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# Protective effect of *Lepidium sativum* against doxorubicin-induced nephrotoxicity in rats

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**ABSTRACT**

Nephrotoxicity is one of the important side effects of anthracycline antibiotics. The aim of this study was to investigate the effects of aqueous extract of *Lepidium sativum* L. against nephrotoxicity induced by doxorubicin (DXN).The rats were divided into control, *Lepidium sativum* (LS) alone, doxorubicin (15 mg/kg, i.p.) and doxorubicin plus LS (200 mg/kg, p.o.) and doxorubicin plus LS (400 mg/kg, p.o.) groups. At the end of the 72 hr, kidney tissues were removed for light microscopy and analysis. The levels of tissues malondialdehyde (MDA), the activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) were determined. Serum creatinine and blood urea nitrogen were also measured. The serum urea and creatinine levels in the DXN alone treated group were significantly elevated ($P<0.001$) with respect to normal group of animals. The levels were reduced in the *Lepidium sativum* (200 and 400 mg/kg, p.o) treated groups. The renal antioxidant enzymes such as superoxide dismutase, catalase activities and level of reduced glutathione were declined; level of malondialdehyde was elevated in the DXN alone treated group. The activities of SOD, CAT and level of GSH were elevated and level of MDA declined significantly in the *Lepidium sativum* (200 and 400 mg/kg) plus DXN. Additionally, histopathological examination and scoring showed that *Lepidium sativum* markedly ameliorated DXN-induced renal tubular necrosis. *Lepidium sativum* can be considered a feasible candidate to protect against nephrotoxicity commonly encountered with doxorubicin treatment.

**Keywords:** *Lepidium sativum* L., doxorubicin, nephrotoxicity, blood urea nitrogen, serum creatinine.

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## INTRODUCTION

Doxorubicin, an anthracyclin antibiotic represents a class of anticancer agents. It shows broad spectrum antitumour activities in certain human cancers including breast cancer, small cell carcinoma of the lung and acute leukaemia [1]. The optimal use of doxorubicin is limited by a number of side-effects, the most important are cardiotoxicity, haematotoxicity [2] and a dose-limiting nephrotoxicity [3]. The exact mechanism of doxorubicin-induce nephrotoxicity is not yet known. However, it has been suggested by many investigators that cellular damage induced by doxorubicin is mediated by the formation of an wx iron anthracyclin free radical [4, 5] which in turn causes severe damage to the plasma membrane [6].

*Lepidium sativum* L. locally known as 'hab arachad' belonging to the family Brassicaceae where LS is largely recommended by traditional herbal healers for hypertension, diabetes control, renal disease and phytotherapy [8]. The seeds and leaves of the plant contain volatile oils [7]. The seeds are consumed in salad and as spice[9]. The plant is also reported to possess haemagglutinating, hypoglycemic, antihypertensive, diuretic and fracture healing property [10,11]. Previous studies have been demonstrated the protective action of LS against carcinogenic compounds [12] and growth inhibition of Pseudomonas aeruginosa, a bacteria strain with a potent antibiotic resistance [13].

## MATERIALS AND METHODS

### Plant Material

Seeds of *L. sativum* were purchased from a commercial supplier, identified and authenticated by Dr. A. M. Mujumdar at Agharkar Research Institiute, Pune (Voucher no. AHMA S-112), where herbarium was deposited. The seeds were dried in shade and then powdered in grinder.

### Drugs

Doxorubicin was obtained as gift sample from Serum Institute of India LTD, Pune, Maharashtra, India.

### Animals

Wistar rats either sex weighing between 150-220 g were used for this study. The animals were obtained from the animal house, SGRS College of Pharmacy, Saswad, Pune, Maharashtra, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee

(SGRS/IAEC/06/2008-09) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), ministry of Social Justice and Empowerment, Government of India, New Delhi.

**Preparation of the extract**

The aqueous extract was prepared in a standardized manner by boiling 1 g of dried powdered seeds of LS in 100ml of distilled water for 10min and left for 15min to infuse. Thereafter, the extract was cooled and filtered before use to remove particular matter. The filtrate was lyophilized and the desired dose (milligram of lyophilized aqueous LS extract per kilogram body weight) was then prepared and reconstituted in 10ml of distilled water per kilogram body weight just before oral administration.

