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Nephroprotective and curative activity of *Lepidium sativum* L. seeds in albino rats using cisplatin induced acute renal failure

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Abstract

The present study was designed to investigate to possible potential nephrocurative & nephroprotective activity of 400mg/kg ethanolic extract of Lepidium sativum L. was use to against cisplatin (5mg/kg, i.p.) induced nephrotoxicity. The experimental protocol designed as the animals were divided into four groups (n=6) like control, model control, curative (400mg/kg) and protective groups (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 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 400mg/kg in curative and protective groups. 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.

Key words: 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, antiasthematic, antiscurbutic, 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 ± 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. One fifth dose of 2000mg/kg was selected regarding toxicity study.

Total duration of study was 16 days. The animals were divided into four groups containing six animals in each group.Group1 served as control and received normal saline throughout the experiment, GroupII (Modal Control) received single dose of cisplatin (5mg/kg i.p.), 1st days, Group III (Protective) received ELS extract (400mg / kg p.o.) for 1st to10th day and11th day, single dose (5mg/kg, i.p.)) of cisplatin was administered, Group IV (Curative) received same dose of cisplatin on day 1 st, and after 6th days ELS extract (400mg / kg p.o.) was administered up to 16 th 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 rmp 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 Na^+K^+ATPase by Bontin methods [16], Ca^{2+}ATPase by Hjerken and Pan [17], Mg^{2+}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) recovered with treatment of 400 mg/kg ethanolic extract of *Lepidium sativum* L. seeds in curative group but less significantly (*P<0.05) in protective groups (Table no.1).The effect ethanolic extract of *Lepidium sativum* L seed on cisplatin nephrotoxicity were evaluated by change of urine volume. The experiment data indicated that significantly (**P<0.01) increased urine volume in model control groups and it was overcome significantly (*P<0.01) and (**P<0.01) with treatment of 400mg/kg same extract in protective and curative group respectively shown less significant (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 1. 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 (400mg/kg)	51.33±1.14*	21.00±0.63**	10.66±0.49**	223.33±6.41**
4.	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<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the model Control*

Table 2. Effect of treatment with ethanolic extract of *Lepidium sativum* seeds on membrane bound enzyme $Na^{+/}K^{+}$ ATPase, Ca^{++} ATPase, and Mg^{++}ATPase in (mM of phosphate librated/mg tissue) of kidney

S. No.	Groups	$Na^{+/}K^{+}$ ATPase	Ca^{++} ATPase	Mg^{++}ATPase
1.	Control	210.17±1.3	102.83±2.31	150.66±0.88
2.	Model control	135.17±1.51	64.33±1.05	81.66±1.05
3.	Protective(400mg/kg)	152.67±2.89**	78.16±2.00**	89.00±2.53*
4.	Curative (400mg/kg)	201.00±1.93**	97.60±1.28**	140.50±1.31**

Each value represents mean± S.D. of six animals
ns, statically different non significant when compare to the model control
**P<0.05, **P<0.01, **P<0.01, **P<0.01 as compared to the model Control*

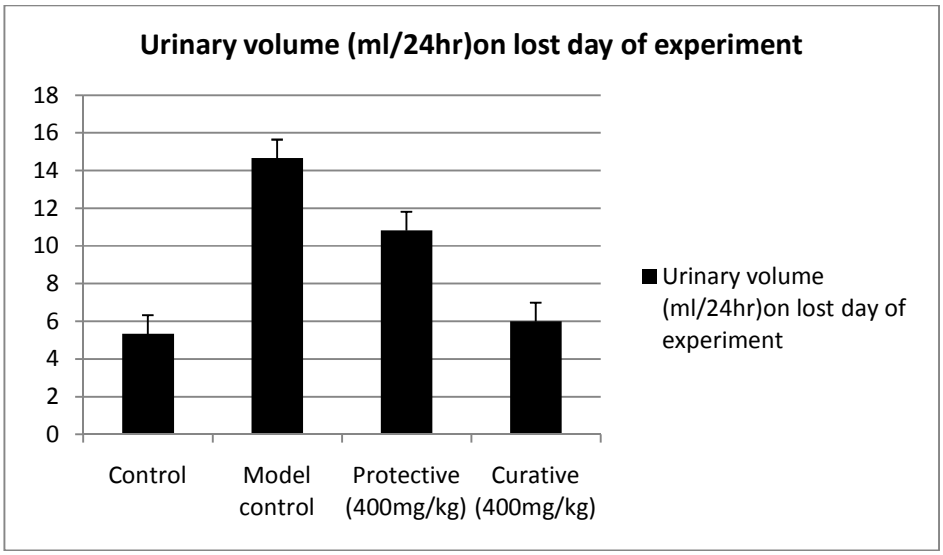

Figure 1. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds on urinary volume (ml/24hr.) in protective and curative groups

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 groups as compare to respective control groups (24.16±1.04and 0.94±0.05) and its was recovered significantly (**P<0.01) in curative and protective groups with same extract but less Significantly (*P<0.05) effect on creatinine recovery in protective groups. 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 recovered significantly in curative and protective groups (Table no.1). It may indicate increase glomerular filtration rate because of increased urea and creatinine level in serum that suggests reduction of glomerular filtration rate [22].

