Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Study on Protective Effect of Omega -3 Fatty Acid Rich Extract of Linium Usitatissimum Seeds on Cisplatin Induced Nephrotoxicity

Priyanka Saini*

Received on: 12-06-2014
Accepted on: 05-07-2014
Published on: 15-08-2014

Corresponding Author:

Priyanka Saini *
Rajiv Gandhi Technical University

*Email Id- b.suribabu20@gmail.com

ABSTRACT

There are many diseases that are still considered as untreatable by most of the physicians, and epilepsy is one of those. Traditional healers claim that they can treat epilepsy by use of some natural components. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of herbal drugs/ products for medicinal benefits. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. One of the important and well-documented uses of plant products is their use as nephro-protective agents. Hence, there is an ever increasing need for safe nephro-protective agent.

Oxidative stress is a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them. Thus due to easy availability of plant and its traditional uses, the present study was designed to investigate the effect of plant extract against Cisplatin induced nephrotoxicity in rats by using biochemical parameters and antioxidant status in kidney of rats after oral administration of 100 and 200 mg/ kg body weight of extract.

Key-words: Omega -3 Fatty Acid, Acid Rich Extract, Linium Usitatissimum Seeds, Cisplatin Induced Nephrotoxicity

Cite this article as:
Priyanka Saini, Study on Protective Effect of Omega -3 Fatty Acid Rich Extract of Linium Usitatissimum Seeds on Cisplatin Induced Nephrotoxicity, Asian Journal of Pharmaceutical Technology & Innovation, 02 (07); 2014.

Introduction

Due to today's life style, human beings are exposed to environmental, occupational and xenobiotics challenges. Enormous free radicals are generated during the exposure to such stressful challenges. Cisplatin is called the "penicillin of cancer" because it is used so widely and it was the first big chemotherapy drug for various types of cancer. Cisplatin forms highly reactive, charged, platinum complexes which bind to nucleophilic groups such as GC-rich sites in DNA, inducing intrastrand and interstrand DNA and DNA-protein cross-links which results in apoptosis and cell growth inhibition[1]. In addition the process of metabolism and excretion of Cisplatin may also generate free radicals. These free radicals bind covalently with the tissue macromolecule leading to the cell necrosis[2]. Its chief dose limiting side effect is nephrotoxicity; 20% of patients receiving high-dose Cisplatin have severe renal dysfunction. The quiescent proximal tubule cells are selectively damaged by Cisplatin.

The mechanism for this renal cell injury has been the focus of intense investigation for many years, and recent studies suggest that inflammation, oxidative stress injury, and apoptosis probably explain part of this injury. Understanding the mechanism(s) for this side effect should allow clinicians to prevent and/or treat this problem better and provides a model for investigating drug-induced nephrotoxicity in general[1,3].

The disproportionate accumulation of Cisplatin in kidney tissue by peritubular uptake in both proximal and distal nephrons contributes to Cisplatin induced nephrotoxicity[4]. In rat, Cisplatin excretion occurs predominantly by glomerular filtration and to a lesser extent through secretion [4, 5].

Omega 3 fatty acids are fatty compounds commonly available in oil of natural origin. A research demonstrate that the use of more quantity of omega-3 fatty acid from foodstuff produces a 25% reduced risk of breast cancer events[6]. Many reporting supports concomitant use of omega 3 fatty acids with chemotherapy to get rid of chemotherapy induced toxicity. The present study was aimed at evaluates nephro-protective activity of *Linium usitatissimum* against Cisplatin induced nephrotoxicity on rats.

Material & Methods

Drugs

Cisplatin (cytoplatin-10) (Cipla) were purchased from the medical store, Bhopal. All other chemicals and reagents used were of analytical grade.

Animals

Male wistar rats, procured from commercial breeder, were kept for a week for acclimatization under environmentally controlled conditions with an alternating 12hrs light/dark cycle (light on 6:00-18:00 hrs) and water provided *ad libitium*. The animals were provided with controlled feed of 50g/cage to study the effect of treatment on food intake.

Rats weighing 150-250 gm were used for the experiments. All animal experiments were carried out according to the guidelines and approval of Institutional Animal Ethics Committee.

Plant Material

Seeds of *Linum usitatissimum* are selected as plant material for further investigation as it is reported to be a good source of Omega 3 fatty acids and was collected locally from market of Bhopal.

Experimental design

PREPARATION OF PLANT EXTRACT

Pet ether (40:60) will be used as solvent for extraction. Maceration will be the preferred method for extraction to avoid degradation of thermo labile material.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening revealed the presence of Carbohydrates, Saponin, Tannins in the extract.

ACUTE TOXICITY STUDIES

The acute oral toxicity study was carried out as per the OECD guideline 423 set by Organization for Economic Cooperation and Development received from Committee for the Purpose of Control and Supervision of Experiments on Animals. Dose of 5 mg/kg to 2000 mg/kg was taken to study the toxicity effect of the extract.

NEPHROTOXICITY STUDY

a) Drug solution preparation

Cisplatin (Cytoplatin-10) 20mg/10ml was used as such (0.5 mg/ml) without any dilution for inducing nephrotoxicity.

