Nephroprotective and Curative Activity of *Lepidium Sativum* L. Seeds in Albino Rats Using Cisplatin Induced Nephrotoxicity

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Summary

The present study was designed to investigate to possible potential nephrocurative & nephroprotective activity of 200mg/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, & protective groups and was administrated vehicle, cisplatin, cisplatin + extract, and extract + cisplatin respectively. After 7th days cisplatin injection blood collected and determine urea and creatinine level in serum of each groups and sacrificed to quantitative estimation of glutathione, lipid peroxidation and superoxide dismutase content in kidney**.** A single dose of cisplatin induced loss in body weight, increased urea & creatinine level in serum in model control, it was recovered significantly (**p<0.01) and (**p<0.01) in curative and protective groups respectively. Whenever increase malondialdehyde, superoxide dismutase and reduction glutathione level in kidney in model control group, it was recovered significantly (**p<0.01) and (**p<0.01) in curative and protective groups respectively. 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 be have nephroprotective and curative activity

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

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Introduction

A large number of medicinal plants, natural products and dietary components have been evaluated as potential chemoprotective 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 peroxides and glutathione (GSH), which protect kidney from free radicals like nitric oxide and superoxide etc. The cisplatin is inhibited the activity of antioxidant enzyme in renal tissue, glutathione 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.-918/ac/05/CPCSEA).

Experimental design

The acute toxicity study of ethanolic extract of *Lepidium sativum* seeds L. were no toxic effect at 2000mg/kg (as per the OECD - 420). The dose was selected one tenth ($1/10^{th}$) of it, for safe treatment. 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.), 1^{st} days, Group III (Protective) received ELS extract (200mg / kg p.o.) for 1^{st} to10^{th} day, single dose of cisplatin was administered on 11^{th} day, Group IV (Curative) received single dose of cisplatin on day 1^{st}, and after 6^{th} days ELS extract (200mg / 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 kidney toxicity. [8&9]. Kidneys were removed, homogenized and centrifuged at 10,000 rpm at 0°C for 20min.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) was measured [14].

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

In present study rat treated with single dose of cisplatin shown marked reduction (184.83 ± 3.710) in body weight as compared to control group (207.50± 2.86) this weight loss was significantly ($P<0.01$) recovered with ethanolic extract of *Lepidium sativum* L. seeds in curative and protective groups (Fig1.). The single dose of cisplatin (5mg/kg) result increased urea (24.60±1.32) and creatinine (2.04±0.17) level in model control compare to respective control. (16.80 ± 0.84, 1.28±0.12) and its was significantly ($P<0.01$) recovered in curative and protective groups. (Fig2 & Fig3). Significantly ($P<0.01$) increase the lipid peroxidation (19.83 ± 2.85) and decrease the level of GSH (41.83 ± 3.25), SOD (5.83 ± 2.31) after cisplatin injection, it was significantly ($P<0.01$) monitored with ethanolic extract of *Lepidium sativum* L. seeds in curative and protective groups (Table 1).

Table1. Effect of ethanolic extract of *Lepidium sativum* L. seeds on Lipid peroxidation and antioxidant enzyme

S.N.	Groups	LOP (nm of MDA/mg) kidney tissue	GSH (µg/gm) kidney tissue	SOD (Unit /gm) kidney tissue
1.	Control	10.66 ± 2.16	63.83 ± 4.66	14.66 ± 2.54
2.	Model control	19.83 ± 2.85	41.83 ± 3.25	5.83 ± 2.31
3.	Preventive	10.67 ± 2.16**	53.33± 4.18**	11.17 ± 1.47**
4.	Curative	9.16 ± 1.94**	61.17 ± 1.94**	5.50 ± 3.93**