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 to increased significant serum creatinine in wistar rats within three to seven days [23, 24]. Our result showed that significantly recovered serum creatinine level in curative and protective groups with treatment of 400mg/kg dose of same extract (Table no.1).

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 monitored significantly (** (P<0.01) in curative and protective groups. However GSH monitored less significant (*P<0.05) same dose in protective group. (Table2). Our present result data shown that significantly monitored GSH, SOD, and CAT and lipid peroxidation. This is indicate that extract have antioxidant activity. 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.

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].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, Ca^{++} ATPase and Mg^{++}ATPase were recovered Significantly (**P<0.01) in curative group and protective groups but Mg^{++}ATPase less significant (*P<0.05) recovered with treatment of same extract. (Fig. 2, 3 & 4). It is indicates that extract may have capacity to deduced cisplatin induced nephrotoxicity.

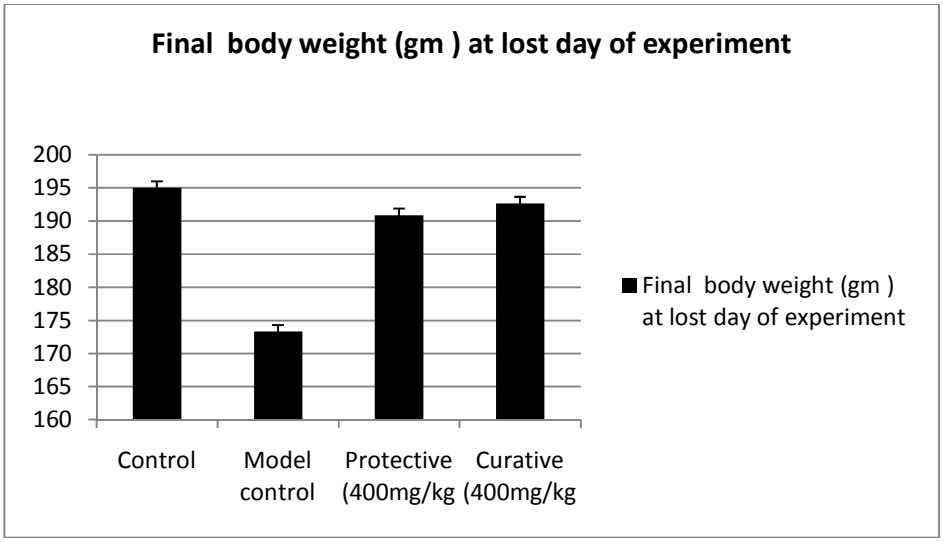

Figure 2. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds on final weight (gm) in protective and curative groups

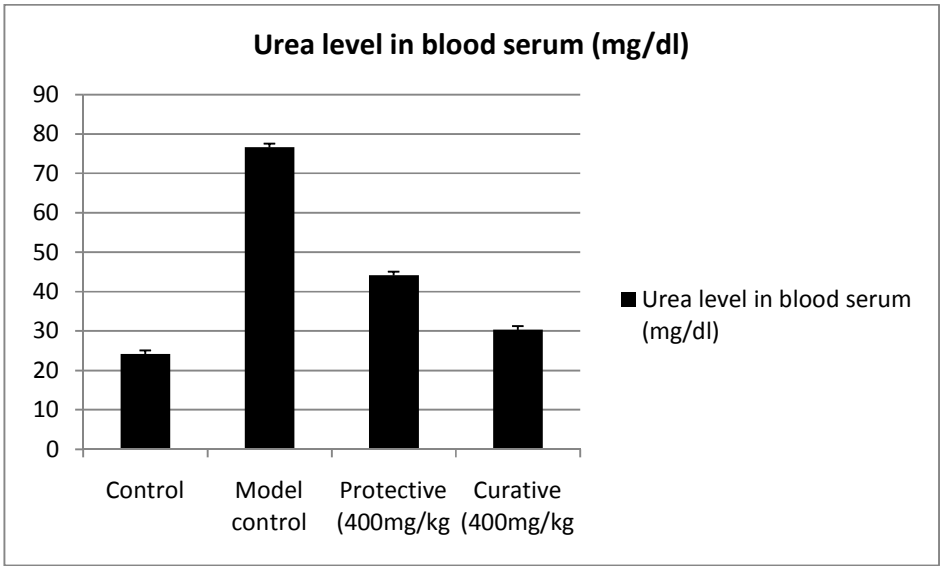

Figure 3. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds on blood serum urea in protective and curative groups