Table 1: grouping of animals for different treatment

Sr. No.	Groups (n=6)	Treatment
Group I	Vehicle treated	Animals were treated with (10ml/kg.)
Group II	Extract treated (A)	*Linseed seeds* (100 mg/kg b.wt.) + Cisplatin (7.5 mg/kg b.wt).
Group III	Extract treated (B)	*Linseed seeds* (200 mg/kg b.wt.) + Cisplatin (7.5 mg/kg b.wt).
Group IV	Standard drug	Cisplatin (7.5 mg/kg b.wt)

The animals were divided into 4 groups of 6 animals each. Group I served as Vehicle treated. Group II and III were administered with various doses of Cisplatin and *Linseed seeds* extract for 6 consecutive days starting from the same of Cisplatin administration. Group IV served as standard drug treated. Cisplatin was given by i.p. single dose on 6^{th} day. Animal was sacrifice 7^{th} day.

b) Method for blood collection

Approximately 1.5 ml of blood was withdrawn from retro-orbital puncture plexus under light ether (anesthesia). Each blood sample was collected in a clean labeled append off containing 10% EDTA. Upper serum layer was collected and analyze for serum creatinine, BUN, urea, uric acid, total protein content

Figure 3: Oral Dosing

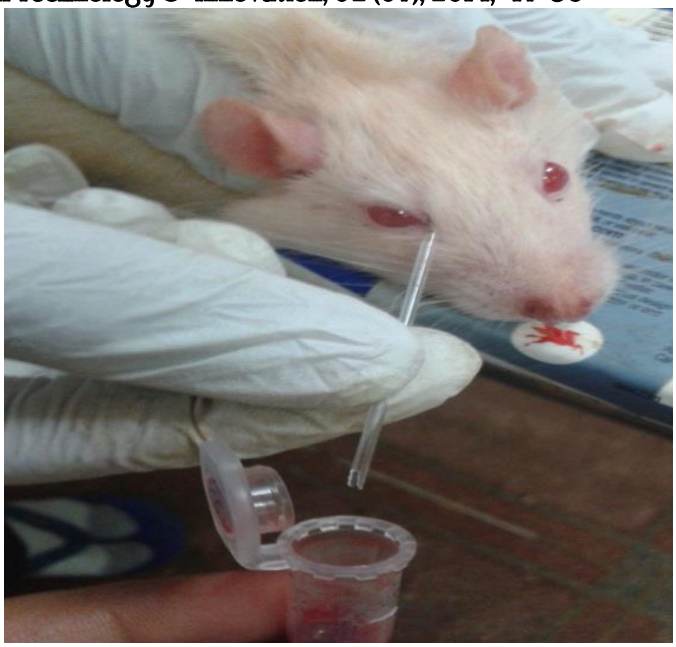


Figure 4: Retro-orbital puncture

c) Kidney function test

Estimation of Serum Creatinine Method

Creatinine reacts with alkaline picrate to produce an orange-yellow color. The absorbance of the orange-yellow color formed is directly proportional to creatinine concentration and is measured phtometrically at 500-520 nm.

Estimation of Serum Urea Method

The estimation of urea in serum involves enzyme catalysis reactions. The rate of decrease in absorbance is monitored at 340 nm and is directly proportional to urea concentration in the sample.

Estimation of Uric Acid Method

Estimation of Uric Acid was done with a modified Trinder peroxidase method using TBHB. The intensity of chromogen (Quinoneimine) formed is proportional to the uric acid concentration in the sample when measured at 505 nm (500-540nm).

Estimation of Total Protein content Method

The peptide bond of protein reacts with copper II ions in alkaline solution to form blue-voilet complex, (biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds. Tartarate is added as stabilizer whilst iodide is used to prevent auto- reduction of the alkaline copper complex. The color formed is the proportional to the protein concentration and is measured at 546 nm (520-560 nm).

Method for collection of kidney

Rats were euthanizing under light anesthesia with diethyl ether and kidneys were dissected out. The kidney was perfused with buffer saline to remove access blood and then right kidney was isolated and stored at -20^{0}C for next day kidney was weighed and homogenize for estimation of GSH, LPO, Catalase and SOD. The left kidney was fixed with 10% neutralized buffered formalin for further histopathological study.

Figure 5: Kidney measurement

Kidney weight

Before kidney homogenate preparation kidney was placed on butter paper and weight on an analytical balance to obtain wet weight.

d) Estimation of antioxidant enzymes

In vivo Biochemical assay for Creatinine, Lipid peroxidase (LPO), Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (enzymes involved in oxidative stress) was performed to determine the % inhibition of these enzymes. Interpretation will be based on standard curve of MDA (Results are expressed in equivalent to MDA).

