by Kakkar et al. method [29]. Sample (0.1 mL) was mixed with sodium pyrophosphate (1.2 mL), phenazine methasulphate (0.1 mL) and nitro blue tetrazolium (0.3 mL). By addition of NADH (0.2 mL) the reaction was started and the mixture was incubated for 90sec at 30 °C. The reaction was halted by addition of glacial acetic acid (0.1 mL), later it was stirred briskly with n-butanol (4 mL). The reaction mixture was allowed to stand for 10 min, centrifuged and the butanol layer was separated. The chromogen colour intensity was measured (against butanol) at 560 nm by spectrophotometer. A system devoid of enzyme activity was defined as enzyme concentration required for decreasing the rate of reaction by 50% in 1 min under the assay conditions.

2.9.4. Estimation of catalase (CAT) in kidney tissue

Catalase (CAT) was assayed calorimetrically by Sinha method [30]. Supernatant homogenate was added to phosphate buffer (1 mL, pH 7) and hydrogen peroxide (0.4 mL, 0.2 M) and by the addition of dichromate acetic acid, reaction was stopped followed by measuring the colour intensity at 620 nm calorimetrically and

expressed as μ moles of hydrogen peroxide consumed per min per mg protein.

2.10. Histopathological studies [31]

Kidneys were isolated after sacrificing the animals. The isolated kidneys were washed with saline, cut into pieces and preserved in 10% neutral formalin solution for two days and then pieces were washed with running water for 12 h followed by dehydration with alcohol. The kidney tissue was cleaned by xylene two times for 15–20min each followed by subjecting to paraffin infiltration in automatic tissue processing unit.

The hard paraffin was heated and melted paraffin was poured in square shaped blocks in which the kidney pieces were dropped quickly and permitted to cool. Microtome was used to cut the blocks to get 5 micrometer thickness sections. These sections were placed on a microscopic slide with precoated sticky substance and the section was dried completely before staining. The acidic stain (eosin) and basic stain (haematoxyllin) were used for staining the sections followed by observing in microscope for any changes in histopathological characteristics.

2.11. Statistical analysis

The experimental data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test by using Graph Pad Prism 5.0 software. Data were expressed as Mean \pm S.E.M. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Phytochemical constituents in ethanolic extract petals of *R. centifolia*

The phytochemical investigation showed the presence of carbohydrates, steroids, triterpenoids, glycosides, saponins, flavonoids, alkaloids, tannins and phenolic compounds.

3.2. Acute oral toxicity

By acute oral toxicity studies, we observed that lethal dose of ethanolic extract of petals of *R. centifolia* was more than 2000 mg/kg body weight. So 1/10th and 1/20th of the 2000 mg/kg body weight was chosen for further studies.

3.3. General observations

Doxorubicin treated animals developed a scruffy fur and red exudates round the eyes and soft watery faeces. At the site of doxorubicin injection necrosis was also observed. These conditions were exacerbated during the last days of study period. However, these changes were less severe in pretreated extract groups.

3.4. Food and water consumption

In doxorubicin administered group, food and water intake was reduced significantly as compared to control group. In treatment groups i.e. ERC ($p < 0.001$), LERC ($p < 0.05$) and HERC ($p < 0.01$) showed significantly improved food and water consumption as compared to doxorubicin group (Fig. 1).

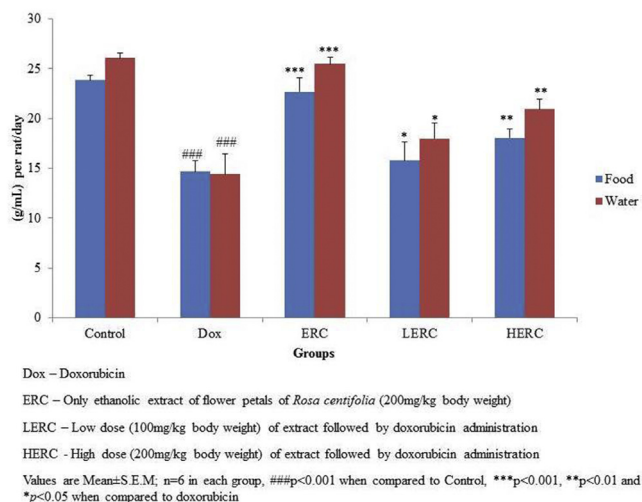


Fig. 1. Effect of ethanolic extract of petals of *Rosa centifolia* on food and water intake.