**Experimental design**

Doxorubicin-induced renal injury five groups of six rats each were used for the study. Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II injected with a single dose of DXN (15 mg/kg body wt., i.p) was kept as control. Groups III and IV were treated with extract of *Lepidium sativum* (200 and 400 mg/kg body wt.) plus DXN. Group V was treated with extract of *Lepidium sativum* (400 mg/kg. Single dose of the extract was administered by oral gavage 1 h before DXN injection. The animals were sacrificed 72 h after the injection of doxorubicin using ether anesthesia; blood was collected directly from heart of each animal. Serum was separated for the estimation of blood urea nitrogen and creatinine.

At the end of the experiments, kidneys were removed rapidly, sectioned for histological analysis. The remaining kidney tissues were homogenized in Tris–HCl buffer (0.05 mol/l Tris–HCl, pH 7.4), using a Polytron homogeniser. The homogenate was centrifuged at 18,000×g (+4°C) for 30 min; the supernatant was utilized for biochemical analysis.

**Biochemical assays**

Serum creatinine (Jaffe's kit) and blood urea nitrogen (DAM kit) concentrations were measured using a diagnostic kit. The concentrations of the malondialdehyde (MDA) were determined according to the method based on the reaction with thiobarbituric acid [14]. Superoxide dismutase (SOD) activity was assayed in cytosolic fraction following the inhibition of pyrogallol autooxidation [15]. Glutathione (GSH) level was measured colorimetrically as protein-free sulfhydryl content using 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) [16]. CAT activity was determined from the rate of decomposition of $H_2O_2$ at 240 nm followed by the addition of tissue homogenate [17].

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## Histopathology

All tissues were formalin fixed and then processed with varying grades of alcohol followed by Xylene and paraffin. Tissues were embedded in paraffin and cut at 5 microns using a microtome (Labcon, HM 22022). The sections were stained with hematoxylin and eosin (H&E) stain. Microscopic analysis was done using Nikon E50i light microscope.

## Statistical analysis

The data was analysed by one way ANOVA followed by post Bonferroni's Multiple Comparison Test using graph pad instat software. The level of significance was $P<0.05$ (Graph pad instat, 2000).

## RESULTS

We found a significant elevation of serum creatinine and urea levels ($p < 0.001$) in the DXN alone treated group compared to the normal group (Table 1). Administration of *Lepidium sativum* (400 mg/kg body wt.) plus DXN significantly ($p < 0.001$) attenuated the increase of serum creatinine and urea levels that have seen with the administration of DXN alone. The levels of urea and creatinine were restored to normal levels in the *Lepidium sativum* plus DXN administered groups.

**Table 1 Effect of aqueous extract of *Lepidium sativum* (LS) on blood urea nitrogen and serum creatinine levels in rat treated with doxorubicin (DXN).**

| Group (n=6) | Treatment (mg/kg) | Blood urea nitrogen (mg/dl) | Serum Creatinine (mg/dl) |
|---|---|---|---|
| Normal | Vehicle | 17.28 ± 1.15 | 0.47 ± 0.04 |
| DXN | 15 (i.p.) | 49.86 ± 0.76*** | 0.98 ± 0.38*** |
| DXN + LS-I | 200 | 39.46 ± 1.33^^ | 0.78 ± 0.07^^ |
| DXN + LS-II | 400 | 28.75 ± 3.57^^^ | 0.57 ± 0.02^^^ |
| LS-II | 400 | 18.36 ± 0.87$^{ns}$ | 0.49 ± 0.01$^{ns}$ |

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Bonferroni's Multiple Comparison Test. *** p<0.001, ns-non significant as compared with normal group, and ^^ p<0.01, ^^^ p<0.001 as compared with doxorubicin group.