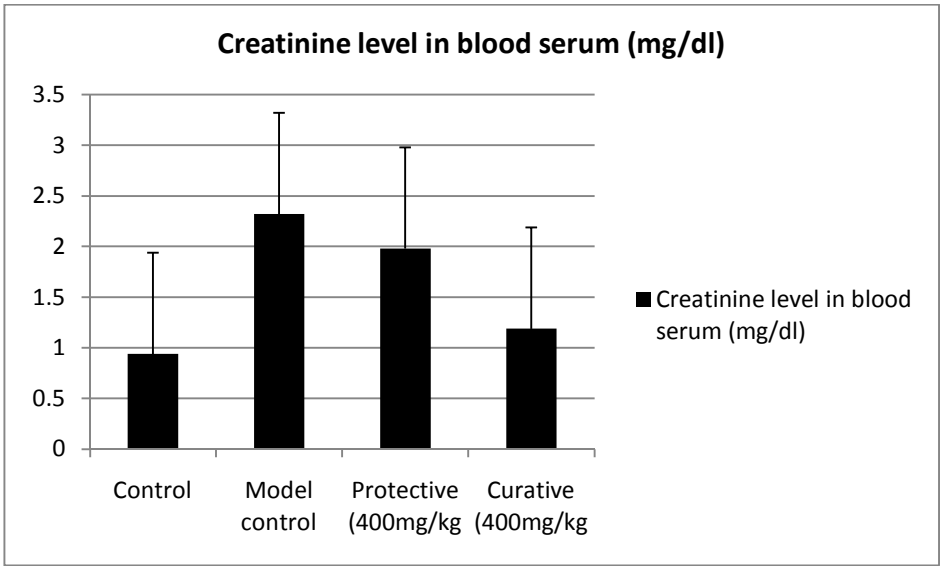

Figure 4. Effect of treatment with ethanolic extract of *Lepidium sativum* (L) seeds on serum creatinine level in protective and curative groups

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 and curative activity. Howerever curative treatment was more significant than protective group.

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REFERENCES

[1] Bergstrom J, Furst P, Norée LO, Vinnars E. *J Appl Physiol* **1974**; 36; 693–6.
[2] Nadkarni′S K.M. *Indian materica medica*. Reprint **1995**; I; 736.
[3] Welbourne TC. *Can J Biochem* **1979**; 57:233–7.
[4] Kirtkar K M, and Basu BD. *Indian medicinal plants*. **2005;** I: 174.
[5] Gonzalez Ricardo, Romay Cheyla, Borrego A, Merino FH, Zamora MZ, and Rojas E. Lipid peroxides and antioxidant enzyme in cisplatin induced chronic nephrotoxicity in rats. Mediators Inflamm. **2005;** 3: 139-143.
[6] Kersten L, Braunlich H, Kepper BK, Gliesing C, Wendelin M, Westphal J. *J appl toxicol* **1998;** 18(2).
[7] Zhang JG, Lindup WE. *Biochem pharmacol* **1993;** 45 (11); 677 – 683.
[8] Kaplan A, Lavemen IS. The tests of renal function, in clinical chemistry. Interpretation and techniques 2nd ed., lea and Febiger, Philadelphia, **1983;** 109-142.

[9] Varley, and Alan HG. Tests in renal disease. In Practical Clinical Biochemistry. 1984; 1: 123.
[10] Sedlak M, and Lindsay R. *Anal Biochem*; **1968;** 25:192–205.
[11] Hartree E. *Anal Biochem* **1972;** 48: 4227.
[12] Didier Portilla, Richard TP, Safar AM. Cisplatin-induced nephrotoxicity Last literature review version. **2008;** 16.3.
[13] Uchiyama M, Mihara M. *Anal Biochem*; 1978; 86: 271–8.
[14] Mishra HP, Fridovich I. *J Bio chem*, **1972;** 247: 3170.
[15] Sinha A. K., *anal biochem*, **1972,** 47,389.
[16] Bontin SL, Presence of enzyme system of mammalian tissue. Wiley inter sci. **1970,** 197,257.
[17] Hjerken S, Pan H. *Biochem Biophys Acta*, **1983,** 728,281.
[18] Ohinishi T, Suzuki Y, Ozawa K. *Biochem biophys ACTA*, **1982,** 684, 64.
[19] Mora l deo antune, LM. Francescato HD, Bianchi M del, *pharmacol.Res*. **2003;** 47, 517-522.
[20] Matsushima H, Yonemura K, Ohishi K, Hishida A. *J Lab Clin Med*, 1, **1998;** 31:518–26.
[21] Daugaard G, Abilgaard U, Holstein-Rathlou NH, Bruunshuus I, Bucher D, Leyssac PP. *Clin Pharmacol Ther* **1988;** 44:164–72.
[22] Naziroglu M, Karaoglu A, Aksoy AO, *Toxicolo*, **2004;** 195: 221-239.
[23] Francescato HDC, Costa RS, Camargo SMR, Zanetti MA, Lavrador MA, Bianchi MLP. *Pharmacol Res* **2001;** 43:77–82.
[24] Antunes LMG, Darin JDC, Bianchi MLP. *Pharmacol Res* **2001**; 43:145–50.
[25] Nelson DL, and Michael M COX. Lehninger principle of biochemistry. 4th edition ***2005*;** 857-858.
[26] Molitoris BA, Dahl R, Geerdes A. *Am J. Physical*, **1992,**265,F488-F495.