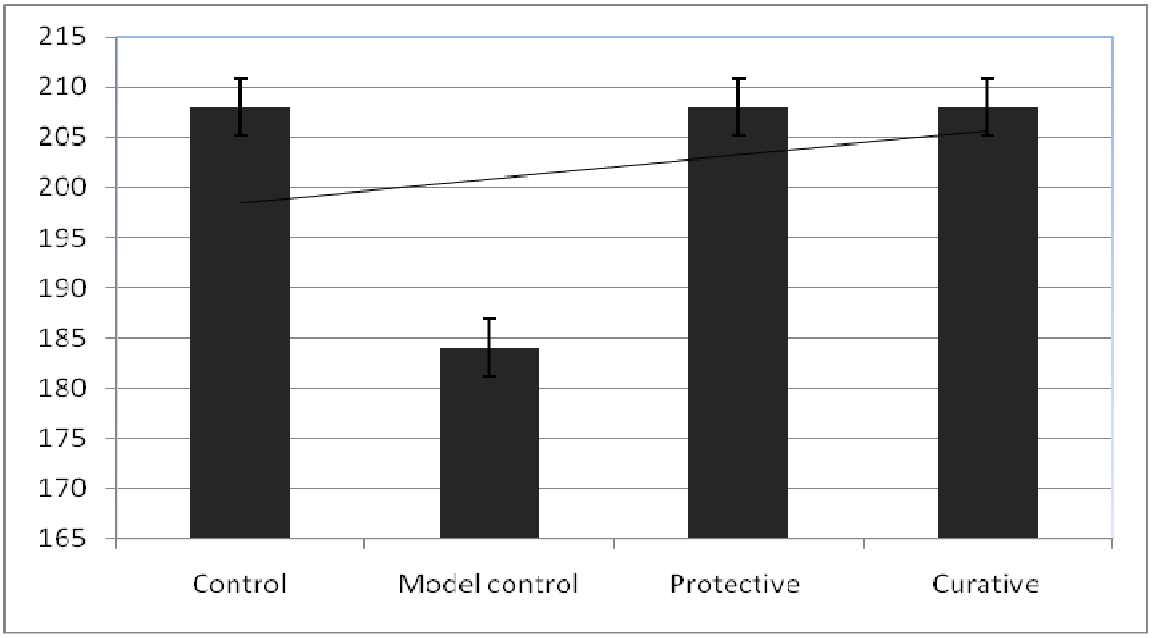

Fig.1. Effect of ethanolic extract of *Lepidium sativum* (L) seeds on body weight of protective and curative groups

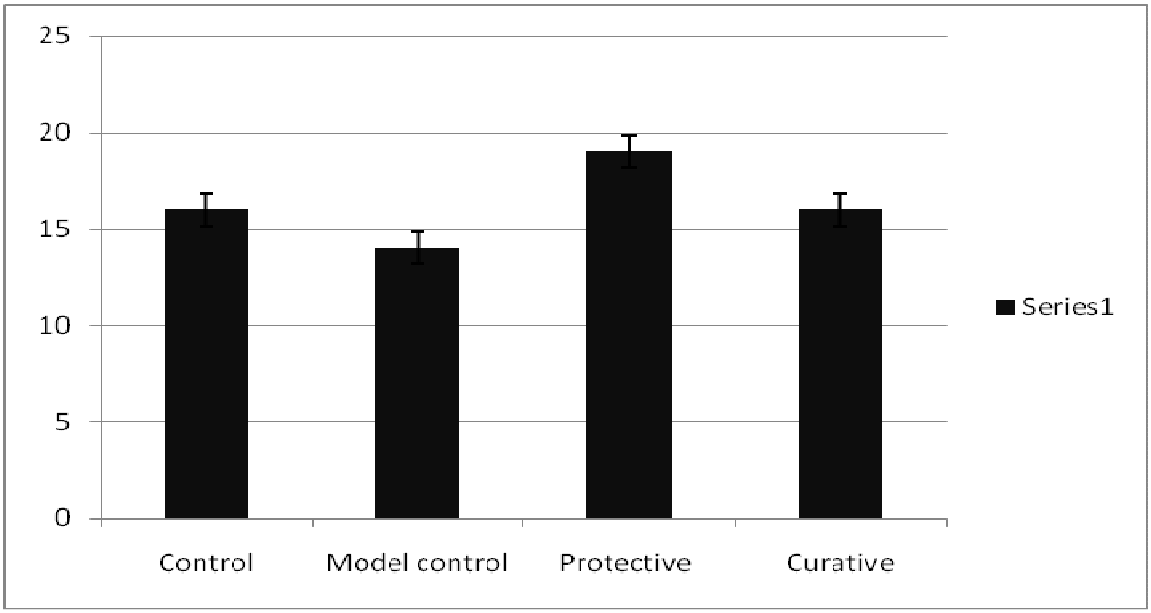

Fig.2. Effect of ethanolic extract of *Lepidium sativum* (L) seeds on urea level in serum of protective and curative groups