3.5. Body weight

In doxorubicin treated group, body weight was significantly reduced as compared to normal group. In treatment groups i.e. ERC ($p < 0.001$) maintained the animal weight and HERC ($p < 0.01$) significantly reduced the weight loss as compared to doxorubicin treated group, whereas LERC showed no significant changes compared to doxorubicin treated group (Table 1).

3.6. Biochemical parameters (serum biomarkers)

Doxorubicin treated animals produced a significant increase in serum enzyme markers such as BUN ($p < 0.001$) and SCr ($p < 0.001$), whereas albumin was reduced ($p < 0.001$) compared to control group. The pretreatment groups i.e. LERC and HERC decreased the levels BUN ($p < 0.05$ and $p < 0.01$), SCr ($p < 0.05$ and $p < 0.05$) respectively and increased the albumin level (only in HERC, $p < 0.01$) as compared with doxorubicin treated group (Table 1).

3.7. Antioxidant enzymes in kidney tissue

Doxorubicin treated animals showed increase in MDA levels ($p < 0.001$) and decrease in GSH ($p < 0.001$), SOD ($p < 0.001$) and CAT ($p < 0.001$) in kidney tissue compared to control group, but

Table 1
Effect of ethanolic extract of petals of *Rosa centifolia* on body weight, serum markers and anti-oxidant enzymes.

Variables	Groups				
	Control	Dox	ERC	LERC	HERC
Body Weight	184.2 ± 1.94	136.5 ± 2.89###	180.2 ± 1.83***	142.7 ± 2.65	155.5 ± 2.37**
Serum Markers					
BUN (mg/dL)	20 ± 4.28	44 ± 6.42###	18 ± 5.54***	38 ± 10.12*	32 ± 9.45**
SCr (mg/dL)	0.62 ± 4.12	2.45 ± 3.54###	0.80 ± 3.65***	1.90 ± 6.75*	1.62 ± 8.15*
Albumin (g/dL)	2.80 ± 0.84	0.80 ± 0.54###	2.62 ± 0.44***	0.82 ± 0.52	1.12 ± 0.24**
Anti-oxidant enzymes					
MDA (n mole/mg of wet tissue)	18.75 ± 2.25	58.88 ± 3.56###	20.51 ± 2.66***	52.44 ± 3.96*	46.14 ± 4.14**
GSH (n mole/mg of wet tissue)	24.57 ± 2.59	10.11 ± 2.35###	22.24 ± 3.55***	15.14 ± 3.66*	18.22 ± 3.48**
SOD (Unit/mg protein)	59.45 ± 2.46	30.22 ± 2.95###	60.44 ± 3.82***	41.12 ± 3.45**	48.56 ± 2.96**
CAT (Unit/mg protein)	55.95 ± 3.66	31.12 ± 3.22###	56.14 ± 3.92***	39.66 ± 2.53*	44.18 ± 3.55**

Values are Mean ± S.E.M; n = 6 in each group, ###p < 0.001 when compared to Control, ***p < 0.001, **p < 0.01 and *p < 0.05 when compared to doxorubicin.
 Dox – Doxorubicin (2.5 mg/kg b.w., i.p.) alternate day in six equal injections for two weeks to become cumulatively 15 mg/kg.
 ERC – Only ethanolic extract of petals of *Rosa centifolia* (200 mg/kg body weight, p.o.).
 LERC – Low dose (100 mg/kg body weight, p.o.) of ethanolic extract of petals of *Rosa centifolia* followed by doxorubicin administration.
 HERC - High dose (200 mg/kg body weight, p.o.) of ethanolic extract of petals of *Rosa centifolia* followed by doxorubicin administration.

pretreatment groups i.e. LERC and HERC had decreased levels of MDA ($p < 0.05$ and $p < 0.01$) and increase in the levels of GSH ($p < 0.01$ and $p < 0.01$), SOD ($p < 0.01$ and $p < 0.01$) and CAT ($p < 0.05$ and $p < 0.01$) respectively as compared to doxorubicin group (Table 1).