**Table 2 Effect of ethanol extract of *Lepidium sativum* (LS) on renal MDA, SOD, CAT and GSH activities in rat treated with doxorubicin (DXN).**

| Group (n=6) | Treatment (mg/kg) | MDA (nmol/g wet tissue) | GSH (µg/g wet tissue) | SOD (U/g wet tissue) | CATALASE (mM $H_2O_2$ consumed/min/g wet tissue) |
|---|---|---|---|---|---|
| Normal | Vehicle | 71.17 ± 2.32 | 173.64 ± 3.56 | 100.42 ± 1.38 | 1158.46 ± 8.05 |
| DXN | 15 (i.p.) | 107.81 ± 4.11*** | 33.97 ± 2.43*** | 53.31 ± 1.96*** | 895.16 ± 4.27*** |
| DXN + LS-I | 200 | 97.87 ± 1.46 | 45.42 ± 1.92^ | 62.29 ± 1.78 | 939.12 ± 7.96^ |
| DXN + LS-II | 400 | 92.60 ± 1.17^^ | 49.75 ± 2.19^^ | 70.78 ± 4.01*** | 961.28 ± 9.11^^^ |
| LS-II | 400 | 72.82 ± 2.48$^{ns}$ | 163.29 ± 3.38$^{ns}$ | 95.85 ± 1.12$^{ns}$ | 1124.84 ± 15.62$^{ns}$ |

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Bonferroni's Multiple Comparison Test. *** p<0.001, ns-non significant as compared with normal group, and ^p<0.05, ^^ p<0.01, ^^^ p<0.001 as compared with doxorubicin group.

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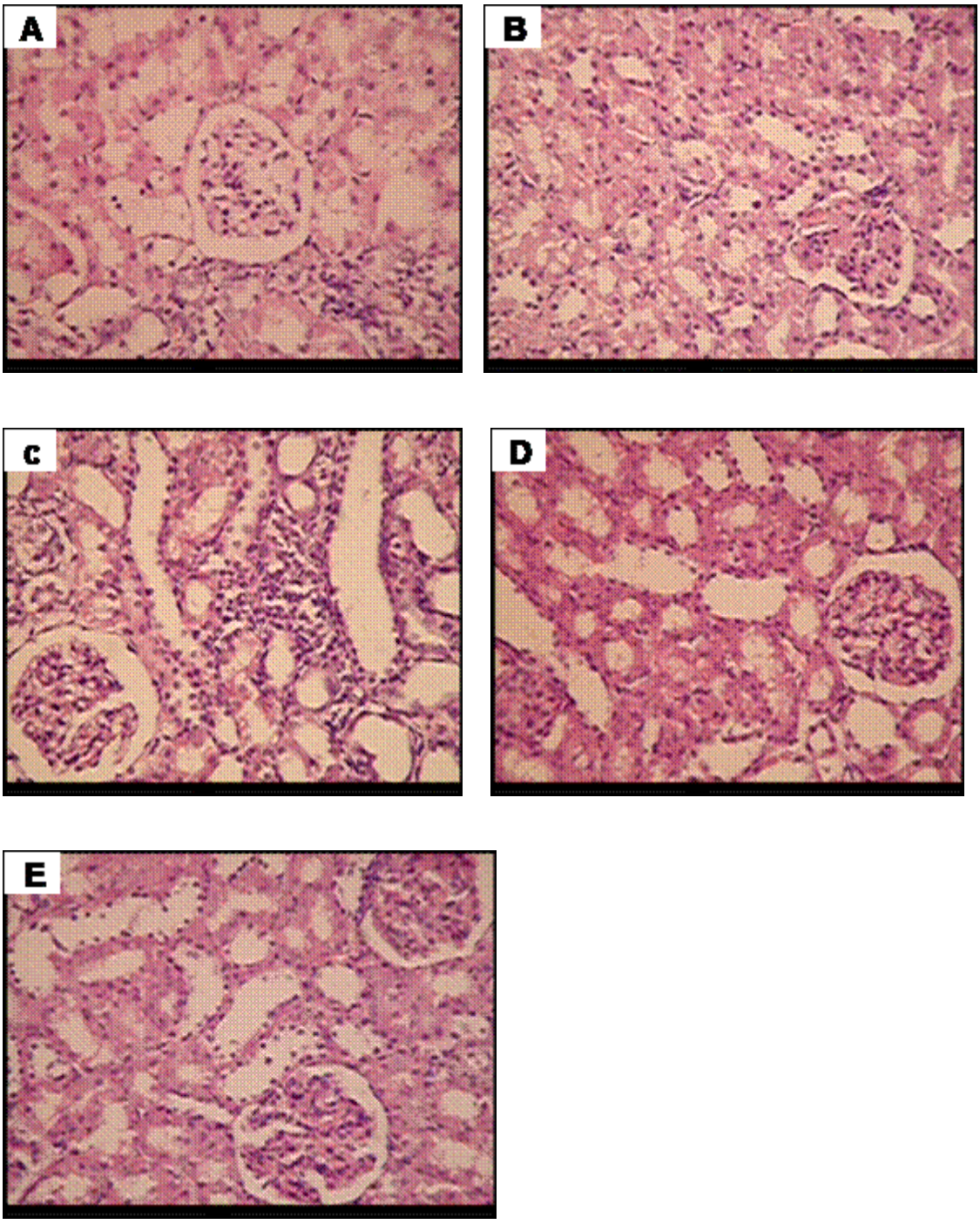


