

***In vivo* antioxidant potential of *lepidium sativum* l. Seeds in albino rats using cisplatin induced nephrotoxicity**

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Abstract

The present study was designed to investigate to possible potential nephrocurative, nephroprotective activity and in vivo antioxidant potential of 200mg/kg and 400mg/kg ethanolic extract of *Lepidium sativum* L. seeds was use to against cisplatin (5mg/kg, i.p.) induced nephrotoxicity. The experimental protocol designed as the animals were divided into six groups (n=6) like control, model control, two curative (200mg/kg and 400mg/kg), and two protective groups (200mg/kg and 400mg/kg, were received vehicle, cisplatin, cisplatin + extract, and extract + cisplatin respectively. After 6th days, blood collected from retro-orbital sinus of rats and determined urea and creatinine level in serum of each group after then rats were sacrificed for quantitative estimation of various enzymes and ATPase content in kidney tissue. A single dose of cisplatin induced loss in body weight, increase urine excretion, increased urea & creatinine level in serum; it was significantly recovered by 200mg/kg and 400mg/kg in curative and protective groups. The enzyme estimation in kidney tissue it found that increase malondialdehyde, superoxide dismutase, catalase and reduced glutathione level, it was significantly monitored by 200mg/kg and 400mg/kg in curative and protective groups. These are defined as vivo antioxidant potential. The level of brush border enzymes like Na⁺ / K⁺ ATPase, Ca⁺⁺ ATPase and Mg⁺⁺ ATPase were found significantly reduced after single dose cisplatin injection. It was overcome by treatment of same extract in curative and protective groups. Finally it is concluded that the present study data conformed nephrotoxicity induced by cisplatin due oxidative stress and ethanolic extract of *Lepidium sativum* L. seeds may have nephroprotective and curative activity.

Keywords: Cisplatin; Nephrotoxicity; urea; creatinine; glutathione; Lipid peroxidation;

Introduction

A large number of medicinal plants, natural products and dietary components have been evaluated as potential nephroprotective agents [1]. 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 [2].

The *Lepidium sativum* L. seeds are used as aperients, diuretic, good anti inflammatory, demulcent, aphrodisiac, carminative, galactagogue, antiasthmatic, antiscorbatic, and stimulant [3&4]. 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 [5]. 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 [6]. Kidneys have some antioxidant enzyme like superoxide dismutase (SOD), lipid peroxidase and glutathione (GSH), and catalase which protect kidney from free radicals like nitric oxide and superoxide etc. 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) [7]. Thus, the purpose of current study was to investigate 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. Its region behind *Lepidium sativum* seeds L. were traditionally used as diuretic and anti inflammatory [4].

Materials and methods

Drug and Reagents

Cisplatin (VHB, Life sciences Inc., India), DTNB (Merck pvt. Ltd., India). Glutathione (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.

Animals

Adult male wistar rats having weight around 180-210 g were maintained at $25 \pm 2^\circ\text{C}$ 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 ($1/10^{\text{th}}$) and fifth ($1/5^{\text{th}}$) 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. Group I served as control and received normal saline throughout the experiment, Group II (Modal Control) received single dose of cisplatin (5mg/kg i.p.), 1st days, Group III (Protective) received ELS extract (200mg / kg p.o.) for 1st to 10th day and 11th day, single dose (5mg/kg, i.p.) of cisplatin was administered, Group IV (Curative) received same dose of cisplatin on day 1st, and after 6th days ELS extract (200mg / kg p.o.) was administered up to 16th days, group V (Protective) received ELS extract (400mg / kg p.o.) for 1st to 10th day, and 11th day, same dose (5mg/kg, i.p.) of cisplatin was administered and Group VI (Curative) received same dose of cisplatin on day 1st, and after 6th days ELS extract (400mg / kg p.o.) was administered up to 16th days.