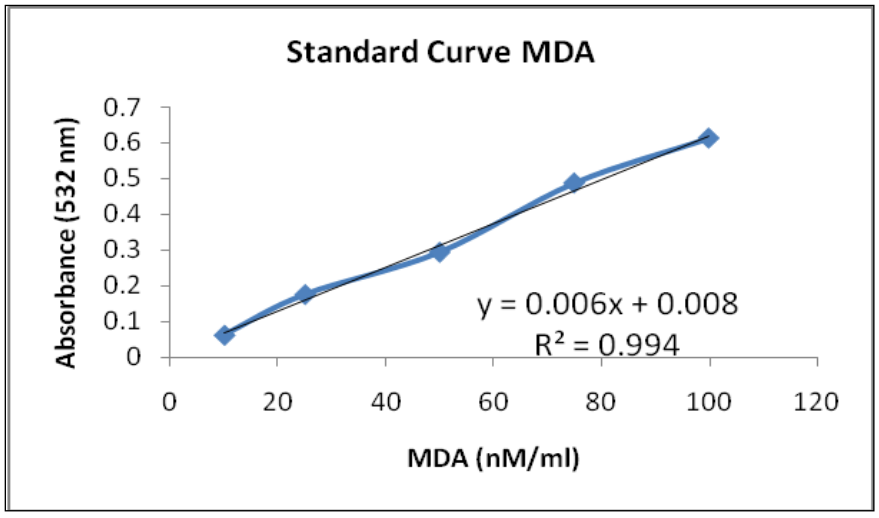


Figure 6: Standard curve MDA

Result & Discussion

1. Physical characterization

From physical examination it was observed that extract was Golden brown, Odourless, Semi solid and lipophilic in nature (Table.2). From phytochemical investigation it was fond that extract was having fatty components and sterols in it (Table.3)

Table 2: Physical characters of extract

Sr. No.	Characters	Observation
1.	Colour	Golden brown
2.	Odour	Odourless
3.	Consistency	Semi solid
4.	Solubility	Soluble in Tween

Table 3: Phytochemical characters of extract

Sr. No.	Test	Observation	Inference
1.	Carbohydrates test	Purple ppt.infernce	Positive
2.	Protien test	Dark blue ppt.was farmed	Negative
3.	Saponin test	White colour appeared	Positive
4.	Glycosides test	Redish brown	Negative
5.	Alkaloids test	Yellow colour appears	Negative
6.	Tannins test	Red colouration with dil. Iodine solution	Positive
7.	Amino acid test	Black ppt	Negative
8.	Flavonoid test	Buff colour appeared	Negative

2. Acute Toxicity Study

To ascertain safety of extract, extract was given up to 2000 mg/kg. Acute oral toxicity study revealed that extract was not having any type of toxicity in acute administration till 2000 mg/kg (Table.4). Thus 2000 mg/kg was considered as Not Observed Adverse Effect Limit (NOAEL). 1/20th and 1/10th of NOAEL, i.e. 100 mg/kg and 200 mg/kg were selected as dose for present investigation.

Table 4: Acute oral toxicity of extract

S.No.	Dose	Number of animals	Mortality
1	5 mg/kg	03	0/3
2	5 mg/kg	03	0/3
3	50 mg/kg	03	0/3
4	50 mg/kg	03	0/3
5	300 mg/kg	03	0/3
6	300 mg/kg	03	0/3
7	2000 mg/kg	03	3/3
8	2000 mg/kg	03	3/3

3. CREATININE Level Analysis

Creatinine level was found to be 1.587 mg/dl in Cisplatin treated group which was significantly higher ($P<0.05$) as compared to vehicle treated group in which level was 0.613 mg/dl. In extract treated animals level of Creatinine was 0.963 and 0.885 mg/dl respectively at 100 mg/kg and 200 mg/kg, which were significantly higher ($P<0.05$) as compared to cisplatin treated animals (Table.5).

Table 5: Creatinine level in blood of animals of different treatment group

Treatment Groups	Creatinine mg/dl#
vehicle treated	0.613
Cisplatin	1.587
Extract (100 mg/kg)	0.963 *
Extract (200 mg/kg)	0.885 *

Data presented in Mean±SD

* P<0.05 as compared to Cisplatin treated group

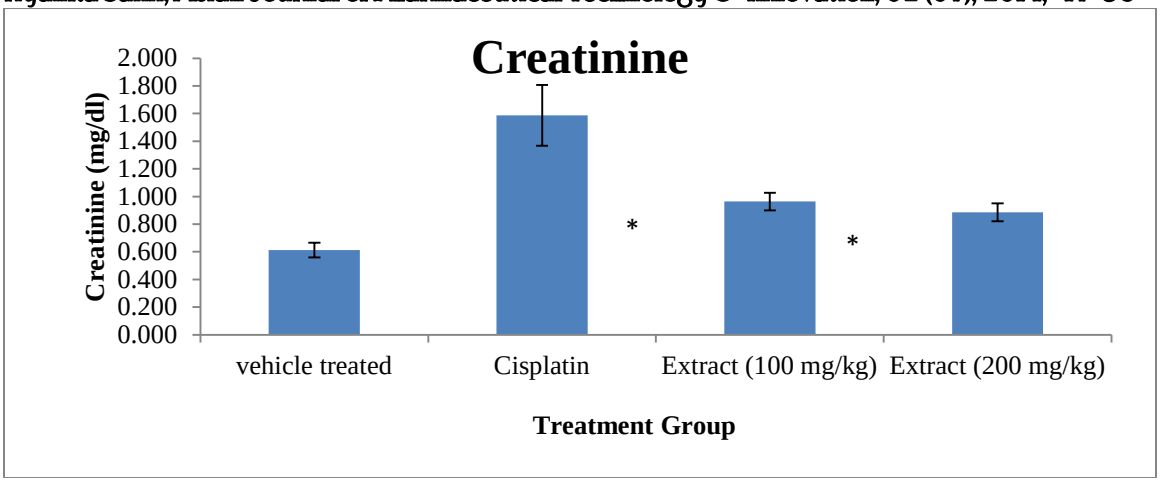


Figure 7: Creatinine level in blood of animals of different treatment group

* P<0.05 as compared to Cisplatin treated group

4. BUN Level Analysis

BUN level was found to be 20.53, 31.78, 26.05, and 22.35 mg/dl in vehicle, Cisplatin, Extract (100 mg/kg), and Extract (200 mg/kg) treated animals (Table.6). Effect of cisplatin was significantly toxic (P<0.05) as compared to vehicle treated animals and extract were protective (P<0.05) as compared to cisplatin treated at the dose of 100 mg/kg and 200 mg/kg.