**Figure 1. Histopathological analysis of kidney in the groups of rats studied: control (A), *Lepidium sativum* L. (B), DXN (C), and DXN + *LS* (400 mg/kg, body weight) (D), DXN + *LS* (400 mg/kg, body weight) (E).**

The activities of renal SOD, CAT, and GSH, MDA level in the DXN alone and DXN plus *Lepidium sativum* administered group were given in Table 2. Renal SOD activity was found to be

decreased significantly ($p < 0.001$) in the DXN alone treated group when compared to the normal group. Administration of single dose of *Lepidium sativum* prior to DXN could significantly protect the DXN-induced decline of SOD activity. Further we found no significant difference ($p > 0.05$) in the SOD activity between the *Lepidium sativum* (200 mg/kg body wt.) plus DXN treated group from that of the DXN alone treated group. The maximum protective effect was obtained in the *Lepidium sativum* at 400 mg/kg body wt. treated group. Administration of *Lepidium sativum* plus DXN significantly ($p < 0.001$) restored the DXN-induced declined activity of catalase to normal. The activity of GSH was decreased significantly ($p < 0.001$) in the DXN alone treated group. Higher dose of *Lepidium sativum* (400 mg/kg body wt.) plus DXN treatment could restore the activity to that of normal group. Similarly the renal GSH concentration was restored to normal in the *Lepidium sativum* (400 mg/kg body wt.) plus DXN treated groups. Administration of *Lepidium sativum* could significantly ($p < 0.001$) protect the DXN-induced elevation of MDA level. The renal GSH activity in the DXN alone treated group was decreased and was found to be enhanced significantly ($p < 0.001$) in the *Lepidium sativum* plus DXN treated groups (Table 2).

There was no abnormal microscopy for the kidney of control and *Lepidium sativum* groups in the light microscopic examination (Fig. 1A and B). On the other hand, light microscopic examination of kidneys of rat revealed glomerulopathy characterized by mild hyperplasia of mesangium, glomerular basement membrane (GBM) thickening and moderate tubular atrophy & dilation was observed in DXN alone (Fig. 1C). In LS (200 mg/kg) treated rats shows mild microscopically changes in hyperplasia of mesangium, glomerular basement membrane (GBM), no tubular atrophy & dilation in LS plus DXN (Fig. 1D). And also no abnormal microscopy for the kidney of doxorubicin plus *LS* (400 mg/kg) groups in the light microscopic examination (Fig. 1E).

## DISCUSSION

Results of the present study indicate that aqueous aqueous extract of *Lepidium sativum* significantly protected DXN-induced nephrotoxicity. Despite the wide use of DXN in the treatment of cancer patients, its mechanism of action is still not well known. However different mechanisms of free radical formation have been described. The first implicates the formation of a DXN semi-quinone free radical by the action of NADPH dependant reductases. In the presence of oxygen this semi-quinone form yields super oxide radicals (O2-). Free radicals can also be produced by a non-enzymatic mechanism that involves reactions of iron–DXN complex that can reduce oxygen to H2O2 and other ROS.[18,19] The dose of DXN used in this study corresponds to the dose that currently being used in clinical practice.[20] In the previous study demonstrated the acute cardio-renal failure in rat after 72 h of a single dose of DXN (10 mg/kg) administration.[21] The results of the renal function test revealed that DXN administration produced intrinsic acute renal failure, which was evident from the elevated levels of serum urea and creatinine. The altered renal damage could be completely restored with the prophylactic oral administration of *Lepidium sativum* at a dose of 400 mg/kg. The antioxidant status of kidney is significantly lowered in the DXN alone treated animals. Therefore the concentration of MDA equivalents, as a result of lipid peroxidation, increased in DXN alone treated animals due

