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EFFECT OF *LEPIDIUM SATIVUM* ON MEMBRANE BOUND PHOSPHATISES (ATPASE) AND HISTOPATHOLOGICAL CHANGE IN RENAL TOXICITY

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Abstract

The present study was designed to determine the possible effect of ethanolic extract of *Lepidium sativum* seeds on alteration membrane bound phosphatises (ATPase) and histopathology of acute renal failure. The 200 mg/kg and 400 mg/kg ethanolic extract of *Lepidium sativum* L. seeds was used to against cisplatin (5 mg/kg, i.p.) induced renal toxicity. The experimental protocol designed as the animals were divided into six groups (n=6) like control, model control, two protective and two curative groups were received vehicle, cisplatin, cisplatin + extract, and extract + cisplatin respectively. After 6th days, the rats were sacrificed for quantitative estimation membrane bound phosphatises (ATPase) and histopathology in kidney tissue. The level of brush border enzymes like Na^{+} / K^{+} ATPase, Ca^{++} ATPase and Mg^{++}ATPase were decreased significantly in model control and. It was overcome by same extract treatment in curative and protective groups, and histopathology changes in model control group like hyaline droplets, tubular dilation was observed, it was cured more in curative groups (200 mg/kg) and protective (400 mg/kg) then the protective (200 mg/kg) and curative groups (400 mg/kg). Finally it is concluded that the present study data conformed acute renal toxicity induced by cisplatin due oxidative stress and ethanolic extract of *Lepidium sativum* L. seeds could contributed protective and curative effect.

Keywords: *Lepidium sativum,* ATPase, histopathology.

Introduction

The *Lepidium sativum* L. (family-Brassicaceae) is a native shrub. The *Lepidium sativum* (L.) seeds contain volatile essential aromatic oils, active principle and fatty oils and carbohydrate, protein, fatty acid, Vitamin: β-carotene, riboflavin, and niacin, and ascorbic acid, Flavonoids, Isothiocynates glycoside [1]. The *Lepidium sativum* L. seeds are used as aperients, diuretic, good anti inflammatory, demulcent, aphrodisiac, carminative, galactagogue, antiasthematic, antiscurbutic, and stimulant [2&3]. Cisplatin (cis-diamminedichloroplatinumII) (CDDP) is one of most potent anticancer drug. it is produced dose limiting nephrotoxicity and high dose of CDDP produce the impairment of kidney, causes decrease in renal blood flow, glomerular filtration rate and increases urea and creatinine level in blood [4]. The cisplatin induced nephrotoxicity was characterized by signs of injury such as changes in urine volume, body weight, increase the products of lipid peroxidation, and change renal clearance [5]. The cisplatin is inhibited the activity of antioxidant enzyme in renal tissue like glutathione, SOD, GSH and Catalase depletion and increase thiobarbuturic acid – reactive substance (TBARS) [6]. Thus, the purpose of current study was to determine the effect whether oral administration of ethanolic extract of *Lepidium sativum* L. (ELS) seeds has any protective and curative effect against cisplatin induced nephrotoxicity in albino rats.

Materials and methods

Drug and Reagents

Cisplatin (VHB, Life sciences Inc., India), Adenosine triphosphate (Merck pvt. Ltd., India).Trichloroacetic acid (Merck pvt. Ltd., India), Thiobarbuturic acid (Loba chemicals pvt.ltd. India).

Plant material

Lepidium sativum L. seeds were purchased from market of Mandsaur city (M.P., India). The plant was identified by Dr. H.S. Chattarjee (Ex professor of botany), P. G. College of Mandsaur, and M.P. And voucher specimen (BRNCP/L/02/2006) was submitted in department of Pharmacognosy; BRNCP, Mandsaur, M.P. The trampled seeds were extracted by soxhlet apparatus using ethyl alcohol as a solvent. The extract was dried by rotator evaporator under reduced pressure.

Photochemical Screening

Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, amino acid & protein and flavonoids (7)

Antioxidant activity

DPPH radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds was determine according to the method reported by Blois [8] and the ferric chloride scavenging assay was performed according to Benzie and Strain [9].

Animals

Adult male wistar rats having weight around 180-210 g were maintained at 25 ± 2°C and kept in well ventilated animal house under photoperiodic condition in large polypropylene cages and were standard food and water *ad libitum*. The experiment was carried out in accordance to the guidelines mentioned in the CPCSEA, and Institutional Animal Ethical Committee approved the experiment protocols (Reg.No.-947/ac/06/CPCSEA).

Experimental design

The acute toxicity study of ethanolic extract of *Lepidium sativum* seeds L. was not occurred at 2000mg/kg (as per the OECD - 420) on male Wistar rats The dose was selected one tenth ($1/10^{th}$) and fifth ($1/5^{th}$) of it, for safe treatment.