Table 6: BUN level in blood of animals of different treatment group

TREATMENT GROUP	BUN gm/dl #
vehicle treated	20.53
Cisplatin	31.78
Extract (100 mg/kg)	26.05
Extract (200 mg/kg)	22.35

Data presented in Mean±SD

* P<0.05 as compared to Cisplatin treated group

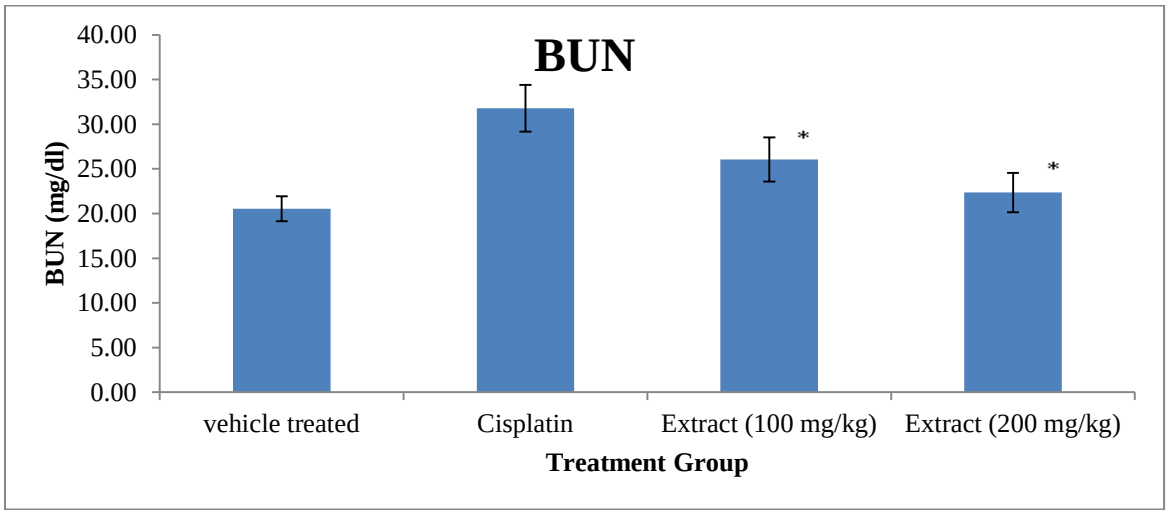


Figure 8: BUN level in blood of animals of different treatment group

* P<0.05 as compared to Cisplatin treated group

5. LPO Level Analysis

Lipid per oxidase (LPO) is the enzyme involved in oxidative degradation of lipids. In the process free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids,

because they contain multiple double bonds in between which lie methylene -CH2- groups that possess especially reactive hydrogens. LPO level in vehicle treated animals was 90.88 nmol/gm, which was found to significantly elevated (P<0.05) to 155.62 nmol/gm in Cisplatin treated animals. In extract treated animals level of LPO decreased significantly (P<0.05) to 129.26 and 117.19 nmol/gm at 100 and 200 gm/kg respectively (Table.7).

Table 7: LPO level in blood of animals of different treatment group

Treatment Groups	LPO(nmol/gm wet tissue)#
vehicle treated	90.88
Cisplatin	155.62
Extract (100 mg/kg)	129.26 *
Extract (200 mg/kg)	117.19 *

*# Data presented in Mean±SD * P<0.05 as compared to Cisplatin treated group*

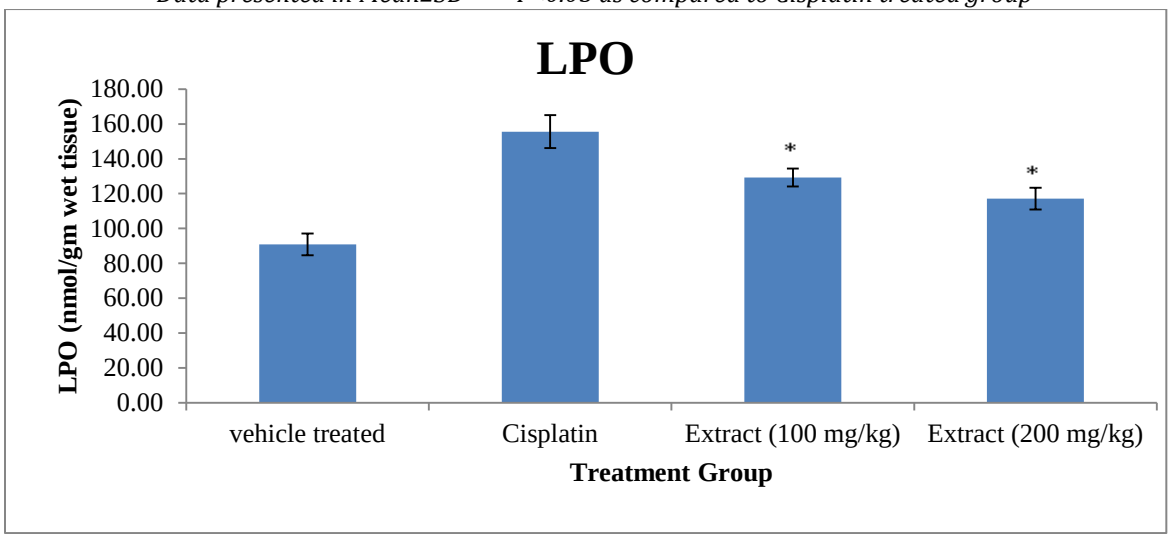


Figure 9: LPO level in blood of animals of different treatment group
** P<0.05 as compared to Cisplatin treated group*