3.8. Histopathological studies

Doxorubicin treated rat renal tissue exhibited tubular damage and moderate inflammation. Whereas normal group showed normal morphological appearances but pretreated groups i.e. LERC and HERC showed minimal tubules damage and less inflammation as compared to doxorubicin treated group (Fig. 2).

4. Discussion

In present study, effect of ethanolic extract of *R. centifolia* petals against doxorubicin induced nephrotoxicity was assessed by general parameters, biochemical parameters and histopathological studies. It is reported that rats treated with doxorubicin show reduced food and water intake by more than 50% within a few days of treatment [32] due to lack of appetite (anorexia) produced as the adverse event of doxorubicin treatment. In present study, the ethanolic extract of petals of *Rosa centifolia* showed statistically significant ($p < 0.01$) increase in the body weight, food and water intake in higher doses compared to doxorubicin treated group. This indicated the reduction of adverse event of doxorubicin by *R. centifolia* plant.

Doxorubicin induced nephrotoxicity was characterized by decrease in glomerular filtration rate (GFR) and increase in the level of BUN and SCr, which are the most sensitive signs of nephrotoxicity concerned in renal injury diagnosis [33]. Our results indicating the increase in BUN and SCr are in accordance with the previous studies [34,35]. In the present study, the extract of *R. centifolia* restored the BUN and SCr levels as compared to doxorubicin treated group and this might be due to its antioxidant property, based on the observations reported by previous studies that recovery from doxorubin induced nephrotoxicity occurs through the anti-oxidant properties of the intervention [35,36].

Doxorubicin treated group showed reduction in plasma albumin concentration, resulting in hypoalbuminemia, which is in accordance with the previous studies [37,38]. In the present study the ethanolic extract of petals of *Rosa centifolia* showed statistically significant ($p < 0.01$) increase in the albumin level compared to doxorubicin treated group.

In doxorubicin treated group, there was decrease in the concentrations of SOD, CAT and GSH enzymes, while increase in the

