ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2014; 5(5): 95 - 102

RESEARCH ARTICLE

Antioxidant and Protective Effects of *Vitex negundo* against Cisplatin induced Nephrotoxicity in Male Albino Rats

M. Janakiraman[*1], K. Jeyaprakash[2], R. L. Rengarajan[3]

[1]*M.Janakiraman, Assistant Professor and Head, Department of Biochemistry, J.J.College of Arts and Science (Autonomous), Pudukkottai-622 422, Tamilnadu, India*
[2]*Head, PG and Research Department of Biochemistry, Rajah Serfoji Govt College (Autonomous), Thanjavur, Tamilnadu, India*
[3]*Research Scholar, Department of Animal Science, Bharathidasan University, Tiruchi, Tamilnadu, India*

Received 23 Jun 2014; Revised 09 Oct 2014; Accepted 21 Oct 2014

ABSTRACT

The aim of the present study was to investigate the renoprotective and antioxidant activities of methanolic extract of leaves of *Vitex negundo* against cisplatin induced nephrotoxicity in male albino rats. Male albino rats were randomly divided into four groups of six rats each group I was served as the normal control group, group II was served as the cisplatin treated group, group III was served as the silymarin treated group and group IV was served as *Vitex negundo* treated group. After 15 days treatment, the kidney of each rat was excised, cleaned, weighed, rinsed in ice-cold saline, homogenized and centrifuged. Collected renal tissue supernatant were used for the analysis of level of malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) and glutathione peroxidase (GPx) in all groups. Histological examination was also carried out in all groups. *Vitex negundo* treated rats revealed significant reduction in MDA level and increased in GPx, CAT and SOD activities in kidney tissue homogenates. Additionally, histopathological examinations revealed markedly ameliorated cisplatin induced toxicity on kidney structure. Our results proved that *vitex negundo* has antioxidant and protective effects against cisplatin induced oxidative stress. Thus, it could be used as a dietary supplementation to reduce toxic side effects of anticancer drugs.

Key words: *Vitex negundo,* Cisplatin, Silymarin, Antioxidant and Nephrotoxicity, Histopathology.

INTRODUCTION

The kidneys are a primary component of the body's defense against toxins and other foreign substances in the environment. Despite the importance of the excretion of metabolic waste products and potential toxins, the threat to life in renal failure typically comes not from the accumulation of metabolic wastes or environmental toxins, but from the loss of the body's ability to balance the daily intake of salts and water by an appropriate rate of excretion. [1]

Nephrotoxicity is a poisonous effect of some substances; both toxic chemicals and medication, on the kidneys. Drugs are a common source of acute kidney injury. Compared with 30 years ago, the average patient today is older, has more comorbidity, and is exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function.[2] Drugs shown to cause nephrotoxicity exert their toxic effects by one or more common pathogenic mechanisms. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. [3] Clinical syndromes of nephrotoxicity can be defined according to the predominant regions of the kidney affected by toxin and reversibility of the injury is likely related to the severity and nature of the injury and also to the duration of toxin exposure. [4] Since these modern medicines have certain serious side effects there is an urgent need to systematically evaluate plants for their activities. In response to this, the medicinal potential of a lot of plants have been explored.

Chemotherapy and radiotherapy are the most common methods of cancer treatment.Cisplatin (Cis-diamino dichloro platinum II) is currently

***Corresponding Author:** M Janakiraman, **Email:** mugavaiprofmj@gmail.com, **Phone No:** +91-9787195178

one of the most important chemotherapeutic drugs used in treatment of a wide range of solid tumors-head, neck, ovarian and lung cancers . However, the clinical usefulness of this drug is limited due to the induction of nephrotoxicity, a side effect that may be produced in various animal models.[5] 20% of the patients receiving high dose cisplatin have severe renal dysfunction. Cisplatin-DNA crosslinks cause cytotoxic lesions in tumors and other dividing cells. DNA damaging agents usually have less toxicity in non-proliferating cells, yet 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. [6] Understanding the mechanisms 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. [7]

A number of herbs are traditionally used in different countries for treating drug- or toxin-induced renal disorders. Many herbs and medicinal plants are rich natural sources of antioxidants. The fact that antioxidants have several preventive effects against different diseases, such as cancer, coronary diseases, inflammatory disorders, neurologic degeneration, and aging, has led to a search for foods rich in antioxidants.[8] Because of poverty and lack of access to modern medicine, about 65–80% of the world's population living in developing countries depends essentially on plants for primary health care.[9] Drug of plant origin is known to play a vital role in the management of kidney diseases.[10] and as a source of safe antioxidants. Kidney failure shall be managed by similar means of non-pharmacological and pharmacological therapeutic interventions. [11]