6. SOD Level Analysis

SOD is a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Metal ion cofactors - Copper, Zinc, Manganese or Iron. Copper/zinc SOD is present in the cytosol. Manganese SOD is present in the mitochondrion. SOD in extracellular fluids, which contains copper and zinc in its active sites The mitochondrial isozyme seems to be the most biologically important of these three. SOD was found to be 24.57, 12.82, 16.65, and 19.05 nMole/gm in vehicle, Cisplatin, Extract (100 mg/kg), and Extract (200 mg/kg) treated animals (Table.8). Effect of cisplatin was significantly toxic (P<0.05) as compared to vehicle treated animals and extract were protective (P<0.05) as compared to cisplatin treated at the dose of 100 mg/kg and 200 mg/kg.

Table 8: SOD level in animals of different treatment group

Treatment Groups	SOD(nmol/gm wet tissue)#
vehicle treated	24.57
Cisplatin	12.82
Extract (100 mg/kg)	16.65 *
Extract (200 mg/kg)	19.05 *

*# Data presented in Mean±SD * P<0.05 as compared to Cisplatin treated group*

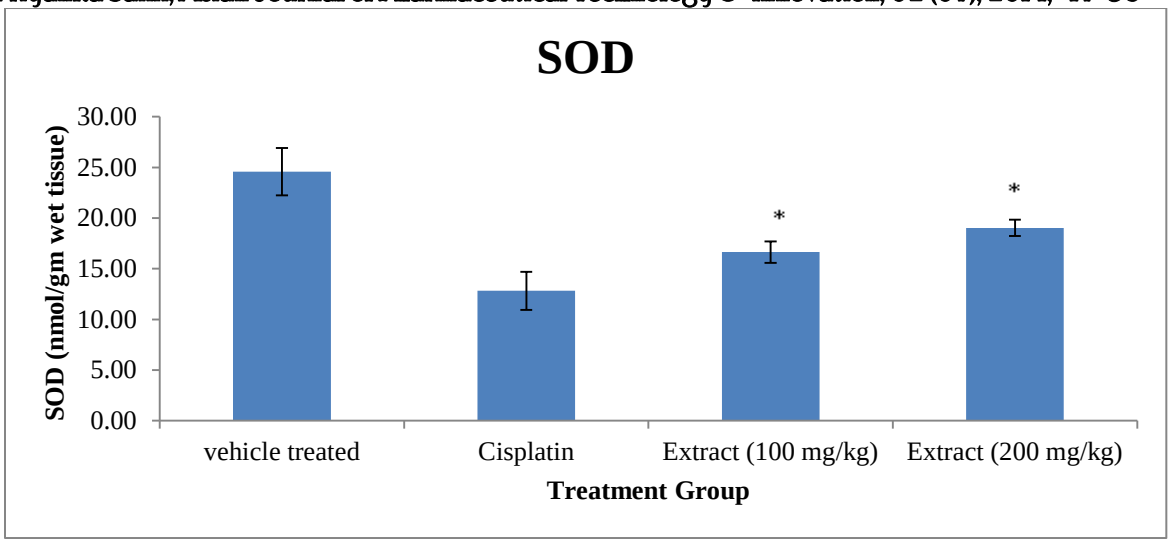


Figure 10: SOD level in blood of animals of different treatment group
** P<0.05 as compared to Cisplatin treated group*