Biochemical assays

After the treatment period, blood was collected from retro-orbital sinus of rat under ether anaesthesia and centrifuged using the table top centrifuge (REMI) at 3000 rpm to get serum. Level of urea and creatinine was estimated using Span diagnostic kit on chemical analyzer (microlab3000) for assessment of renal toxicity. [8&9], After then Kidneys were removed, homogenized and centrifuged at 10,000 rpm at 0°C for 20 min. the supernatant was used for estimation of different antioxidant level by calorimetric method using spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam), Glutathione reductase (GSH) estimated by Sedlak and Lindsay method [10 & 11], Lipid peroxidation by thiobarbuturic acid-reactive substances (TBARS) methods [12&13], Superoxide dismutase (SOD) by method developed by Misra and Fridovich (1972). [14], Catalase (CAT) by colorimetric assay [15], and the sediment of the centrifuge was used for estimation of the $\text{Na}^+\text{K}^+\text{ATPase}$ by Bontin methods [16], $\text{Ca}^{2+}\text{ATPase}$ by Hjerken and Pan [17], $\text{Mg}^{2+}\text{ATPase}$ by Ohinishi *et al.* method [18].

Statistical analysis

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

Results and Discussion

In present study rat treated with single dose of cisplatin shown marked reduction of body weight (173.33 ± 4.21) in model control group as compared to control group (195.00 ± 4.28). it was significantly ($**P < 0.01$) and ($*P < 0.05$) recovered with treatment of 400mg/kg ethanolic extract of *Lepidium sativum* L. seeds in curative and protective groups respectively but less significantly ($*P < 0.05$) 200mg/kg dose curative and not significant protective group of same extract. (Table no.1). However significantly ($**P < 0.01$) increased urinary volume (14.66 ± 0.88) in model control group (5.33 ± 0.33). It was overcome significantly ($**P < 0.01$) in 400mg/kg curative and protective groups and 200mg/kg in curative but less significantly ($*P < 0.05$) in protective group (fig.1). The loss body weight and increase urinary volume of animal after injection cisplatin may due to gastrointestinal toxicity and by reduced ingestion of food [19]. Cisplatin treated group had an increase urinary volume. That is agreement with Matsushima *et al* [20]. In present phytochemical study of the ethanolic extract of *Lepidium sativum* L. seeds have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, and amino acids like glutamine, Cysteine, and Glycine. That may help to reduce gastrointestinal toxicity cause to recover of body weight and urinary volume.

Table no.1. Effect of treatment with ethanolic extract of *Lepidium sativum* seeds on body weight, serum urea, serum creatinine

S. No.	Groups	Final body weight (gm) at lost day of experiment	Urea level in serum (mg/dl)	Creatinine level in blood serum (mg/dl)
1.	Control	195.00±4.28	24.16±1.04	0.94±0.05
2.	Model control	173.33±4.21	76.66±2.24	2.32±0.10
3.	Protective(200mg/kg)	179.17±3.74 ^{ns}	60.83±2.76**	2.00±0.04*
4.	Curative (200mg/kg)	188.33± 2.591*	40.83±0.90**	1.81±0.09**
5.	Protective(400mg/kg)	190.90±4.35*	44.16±1.74**	1.98±0.04*
6.	Curative (400mg/kg)	192.67±4.05**	30.33±0.95**	1.19±0.10**

Each value represents mean± S.D. of six animals.

ns, statically different non significant when compare to the model control .

**P<0.01, * P<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the model Control.

After injected the single dose of cisplatin (5mg/kg) result increased urea (76.66±2.24) and creatinine (2.32±0.10) level in model control compare to respective control group (24.16±1.04 and 0.94±0.05) and its was recovered significantly (**P<0.01) in curative and protective groups with both 200mg/kg and 400mg/kg treatment of same extract but less significantly (*P<0.05) effect on creatinine recovered in protective groups of both 200mg/kg and 400mg/kg of extract (Table no.1).