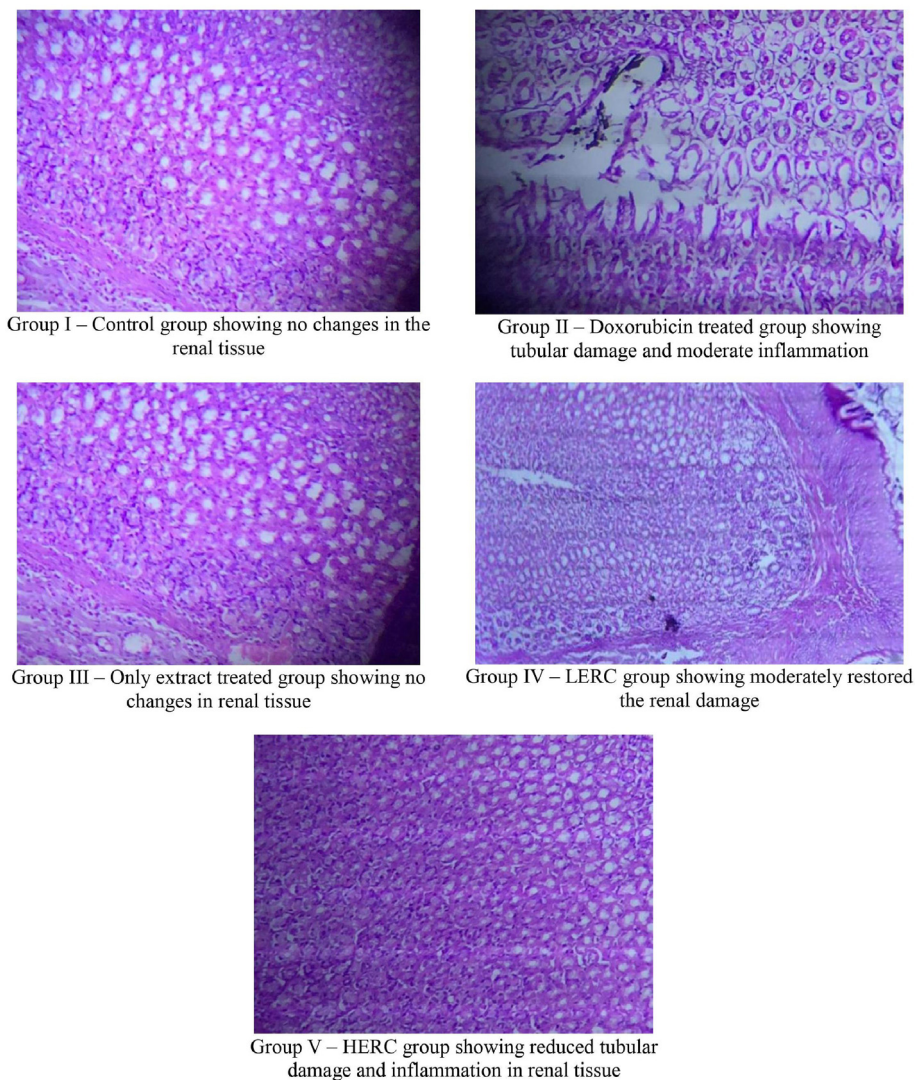


Fig. 2. Effect of ethanolic extract of petals of *Rosa centifolia* on histopathology of kidney tissue.

concentration of MDA. These observations are in accordance with the earlier studies [39]. Several articles have reported the occurrence of increased oxidative stress as a result of decrease in antioxidant enzymes, leading to a sequence of reactions, ultimately causing doxorubicin induced heart muscle and kidney damage [40]. Accumulation of Doxorubicin in glomerulus damages kidney but the exact mechanisms are not yet elucidated [41]. Literature survey reveals that doxorubicin stimulates semi quinone radical formation, which combines with O_2 and produces other free radicals at preliminary stage, leading to locally in-filtered neutrophils and glomerular mesangial cells continue to produce free radical [42,43].

Superoxide dismutase enzyme catalyzes the dismutation of molecular oxygen to hydrogen peroxide and molecular oxygen (O_2), while glutathione peroxidase and catalase enzymes catalyze the degradation of hydrogen peroxide to O_2 and H_2O [44]. In detoxification of xenobiotic compounds, these enzymes play an important role as it produces anti-oxidation of free radicals. Low levels of these are associated with excessive oxidative stress [45]. In the present study the ethanolic extract of petals of *Rosa centifolia* showed statistically significant increase in the superoxide dismutase ($p < 0.01$), glutathione ($p < 0.01$) and catalase ($p < 0.01$) enzymes indicating that the nephroprotective property of ethanolic extract of petals of *Rosa centifolia* might be due to degradation of the free radicals. As

Deman et al. [43] reported that enhanced concentrations of reduced glutathione particularly in renal cortex supported the involvement free radicals in doxorubicin nephrotoxicity.

In doxorubicin treated group malondialdehyde level was increased compared to control group resulting in increased lipid peroxidation. It is the end product of polyunsaturated fatty acids peroxidation in the cells. Increased production of malondialdehyde occurs when there is increase in free radicals and it is commonly considered as a biomarker of oxidative stress [46]. In the present study, the ethanolic extract of petals of *Rosa centifolia* showed statistically significant ($p < 0.01$) decrease in the malondialdehyde concentration compared to doxorubicin treated group and indicated the ethanolic extract of petals of *Rosa centifolia* was reduced the lipid peroxidation as well as reduced the free radical generation and oxidative stress and this might be responsible for its nephroprotective activity.

In histopathologic studies of kidney tissues, of doxorubicin treated rats showed tubular damage, inflammation and degenerative changes in the renal tubules and glomeruli compared to control group and these changes are in accordance with the previously reported studies [47,48]. In the present study ethanolic extract of petals of *Rosa centifolia* was ameliorated the histopathological damage caused by doxorubicin.

5. Conclusion

The present study signifies the ethanolic extract of petals of *R. centifolia* reduced the nephrotoxicity induced by cumulative administration of doxorubicin in rats. The study revealed that ethanolic extract of petals of *Rosa centifolia* may be considered as useful in combination with doxorubicin. However, further elucidation of the cellular and molecular mechanisms would provide the robust evidence for nephroprotective effects of ethanolic extract of petals of *Rosa centifolia*.

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Conflict of interest

None.

Author contributions

S.K. Nimbale: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation. **Pramod C. Gadad:** Writing - original data, Writing - Review & Editing, Supervision. **Basavaraj C. Koti:** Conceptualization, Supervision, Validation.