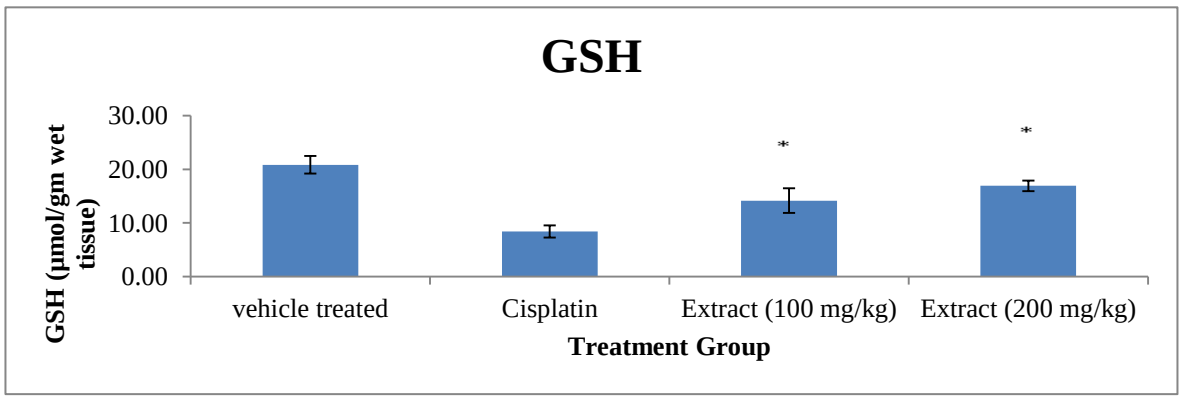
7. GSH Level Analysis

Biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. There are several isozymes encoded by different genes, which vary in cellular location and substrate specificity. (GPx1 to GPx8). Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. By increasing one's glutathione (GSH) levels, you help the productivity of the glutathione peroxidase (GPx) and vice versa. GSH was observed to to be 20.83, 8.40, 14.17, and 16.94 µmol/gm in vehicle, Cisplatin, Extract (100 mg/kg), and Extract (200 mg/kg) treated animals (Table.9). Cisplatin was exerting significantly oxidative stress (P<0.05) as compared to vehicle treated animals and extract were protective (P<0.05) as compared to cisplatin treated at the dose of 100 mg/kg and 200 mg/kg.

Table 9: GSH level in animals of different treatment group

Treatment Groups	GSH(µmol/gm wet tissue#)
vehicle treated	20.83
Cisplatin	8.40
Extract (100 mg/kg)	14.17 *
Extract (200 mg/kg)	16.94 *

*# Data presented in Mean±SD * P<0.05 as compared to Cisplatin treated group*



** P<0.05 as compared to Cisplatin treated group*

8. CATALASE Level Analysis

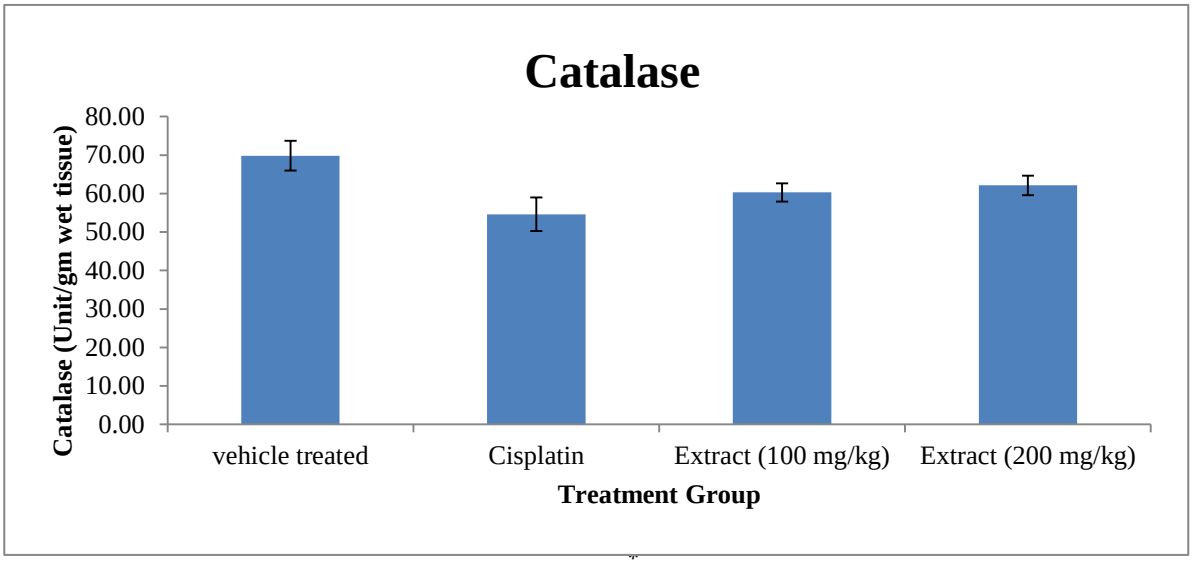
Catalases are enzymes that catalase the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. This protein is localized to peroxisomes in most eukaryotic cells. Its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Catalase was estimated to be 20.83, 8.40, 14.17 and 16.94 unit/gm in vehicle, Cisplatin, Extract (100 mg/kg), and Extract (200 mg/kg) treated animals (Table.10). Cisplatin was exerting significantly oxidative stress (P<0.05) as compared to vehicle treated animals and extract were protective (P<0.05) as compared to cisplatin treated at the dose of 100 mg/kg and 200 mg/kg.

Table 10: Catalase level in animals of different treatment group

Treatment Groups	Catalase(unit/gm wet tissue)#
vehicle treated	20.83
Cisplatin	8.40
Extract (100 mg/kg)	14.17 *
Extract (200 mg/kg)	16.94 *

 # Data presented in Mean±SD

** P<0.05 as compared to Cisplatin treated group*



P<0.05 as compared to Cisplatin treated group

HISTOPATHOLOGICAL STUDY OF KIDNEY

In histological examination in control group normal architecture of kidney was found to be abnormal as compared to vehicle treated animals. Nephrons, Glomeruli, Bowman's capsule, efferent and afferent duct were observed. The histological observation of Cisplatin treated animals revealed marked dilation of proximal convoluted tubules with damage in epithelium and desquamation of tubular epithelium. In extract treated animals at both doses some short of protection was observable. In which 200 mg/kg was found to be more protective as compared to 100 mg/kg.