Vitex negundo L. (Tamil- vella nochi, Hindi- Nirgundi) belongs to the family Lamiaceae. It is an aromatic large shrub. It commonly bears tri-or penda-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. [12] It is almost found throughout India. Phytochemical analysis of plant showed that its leaves contains alkaloid (nishundine), flavonoids like flavones, luteolin-7-glucoside, casticin, iridoid glycosides, an essential oil and other constituent like vitamin C, carotene, benzoic acid, β-sitosterol and C-glycoside.[13] Traditionally it is used as vermifuge, in headache, catarrh, acute rheumatism, expectorant, fever, sinusitis, to increase memory, as hypolipidaemic, in bodyache.[14] It has got antioxidant, anti-inflammatory and immunomodulatory and anticonvulsant activities.[15] As the leaves of *Vitex negundo* L. possess anti-inflammatory and antioxidant properties, this study has been undertaken to evaluate the effect on experimentally induced cisplatin and to find its probable mechanism of action including its antioxidant potential.

MATERIALS AND METHODS

Plant Material

The leaves of *Vitex negundo* were collected from Manapparai, near Trichy district, Tamilnadu, India in the month of January. The botanical identity of the plant material was authenticated by Dr.G.V.S.Murthy, Botanical survey of India, Coimbatore, Tamilnadu, India and a voucher specimen of the plant material was deposited in the department under the number BSI/SRC/5/23/2014-2015/TECH/540 for further study.

Drugs and Chemicals

Cisplatin vial (Pharmacia India) was used to induce nephrotoxicity and Silymarin (Ranbaxy Ltd) was used as standard drug were procured from medical shop, Trichy. All other chemicals and reagents used in the study were obtained commercially and were of analytical grade.

Preparation of Plant Extract

Vitex negundo leaves are dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. The powder was then subjected to continuous hot extraction process using Sox let apparatus at 60^{o}C with methanol (90%) for 72hrs. After extraction, the solvent was removed by rotary evaporator at 200^{o}C. The extract was concentrated and stored in desiccator.

Experimental Animals

Male albino rats, weighing 150-200g were obtained from the Department of Animal Science, Bharathidasan University, Trichy.They were housed in clean polypropylene cages under standard conditions of humidity (45±4%), temperature (25±20^{o}C), and light (12 h light/12 h dark cycle), and fed with standard diet and water ad libitum.This study was approved by the Institutional Animal Ethics Committee IAEC (1416/PO/a/11/CPCSEA).

Experimental Design

After one week of acclimatization period, male albino rats were divided randomly into four groups of six animals each. Group I: Normal control rats were treated with oral dose of distilled water for 15 days. Group II: Rats were treated with single i.p.dose of cisplatin 16 mg/kg of body weight on day1. Group III: Rats were treated with oral dose of silymarin 50mg/kg body weight from 2^{nd} day to 15^{th} day for 14 days with single i.p.dose of cisplatin 16 mg/kg of body weight on day1. Group IV: Rats were treated with oral dose of methanolic extract of *vitex negundo* 200 mg/ kg of body weight from 2^{nd} day to 15^{th} day for 14 days along with single i.p.dose of cisplatin 16 mg/kg of body weight on day1. At the end of the experimental period, rats were sacrificed for the studies of enzymatic antioxidants and histopathological studies in all groups.

Preparation of Kidney homogenate

Prior to termination of the experiment on the 15^{th} day, the rats were fasted overnight. On the 16^{th} day the fasted rats were sacrificed under chloroform anesthesia. The kidney was quickly removed, washed in ice cold, isotonic saline and blotted on ash-free filter paper, The tissues were then homogenized in 0.1 M Tris-HCl buffer, pH 7.4 using a Teflon homogenizer at 4°C, the crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 min in cold centrifuge, the supernatant was kept at -20°C for assay of antioxidant enzyme activity. [16]

Assessment of Renal Function

Lipid Peroxidation Analysis

Lipid peroxidation was evaluated as malondialdehyde (MDA) production as described by Heath and Backer. [17] The animals were sacrificed by decapitation on 16^{th} day. The kidneys were dissected out, immediately placed in ice cold saline to prevent contamination with blood and they were pressed on blotted paper, weighed and homogenized in 1.5% KCl with the help of Teflon homogenizer. To 1 ml of homogenate, 2.5 ml of trichloroaceticacid (TCA, 20%) was added and centrifuged at 3500 rpm for 10 min. The resulting pellet was dissolved in 2.5 ml of 0.05 M H_2SO_4 and then 3 ml of thiobarbituric acid was added and incubated at 37^oC for 30 minutes. The contents were then extracted into 4ml of n-butanol and the absorbance was measured spectrophotometrically at 530 nm.