Total duration of study was 16 days. The animals were divided into six groups containing six animals in each group. Group1 served as control and received normal saline throughout the experiment, Group II (Modal Control) received single dose of cisplatin (5 mg/kg i.p.), 1^{st} days, Group III (Protective) received ELS extract (200 mg / kg p.o.) for 1^{st} to10^{th} day and 11^{th} day, single dose (5 mg/kg, i.p.)) of cisplatin was administered, Group IV (Curative) received same dose of cisplatin on day 1^{st}, and after 6^{th} days ELS extract (200 mg / kg p.o.) was administered up to 16 th days, group V (Protective) received ELS extract (400 mg / kg p.o.) for 1^{st} to10^{th} day, and11^{th} day, same dose (5mg/kg, i.p.)) of cisplatin was administered and Group VI (Curative) received same dose of cisplatin on day 1^{st}, and after 6^{th} days ELS extract (400 mg / kg p.o.) was administered up to 16 th days. (10 & 11**)**

Biochemical assays

After then Kidneys were removed, homogenized and centrifuged at 10,000 rpm at 0°C for 20 min. sediment of the centrifuge was used for estimation of the Na^+K^+ATPase by Bontin methods [12], Ca^{2+}ATPase by Hjerken and Pan [13], Mg^{2+}ATPase by Ohinishi *et al*. method [14], and histopathology 15].

Statistical analysis

Results were expressed as one way analysis of variance (ANOVA) followed by Dennett's test and $P< 0.05$ was considered as significant.

Results and Discussion

The level of brush border enzymes like Na^+/K^+ ATPase, Ca^{++} ATPase and Mg^{++}ATPase were found to reduced significantly (**P<0.01) in model control group animals as compared with control group. The Na^+/K^+ ATPase and Ca^{++} ATPase were significantly (**P<0.01) monitored with dose 200 mg/kg in curative and 200 mg/kg and 400 mg/kg protective groups but less significant (*P<0.05) with dose 200 mg/kg of protective groups. The Mg^{++}ATPase was also increased significantly (**P<0.01) 200 mg/kg and 400 mg/kg curative groups and less significant (*P<0.05) dose 200 mg/kg and 400mg/kg in protective groups. (Table 1). After damage of kidney, pathophysiological change in occur in proximal tubules cisplatin toxicity by formation of reactive species which cause the redistribution of brush border enzyme (16) and histopathology changes in model control group like hyaline droplets and tubular dilation were observed, it was recovered more in curative groups (200 mg/kg) and protective (400 mg/kg) then the protective (200 mg/kg) and curative groups (400 mg/kg) shown fig.1. This is indicate that extract have antioxidant potential whereas in present phytochemical study of the extract have revealed the presence of Flavonoids, and amino acids like glutamine, Cysteine, and Glycine and fig no. 2). The tannin (Phenolic compound), Flavonoids have antioxidant activity and Glutamate, Cysteine, Glycine were used to synthesis of the endogenous glutathione [17]. Free radical scavenging activities of the ethanolic extract of *Lepidium sativum* L. seeds was assessed by the DPPH assay and Fecl3 assay for estimation of in - vitro antioxidant potential. DPPH radical and Fecl3 free radical concentration were significant decreased due to scavenging potential of the extract. The results show that ethanolic extract *Lepidium sativum* has the highest DPPH scavenging and percentage DPPH radical of inhibition (IC50 value 18.46±0.27 µg/ml) and Fecl3 scavenging and percentage DPPH radical of inhibition (IC50 value 9.11±0.4 µg/ml.) (fig.1, fig 2). It is indicated that ethanolic extract *Lepidium sativum* has good antioxidant potential. So it helps to protected and cured the renal toxicity induced by oxidative stress.

Conclusion

Finally it is concluded that the present study data conformed acute renal toxicity induced by cisplatin due oxidative stress and ethanolic extract of *Lepidium sativum* L. seeds could contributed protective and curative effect.

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Table no. 1. Na^+K^+ATPase, Ca^{++} ATPase and Mg^{++}ATPase in kidney tissue of various groups: (Dunnett multiple comparison test)

S.No.	Groups	Na^+/K^+ ATPase (mM of phosphate librated/mg tissue)	Ca^{++} ATPase (mM of phosphate librated/mg tissue	Mg^{++}ATPase (mM of phosphate librated/mg tissue)
1.	Control	210.83±1.64	102.83±2.31	150.67±0.88
2.	Model control	135.17±1.51 [a]	64.33±1.05 [a]	81.66±1.05 [a]
3.	Protective (200mg/kg)	143.83±1.90 [b′]	71.50±2.02 [b′]	88.67±2.56 [b′]
4.	Curative (200mg/kg)	177.50±1.45 [b]	89.00±1.03 [b]	122.33±1.83 [b]
5.	Protective (400mg/kg	152.67±2.89 [b]	78.16±2.00 [b]	89.00±2.53 [b′]
6.	Curative (400mg/kg	201.00±1.93 [b]	97.60±1.28 [b]	140.50±1.31 [b]