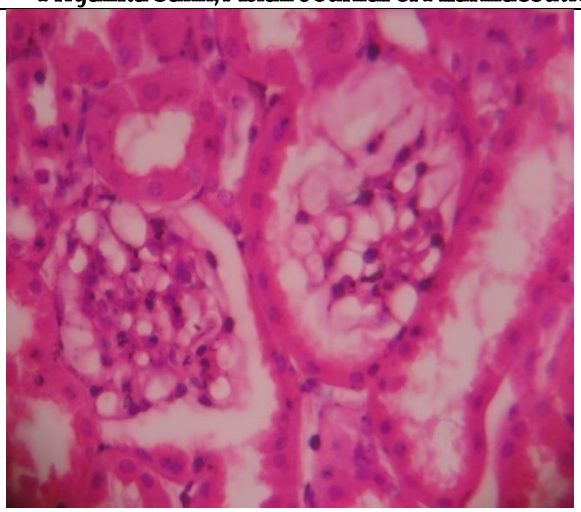


Figure13: Vehicle treated

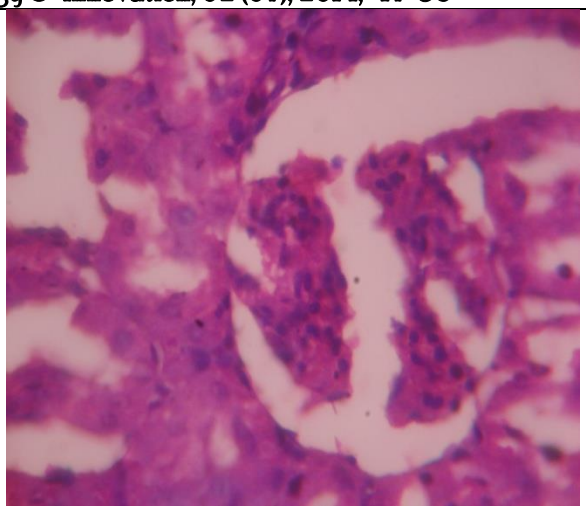


Figure14: Control treated

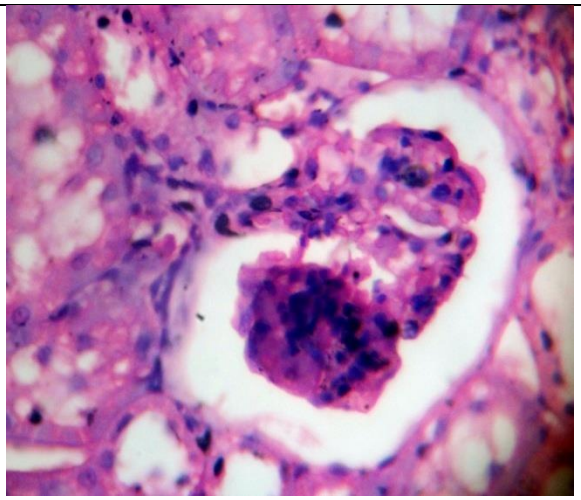


Figure15: 100 mg/kg extract treated

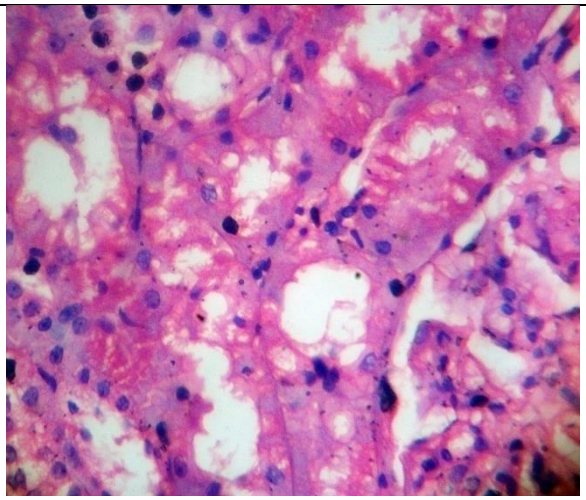


Figure16: 200 mg/kg extract treated

CONCLUSION

Since ancient time plants are considered to be rich source of bioactive components. These bioactive components are used now a days for treatment of various ailments. Nephro-protective agents are the substances which possess protective activity against Nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances. Early literatures have prescribed various herbs for the cure of renal disorders. Co-administration of various medicinal plants possessing nephro-protective activity along with different nephrotoxic agents which may attenuate its toxicity. In present study pet ether extract of *L. Usitatissimum* was investigated for its protective potential against Cisplatin induced nephrotoxicity.

Cisplatin is a potent antitumor drug, but its clinical use is limited due to renal toxicity. Cisplatin decreases antioxidants and antioxidant enzymes leading to enhanced generation of reactive oxygen metabolites and lipid peroxidation[7, 8]. It is reported that many Indian medicinal plants show beneficial effects against renal injury[9].

An early report indicated that nephrotoxicity might occur in as many as 50 to 75% of patients receiving this drug, and is dose limiting. It is used intensively in man, being effective in ovarian & bladder

carcinoma, neuroblastoma, head and neck carcinoma, and lymphoma as well as thyroid endometrial neoplasm. However, the most significant activity is observed in testicular cancer. The clinical use of Cisplatin is often complicated by nephrotoxicity, ototoxicity, gastrointestinal disturbances like nausea, vomiting and myelosuppression.