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References

- Blum RH, Carter SK. Adriamycin. A new anticancer drug with significant clinical activity. *Ann Intern Med* 1974;80(2):249–59.
- Gillick J, Giles S, Bannigan J, Puri P. Cell death in the early adriamycin rat model. *Pediatr Surg Int* 2002;18:576–80.
- Yilmaz S, Atesahin A, Sahna E, Karahan I, Ozer S. Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. *Toxicology* 2006;218:164–71.
- Saad SY, Najjar TA, Al-Rykaby AC. The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. *Pharmacol Res* 2001;43:211–8.
- Doroshov JH. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. *Canc Res* 1983;43(2):460–72.
- Rajaprabhu D, Rajesh R, Jeyakumar R, Buddhan S, Ganesan B, Anandan R. Protective effect of *Picrorhizakarroa* on antioxidant defense status in adriamycin-induced cardiomyopathy in rats. *J Med Plants Res* 2007;1:80–5.
- Olson RD, Mushlin PS. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. *Faseb J* 1990;4(13):3076–86.
- Gille L, Nohl H. Analyses of the molecular mechanism of adriamycin-induced cardiotoxicity. *Free Radic Biol Med* 1997;23(5):775–82.
- Vikrant A, Gupta VK. Chemistry and Pharmacology of plant cardioprotectives: a review. *Int J Pharm Sci Res* 2011;2(5):1156–67.
- Tilak-jain JA, Paul T, Devasagayam A. Cardioprotective and other beneficial effects of some Indian medicinal plants. *J Clin Biochem Nutr* 2006;38:9–18.
- Shinde N, Jagtap A, Undale V, Kakade S, Kotwal S, Patil R. Protective effect of *Lepidium sativum* against doxorubicin-induced nephrotoxicity in rats. *Res J Pharmaceut Biol Chem Sci* 2010;1(3):42–9.
- Mahalaxmi M, Kamble S, Gadhi P, Kasture S. Protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats. *Food Chem Toxicol* 2010;1(48):436–40.
- El-Sayed EM, Mansour AM, El-Sawy WS. Protective effect of proanthocyanidins against doxorubicin-induced nephrotoxicity in rats. *J Biochem Mol Toxicol* 2017;31(11):1–6.
- Chauhan Meenakshi. Rose (*Rosa centifolia*) – medicinal properties, benefits, Dosage [updated 2019 April 17; cited 2020 Jan 12]. Available from: <https://www.planetayurveda.com/library/rose-rosa-centifolia/>.
- Helen A, Rajasree CR, Krishnakumar K, Augusti KT, Vijayammal PL. Antioxidant role of oils isolated from garlic (*Allium sativum* Linn) and onion (*Allium cepa* Linn) on nicotine-induced lipid peroxidation. *Vet Hum Toxicol* 1999;41(5):316–9.
- Balaseshthil S, Arivazhagan S, Nagini S. Garlic enhances circulatory antioxidants during 7,12-dimethylbenz[*a*]anthracene-induced hamster buccal pouch carcinogenesis. *J Ethnopharmacol* 2000;72(3):429–33.
- Subramanian M, Sreejayan, Devasagayam TPA, Singh BB. Diminution of singlet oxygen-induced DNA damage by curcumin and related antioxidants. *Mutat Res* 1994;311(2):249–55.
- Sreejayan N, Rao MNA, Priyadarsini KI, Devasagayam TPA. Inhibition of radiation-induced lipid peroxidation by curcumin. *Int J Pharm* 1997;151(1):127–30.
- Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *J Agric Food Chem* 2000;48(2):180–5.
- Jitendra J, Vineeta T, Ashok K, Brijesh K, Singh P. *Rosa centifolia*: plant review. *Int J Res Pharm Chem* 2012;2(3):794–6.
- Liao CH, Ho CT, Lin JK. Effects of garcinol on free radical generation and NO production in embryonic rat cortical neurons and astrocytes. *Biochem Biophys Res Commun* 2005;329(4):1306–14.
- Grover J. Hepato and cardio protective action of tulsi (*Ocimum sanctum* Linn). *Eur J Pharmacol* 1990;183(3):872.
- Gupta S, Sharma M, Kishore K, Arya D. *Ocimum sanctum* provides cardioprotection in isoproterenol induced myocardial infarction in rats. *J Mol Cell Cardiol* 2001;33:A42.