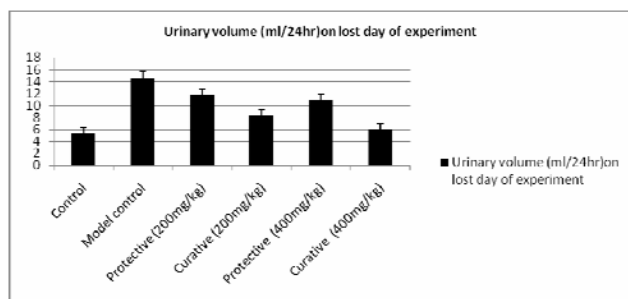


Figure 1. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds urinary volume (ml/24hr.) in protective and curative group. Each group represents mean± S.D. of six animals, ** P<0.01, P<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the Model Control

The change of renal function observed in the rat correlate well with the nephrotoxicity effect with man (21). The single dose of cisplatin (5mg/kg) result increased urea and creatinine level in model control compare to control. It was significantly recovered in curative and protective groups (Table no.1). The increased urea and

creatinine level suggests the reduction of glomerular filtration rate (22). But protective and curative treatment of ethanolic extract of *Lepidium sativum* seeds L. with cisplatin significantly reduced the level of urea and creatinine that indicates increase glomerular filtration rate.

Jeong et al (19) observed that a single injection of cisplatin dose 5mg/kg body weight in rabbit caused a mark reduction of glomerular filtration rate, which is accompanied by increase in serum creatinine level indicating induction of acute renal failure. According to previous findings, we conformed that a single dose cisplatin induced a significantly serum creatinine in wistar rats three to seven days after administration (23, 24). Our result shown that significantly recovered of decreased urea and creatinine serum level in curative and protective groups with treatment of both 200mg/kg and 400mg/kg of same extract but less significantly creatinine recovery in protective groups of both 200mg/kg and 400mg/kg of extract.

In aspect of kidney tissue estimation, it is shown as significantly (**P<0.01) increase the lipid peroxidase (24.50±0.61) and decrease the level of GSH (45.33±1.66), SOD (07.16±0.60) and CAT (201.67±3.33) after single dose injection of cisplatin in model control group. The lipid peroxidase, SOD and CAT were significantly ** (P<0.01) monitored with dose 200kg/kg in curative groups and 400mg/kg in curative and protective groups. However less significant

Table no.2. Effect of treatment with ethanolic extract of *Lepidium sativum* seeds on the lipid peroxidation and antioxidant enzyme of kidney

S. No.	Groups	µmol GSH/gm. Kidney tissue	n Mol MDA/gm. ml	(Unit SOD /gm) kidney tissue	CAT (µ mole of H2O2/gm kidney tissue)
1.	Control	69.50±1.54	14.00±0.57	21.83±0.94	323.33±1.75
2.	Model control	45.33±1.66	24.50±0.61	07.16±0.60	201.67±3.33
3.	Protective(200mg/kg)	48.16±1.24 ^{ns}	21.50±0.76*	10.00±0.51*	219.17±6.24*
4.	Curative(200mg/kg)	59.66±1.28**	17.16±0.60**	15.33±0.88**	301.83±2.63**
5.	Protective(400mg/kg)	51.33±1.14*	21.00±0.63**	10.66±0.49**	223.33±6.41**
6.	Curative (400mg/kg)	67.83±1.07**	15.33±0.76**	16.80±0.56**	315.50±1.40**

Each value represents mean± S.D. of six animals.

ns, statically different non significant when compare to the model control.

**P<0.01, *P<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the model Control.

(*P<0.05) dose 200mg/kg in protective group. But in expect of GSH level, 200mg/kg and 400mg/kg dose were significantly (**P<0.01) monitored in curative group, however less significant 400mg/kg dose in protective groups and not significant in dose 200mg/kg in protective group. (Table2 & fig. 2). Our present result data shown that significantly monitored GSH, SOD, and CAT and lipid peroxidation. 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. The tannin (Phenolic compound), Flavonoids have antioxidant activity and Glutamate, Cysteine, Glycine were used to synthesis of the endogenous glutathione [25]. It's all may contribute synergistic reason to increase GSH level in kidney tissue significantly. It is represented that ethanolic extract of *Lepidium sativum* L may have antioxidant potential.