[a]= **P<0.01 as compared to the Control, [b′]=*P<0.05, [b]= * P<0.01, [b′]=*P<0.05,[b]=**P<0.01 as compared to the model Control

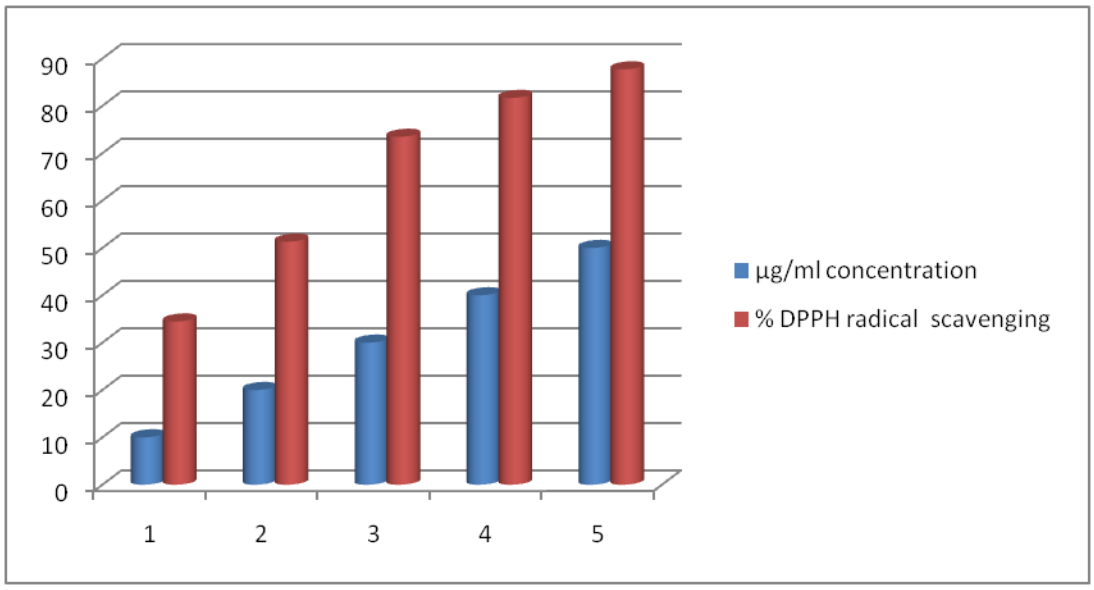


Fig.1. % DPPH radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds

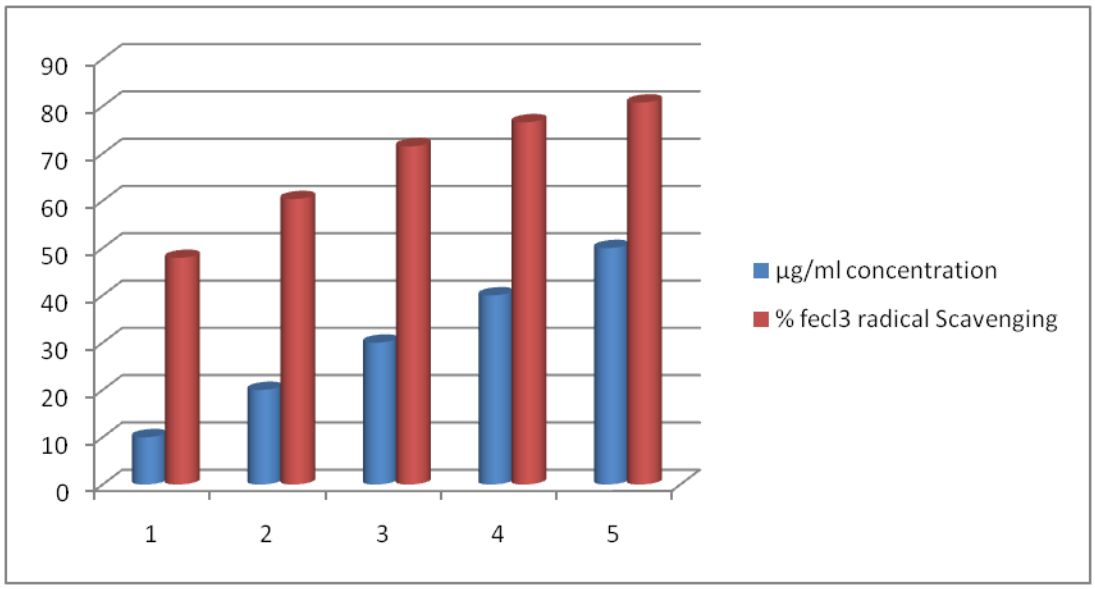
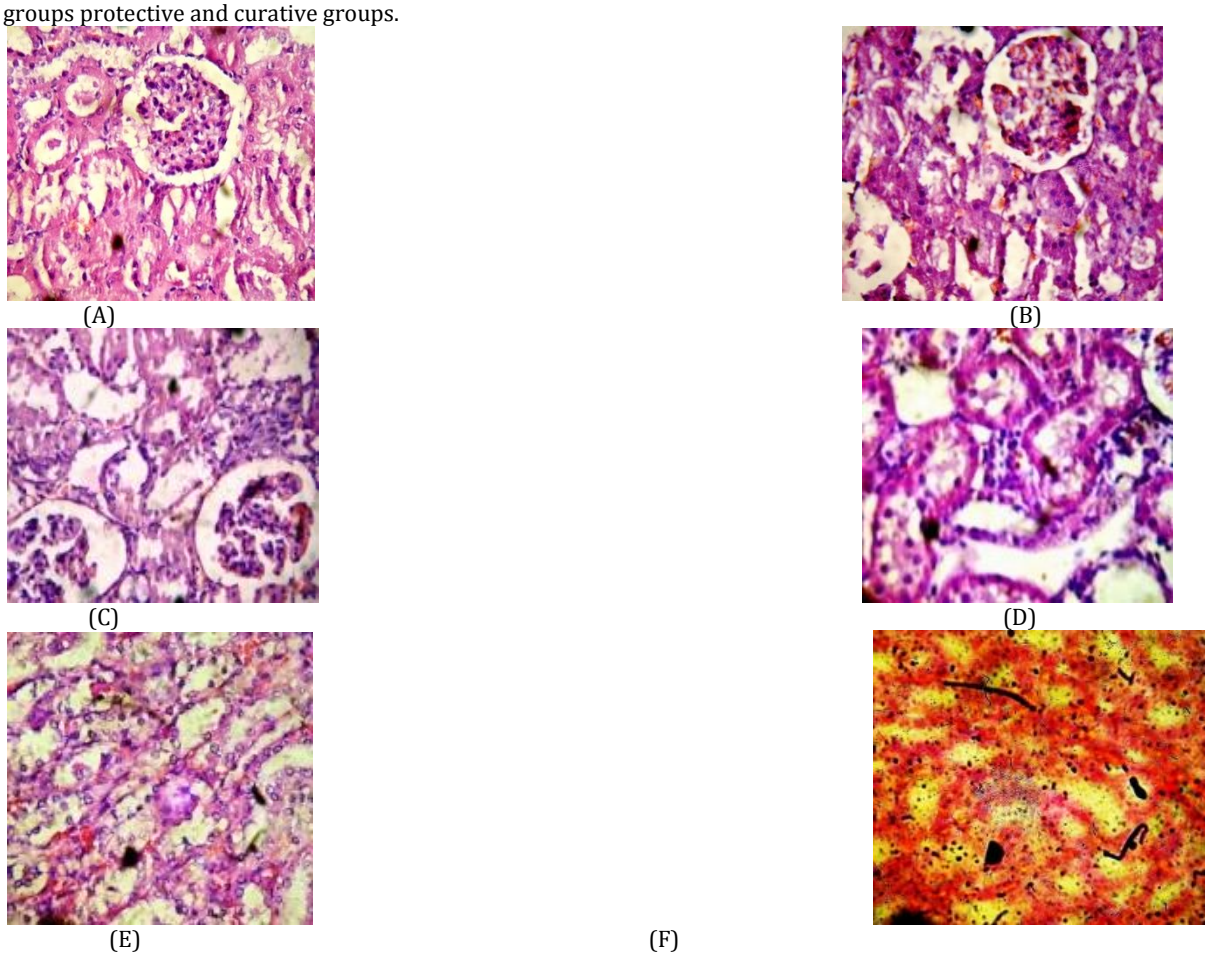


Fig.2. % Fecl3 radical scavenging activity of ethanolic extract of *Lepidium sativum* seeds

Histopathology: Cisplatin treated group shown hyaline droplets, tubular dilation and cellular infiltration that is cured in groups protective and curative groups.



(A) (B)

(C) (D)

(E) (F)

Fig3. Histopathological evidence of cisplatin - induced proximal tubular toxicity. Representative histopathology stained sections histological sections from kidney of (A) vehicle-treated (magnification 40×), (B) cisplatin-treated rats (5 mg/kg, 6 days) (magnification 40×), (C) protective *Lepidium sativum* treated with 200 mg/kg rats (magnification 40×), and (D) curative *Lepidium sativum* treated with 200 mg/kg rats (magnification 40x). (E) Protective *Lepidium sativum* treated with 400 mg/kg days (F) curative *Lepidium sativum* treated with 400 mg/kg rats.