- Harborne J. *Phytochemical Methods, A guide to modern techniques of plant analysis*. 3rd ed. London (UK): Chapman & Hall; 2007. p. 16–32.
- OECD Guidelines 423 for testing of chemicals. 2001. p. 1–26.
- Mansour MA, El-Kashef HA, Al-Shabanah OA. Effect of captopril on doxorubicin-induced nephrotoxicity in normal rats. *Pharmacol Res* 1999 Mar;39(3):233–7.
- Ellman George L. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70–7.
- Niehaus WG, Samuelsson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 1986;6:126–30.
- Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984;21(2):130–42.
- Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972;47(2):389–94.
- Agadihiremath VS, Sumeet G, Agadihiremath T, Basavaraj CK, Donnahalli VM. Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. *Indian J Pharmacol* 2012;44(1):73–7.
- Gilliam G, St Clair Dk LAA. Chemotherapy-induced weakness and fatigue in skeletal muscle: the role of oxidative stress. *Antioxidants Redox Signal* 2011;15(9):2543–63.
- Refaie MM, Amin EF, El-Tahawy NF. Possible protective effect of diacerein on doxorubicin-induced nephrotoxicity in rats. *J Toxicol* 2016;9507563.
- Rashid S, Ali N, Nafees S, Ahmad ST, Arjumand W, Hasan SK, et al. Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. *Toxicol Mech Methods* 2013 Jun;23(5):337–45.
- Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of Solanum torvum on doxorubicin-induced nephrotoxicity in rats. *Food Chem Toxicol* 2010;48:436–40.
- Ajith TA, Aswathy MS, Hema U. Protective effect of *Zingiber officinale* roscoe against anticancer drug doxorubicin-induced acute nephrotoxicity. *Food Chem Toxicol* 2008;46:3178–81.
- Rashikh A, Pillai KK, Ahmad SJ, Akhtar M, Najmi AK. Aliskiren alleviates doxorubicin-induced nephrotoxicity by inhibiting oxidative stress and podocyte injury. *J Renin-Angiotensin-Aldosterone Syst JRAAS* 2013;14:14–22.
- Mansour M, El-Kashef H, Al-Shabanah O. Effect of captopril on doxorubicin-induced nephrotoxicity in normal rats. *Pharmacol Res* 1999;39:239–45.
- Koti BC, Nagathan S, Vishwanathswamy A, Gadad PC, Thippeswamy A. Cardioprotective effect of Vedic Guard against doxorubicin-induced cardiotoxicity in rats: a biochemical, electrocardiographic, and histopathological study. *Phcog Mag* 2013;9(34):176–81.
- Kumar D, Kirshenbaum LA, Danelisen I, Singal PK. Apoptosis in adriamycin cardiomyopathy and its modulation by probucol. *Antioxidants Redox Signal* 2001;3:135–45.
- Liu Hai-Xia, Jin Li, Li Qi-Xiong. Therapeutic effect of valsartan against doxorubicin-induced renal toxicity in rats. *Iran J Basic Med Sci* 2019;22(3):251–4.
- Shah SV. Role of reactive oxygen metabolites in experimental glomerular disease. *Kidney Int* 1989;35:1093–106.
- Demian A, Ceysens B, Pauwels M, Zhang J, Houte KV, Verbeelen D, et al. Altered antioxidant defence in a mouse adriamycin model of glomerular sclerosis. *Nephrol Dial Transplant* 2001;16(1):147. 5.
- Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK. Kidney ischemia-reperfusion: modulation of antioxidant defenses. *Mol Cell Biochem* 2000;205:1–11.
- Bray TM, Taylor CG. Tissue glutathione, nutrition, and oxidative stress. *Can J Physiol Pharmacol* 1993;71(9):746–51.
- Gawet S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad Lek* 2004;57(9–10):453–5.
- Ayla S, Seckin I, Tanriverdi G, Cengiz M, Eser M, Soner BC, et al. Doxorubicin induced nephrotoxicity: protective effect of nicotinamide. *Int J Cell Biol* 2011;2011:390238.
- Azza AK, Mohamed AM, Marwa MM, Rehab AR, Aly MA. Effect of coenzyme-Q10 on doxorubicin-induced nephrotoxicity in rats. *Adv Pharmacol Sci* 2012: 981461.