From physical examination it was observed that extract was Golden brown, Odourless, Semi solid and lipophilic in nature (Table.2). From phytochemical investigation it was fond that extract was having fatty components and sterols in it (Table.3)

Experimental studies have shown that there is an abrupt fall in the effective renal plasma flow within 3 hrs of the i.p. dose of Cisplatin. It is known to be filtered by the glomeruli and concentrated in the glomerular filtrate from which it is activated in the presence of a low intra cellular chloride concentration. The low intracellular concentration of chloride facilitates the displacement of chloride by the water molecule yielding a positively charged, hydrated and hydroxylated complex. Hydration of Cisplatin induces formation of

monochloromonoaquodiaminoplatin or diaquodiammineplatin. These agents alkylate the purine and pyrimidine bases of nuclear material[10]. Renal damage is seen in proximal tubular S3 portion, the distal tubule and collecting duct. Other proposed explanation of the nephrotoxicity of Cisplatin include the possibility that it include generate reactive metabolites that bind covalently to tissue macromolecules. The nephrotoxic effects might also be due to sulphydryl binding of heavy metal. A reduction in sulphydryl groups in the rat renal cortex has been demonstrated; this occurred before any significant change in renal function could be detected, suggesting that this biochemical change may be a primary event. Cell fractionations have shown that the greatest decline of sulphydryl groups occurs in the mitochondrial & cytosol fractions; these also had the highest concentrations of platinum[10]. A recent study found that Cisplatin induced proximal tubule injury could be ameliorated by the administration of hydroxyl radical scavengers. In these studies Cisplatin (5mg/kg BW) caused lipid peroxidation. The hydroxyl radical scavenger prevented acute renal failure by altering tubule damage & enhancing the regenerative response of damaged tubule cells protection from Cisplatin toxicity has generally focused on providing free radical scavengers[11].

From present investigation it can be concluded that pet ether extract of *L. usitatissimum* possess significant protective potential against Cisplatin induced nephrotoxicity and this can be attributed to presence of omega 3 fatty acid and its potential to modulate enzymes involved in oxidative stress. In future study is required to confirm mechanism of action behind the said activity.

REFERENCES

1. http://www.cancer.gov/drugdictionary?CdrID=39515
2. Vijay KK, Naidu MUR, Shifow AA, Ratnakar KS, Nephroprotective and antioxidant activity of ethanolic extract of the bark of *Madhucalongifolia* (Koenig), Indian J Pharmacol. 2000; 32: 108.
3. Schrier RW, Wang W, Poole B, Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. J Clin Invest 2004; 114: 5–14.
4. Arany I, Safirstein RL, "Cisplatin", SeminNephrol. 2003; 23: 460-4.
5. Kroning R, Lichtenstein AK ,Nagami GT, Sulfur containing amino acids decrease cisplatin cytotoxicity and uptake in renal tubule epithelial cell lines. Cancer Chemother Pharmacol. 2000; 45: 43-9.
6. Xin Yao, Kessarin Panichpisal, Neil Kurtzman, Kenneth Nugent, Cisplatin Nephrotoxicity: A Review, Am J Med Sci. 2007; 334(2): 115–124.
7. Baumann, K and Hannemann. J. Toxicol. 1988; 51: 119- 132.
8. Sadzuka,Y., Shoji, T. and Takino, Y. Toxicol. Lett. 1992; 62: 293-300.
9. Ali, B.H. and Al Moundhri, MS. Food Chem. Toxicol. 2006; 44: 1173-1183.
10. Davison A M, Cameron J S, Grunfeld J P, Kerr D N S. Oxford Textbook of Clinical Nephrology 2(3), Published by Oxford University Press, 1988; 2650-53.
11. Brenner B M, The Kidney, Published by W B Saunders Co. USA. 6: 1563-1564.
12. Kroning R, Lichtenstein AK ,Nagami GT, Sulfur containing amino acids decrease cisplatin cytotoxicity and uptake in renal tubule epithelial cell lines .Cancer Chemother Pharmacol 2000;45:43-9.
13. Gately DP, Howell SB. Cellular accumulation of the anticancer agent cisplatin: a review. Br J Cancer 1993; 67: 1171–6.
14. Patterson RE. Flatt SW, Newman VA, Natarajan L, Rock CL, Thomson CA, Caan BJ, Parker BA. Marine Fatty Acid Intake is Associated with Breast Cancer Prognosis. J Nutrition, 2010; 141 (2): 201–206.
15. Haliga R, Mocanu V, Păduraru I, Stoica B, Oboroceanu T, Luca V., Effects of dietary flaxseed supplementation on renal oxidative stress in experimental diabetes. Rev Med Chir Soc Med Nat Iasi. 2009; 113(4): 1200-4.
16. Barakat LA, Mahmoud RH., The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats., N Am J Med Sci. 2011; 3(9): 411-7.
17. Ogborn MR, Nitschmann E, Bankovic-Calic N, Weiler HA, Aukema HM., Effects of flaxseed derivatives in experimental polycystic kidney disease vary with animal gender. Lipids. 2006; 41(12): 1141-9.
18. Sankaran D, Bankovic-Calic N, Peng CY, Ogborn MR, Aukema HM., Dietary flax oil during pregnancy and lactation retards disease progression in rat offspring with inherited kidney disease. Pediatr Res. 2006; 60(6): 729-33.
19. Stuglin C, Prasad K., Effect of flaxseed consumption on blood pressure, serum lipids, hemopoietic system and liver and kidney enzymes in healthy humans. J Cardiovasc Pharmacol Ther. 2005; 10(1): 23-7.