to the decreased SOD, CAT activities and GSH level. A high dose of *Lepidium sativum* plus DXN could restore the kidneys antioxidant status and completely protect against renal damage. The importance of thiol mediated detoxification of anticancer drugs that produce toxic electrophilies has been of considerable interest to many investigators. GSH, a non-protein thiol in the cell is involved in the xenobiotic metabolism. The enhanced GSH level in *Lepidium sativum* treated animals partially explains its mechanism of protection. The elevated levels of GSH could effectively provide thiol group for the possible GSH mediated detoxification reactions of GPx and GST. Administration of *Lepidium sativum* enhanced the activities of renal GSH. Moreover, the enhanced SOD activity in the *Lepidium sativum* treated group might be involved in the scavenging of O2- generated from the DXN. Thus the enhanced renal antioxidant status results from the treatment of *Lepidium sativum* could explain the nephroprotective effect. The in vitro study using *Lepidium sativum* had reported the significant antioxidant activities.[22]

## CONCLUSION

In conclusion, the overall results of this study have clearly shown LS to offer protection against the deleterious renal side-effects of doxorubicin. In the near future, LS could constitute a lead to discovering a novel drug which will be useful in treatment of drug-induced nephrotoxicity.

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## REFERENCES

[1] Blum RH, Carter SK. Ann Intern Med 1974; 80: 249-259.
[2] Al-Harbi M, Al-Garably M, Al-Shabanah O, Al-Bekairi A, Osman A, Tawfik H. Cancer Chem Pharmacol 1992; 31: 200-204.
[3] Jovanovic D, Djukanovic I, Susic D, Funduk G, Jovanovic Z, Dragojlovic Z, Boydanovic G, Starcevic Bonzovic Z. Srp Arh Celok Lek 1996; 124 (1): 47-49.
[4] Olsen H, Capen C. Toxicol Appl Pharmacol 1978; 44:606-616.
[5] Powis, G. Free Radical Biol Med 1989; 6: 63-101.
[6] Algria A, Rodriguez M, Hermandez J. Biochem Biophys Acta 1990; 1035: 51-5.
[7] Abuelgasim AI, Nuha HS, Mohammed AH. Res J Ani Vet Sci 2008; 3: 20-23.
[8] Jouad H, Haloui M, Rhiouani H, El Hilaly J, Eddouks M. J Ethnopharmacol 2001; 77: 175-182.
[9] Maier UH, Gundlach H, Zenk MH. Phytochemistry 1998; 6: 1791-1795.
[10] Ziska P, Kind A, Franz H Acta.Histochem 1982; 71 (1): 29-33.
[11] Eddouks M, Maghrani M, Zeggwagh NA; Michel JB. J Ethnopharmacol 2005; 97(2): 391-395.

[12] Maghrani M, Zeggwagh NA, Michel JB, Eddonks M. J Ethnopharmacol 2005; 100 (1-2): 193-197.
[13] Atasan SK. International J Crud Drug Res 1989; 27(4): 235-239.
[14] Ohkawa H, Ohishi N, Yagi K. Annal Biochem 1979; 95: 351-358.
[15] Misra HP, Fridovich I. J Biol Chem 1972; 247: 3170-3175.
[16] Moron MA, Depierre JW, Mannervik B. Biochemica et Biophysica Acta 1979; 582: 67-78.
[17] Aebi H. Methods Enzymol 1984; 105: 121-126.
[18] Singal, PK, Li T, Kumar D, Danelisen I, Hiskovic N. Mol Cell Biochem 2000; 207: 77-86.
[19] De Beer EL, Bottone AE, Voest EE. Eur J Pharmacol 2001; 415: 1-11.
[20] Chabner BA, Ryan DP, Paz-Ares L, Garcia-Carbonevo R, Calabresi P. Antineoplastic agents. In: Hardman, J.G., Limbird, L.E., Gilman, A.G. (Eds.), Goodman and Gilman's the Parmacological Basis of Therapeutics. McGraw-Hill Companies Inc., USA, 2001; pp 1389-1459.
[21] Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S. Toxicol 2006; 218: 164-171.
[22] Jain V, Singh D, Saraf S. 2006. http://www.cababstractsplus.org/Abstracts.htm. Accessed 10 January, 2009.