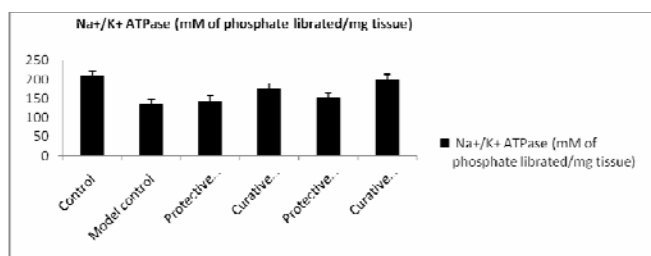


Figure.3. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds Na⁺/K⁺ ATPase (mM of phosphate liberated/mg tissue) in protective and curative group. Each group represents mean± S.D. of six animals, *P<0.01, * P<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the Model Control

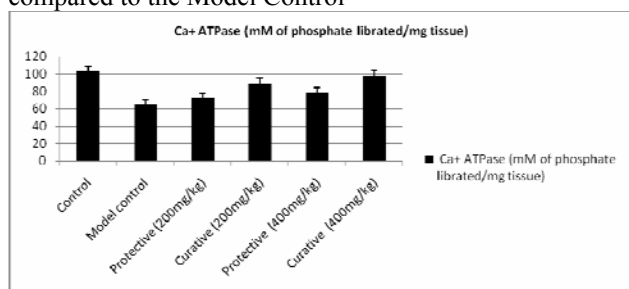


Figure.4. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds Ca⁺⁺ ATPase (mM of phosphate liberated/mg tissue) in protective and curative groups. Each group represents mean± S.D. of six animals, *P<0.01, **P<0.01, **P<0.01, *P<0.01, *P<0.01 as compared to the model Control.

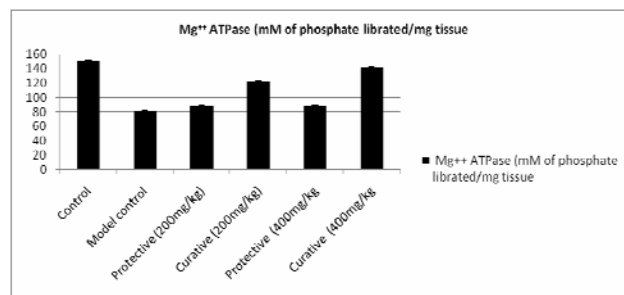


Figure 5. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds (Mg⁺⁺ATPase) kidney tissue (mM of phosphate liberated/mg tissue) in protective and curative groups. Each group represents mean± S.D. of six animals, **P<0.01, *P<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the Model Control.

The level of brush border enzymes like Na⁺/K⁺ ATPase, Ca⁺⁺ ATPase and Mg⁺⁺ATPase were found to reduced significantly (**P<0.01) as compared to model control group animals, the Na⁺/K⁺ ATPase and Ca⁺⁺ ATPase were Significantly (**P<0.01) recovered with dose 200mg/kg in curative and 200mg/kg and 400mg/kg protective groups but less significant(*P<0.05) with dose 200mg/kg of protective groups. The Mg⁺⁺ATPase was recovered significantly (**P<0.01) 200mg/kg and 400mg/kg curative groups and less significant (*P<0.05) dose 200mg/kg and 400mg/kg in protective groups. (Fig 3, 4 &5). 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 (26). In present result data reveal that extract may have antioxidant and anti-inflammatory activity.

Conclusion

Finally it is concluded that the present study data conformed nephrotoxicity induced by cisplatin due oxidative stress and ethanolic extract of *Lepidium sativum* L. seeds may have nephroprotective, curative and in vivo antioxidant potential.

Authors' contributions

Mr. Yogesh chand yadav is main author. He had done whole work and written research paper, Dr.

D. N. Srivastav and Dr. Vipin saini were guided to work and writing a paper, Dr. A.K. Seth and Dr. Balaraman were evaluated ANOVA *Statistical* analysis, where Mr. Tejas K. Ghelani was help in biochemical assays.

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