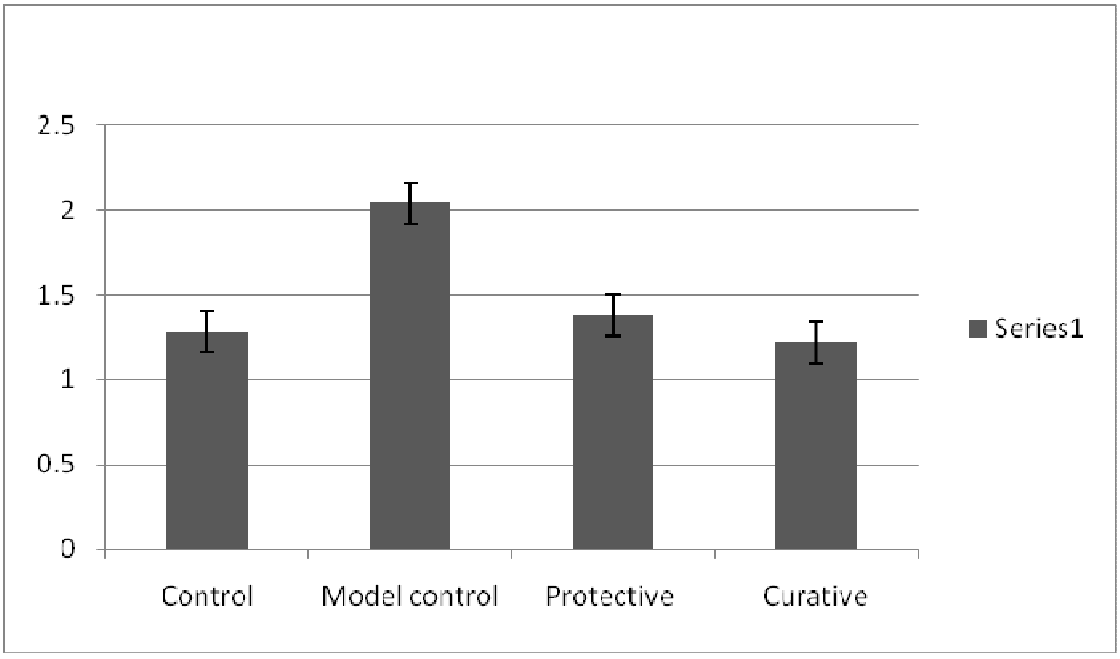

Fig.3. Effect of ethanolic extract of *Lepidium sativum* (L) seeds on creatinine level in serum of protective and curative groups

Discussion

There are a large number of synthetic drugs induce nephrotoxicity and acute renal failure (15). The acute renal failure is reversible loss of renal functions. It may be recovered by herbal medicine. In kidney having some naturally occurring antioxidant enzyme like Superoxide dismutase (SOD), lipid peroxides and glutathione (GSH), which protect kidney from free radicals, induce oxidative renal impairment (5).

After single dose cisplatin administration, reduction of body weight. It was observed in model control group but in curative and protective groups, were significantly recovered (**p< 0.001), (**p<0.01) respectively (Fig.1). Weight loss during cisplatin treatment may be due to gastrointestinal toxicity and by reduced ingestion of food (16). The single dose of cisplatin (5mg/kg) result increased urea and creatinine level in model control compare to control. It was significantly (P<0.01) recovered in curative and protective groups (Fig.2&3). The increased urea and creatinine level suggests the reduction of glomerular filtration rate (17). 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.

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. The tannin (Phenolic compound), Flavonoids may have antioxidant activity whenever Glutamate, Cysteine, Glycine are intermediates for synthesis of the endogenous antioxidant glutathione [18]. It's all may contribute synergistic reason to increase significantly (P<0.01) GSH level and decrease significantly (P<0.01) lipid peroxidation in protective and curative group (Table2.) however after cisplatin treatment decreased GSH and increased lipid peroxidation level in model control that indicates production of free radicals involvement of oxidative stress due to cisplatin induced nephrotoxicity (19).

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 be have nephroprotective and curative activity.

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