Enzymatic Antioxidants Analysis

Superoxide dismutase (SOD) was assayed in the renal tissue supernatant by the method of kakkar ed al.[18] Catalase (CAT) activity was assayed in the renal tissue supernatant by the method of sinha et al.[19] Glutathione peroxidase (GPx) activity was assayed in the renal tissue supernatant by the method of Rotruck *et al*.[20]

Histopathological Analysis

After the animals were sacrificed, the kidney samples were excised from the control and treated groups of animals and washed them with normal saline. They were fixed in 10% buffered formalin for 24 h and embedded in paraffin wax. Cross-sections of the kidney tissue (5-6µm thick) were prepared and stained with haematoxylin-eosin dye. The sections were evaluated by microscopical examination. [21]

Statistical Analysis

The Results were expressed as the mean value ± SD. Within group comparisons were performed by the analysis of variance using ANOVA test. Significant difference between normal control and experimental groups were assessed by student's t - test. A probability level of less than 5% ($P<0.05$) was considered as significant.

RESULTS

Effect of *Vitex negundo* on Renal Lipid Peroxidation in Cisplatin induced Nephrotoxic Rats.

The level of MDA in the kidney homogenates of group I, II, III and IV animals are shown in (**Table 1 & Fig 1**). Lipid peroxidation level was significantly increased in cisplatin treated group II when compared to normal control group I.Treatment with *vitex negundo*(Group IV) significantly ($p < 0.05$) decreased the level of kidney tissue lipid peroxidation level when compared to the level of cisplatin treated group II. The silymarin treated group III also significantly ($p < 0.05$) decreased the kidney tissue lipid peroxidation level when compared to cisplatin treated group II.

Effect of *Vitex negundo* on Renal Enzymatic Antioxidants in Cisplatin induced Nephrotoxic rats.

The activities of enzymatic antioxidants in the kidney homogenates of all the groups of animals are shown in (**Table 1 & Fig 2A, 2B & 2C**). Renal antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) level significantly decreased in

cisplatin treated group II when compared to normal control group I. Treatment with *vitex negundo* group IV significantly ($p < 0.05$) increased the level of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) level when compared to cisplatin treated group II. The silymarin treated group III also significantly ($p < 0.05$) increased the antioxidant enzyme level when compared to cisplatin treated group II.

Effect of *Vitex negundo* on Histopathological Examination in Cisplatin induced Nephrotoxic Rats.

Group I: Sections of a normal control rat kidney shows that the glomeruli **(G)** are abundant in the cortex and they are morphologically normal. The tubules **(T)** are normal and they are lined by cells with abundant bright eosinophilic cytoplasm **(arrows).**the blood vessels and the interstitium are also normal (**Fig 3A**).

Group II: Sections of cisplatin treated rat kidney shows that the glomeruli **(G)** are abundant in the cortex and they are morphologically abnormal. The tubules **(T)** show pathological changes characterized by sloughing off of the lining epithelium (**arrows).** The blood vessels and the interstitium are also normal (**Fig 3B**).

Group III: Sections of silymarin treated rat kidney shows that the glomeruli **(G)** are abundant in the cortex and they are morphologically normal. While some of the tubules **(T)** shows pathological changes characterized by sloughing off of the lining epithelium (**down arrows),** others show cells with abundant bright eosinophilic cytoplasm **(right arrows).** The blood vessels and the interstitium are also normal. Thus, there appears to be partial reversal of the tubular pathological changes induced by cisplatin(**Fig 3C**).

Group IV: Sections of *vitex negundo* treated rat kidney shows that the glomeruli **(G)** are abundant in the cortex and they are morphologically normal. All of the tubules **(T)** show lining epithelium **(arrows)** whose cells shows normal abundant bright eosinophilic cytoplasm**.** The blood vessels and the interstitium are normal. Thus,vitex negundo brought complete reversal of the tubular pathological changes induced by cisplatin(**Fig 3D**).

Table 1: Effect of *Vitex negundo* on Renal Enzymatic Antioxidants and MDA in cisplatin induced nephrotoxic rats

Groups	Treatments	SOD	CAT	GPx	MDA
I	Normal control(Normal saline 2ml/kg b.w) for 15 days	14.20±0.08	18.11±0.05	12.30±0.17	13.66±0.08
II	Treated with cisplatin (16mg/ kg b.w i.p) single dose	06.79±0.12*	10.20±0.03*	05.41±0.14*	25.73±0.12*
III	Treated with silymarin (50mg /kg b.w) in cisplatin induced rats for 15 days	12.10±0.04**	17.13±0.06**	10.22±0.08**	14.32±0.02**
IV	Treated with *Vitex negundo* (200mg/kg b.w) in cisplatin induced rats for 15 days	11.31±0.03**	16.31±0.11**	09.21±0.05**	18.71±0.01**

All values were expressed as Mean±SD (n=6). Statistically significant of *P < 0.05 compared to normal control group (I) and **P < 0.05 compared to cisplatin treated group (II). SOD (Superoxide dismutase) = units/min/mg protein, CAT (Catalase) =µmoles/min/mg protein, GPx(Glutathione peroxidase) = µmoles/min/mg protein, MDA(Malondialdehyde).= nmoles/min/mg protein.

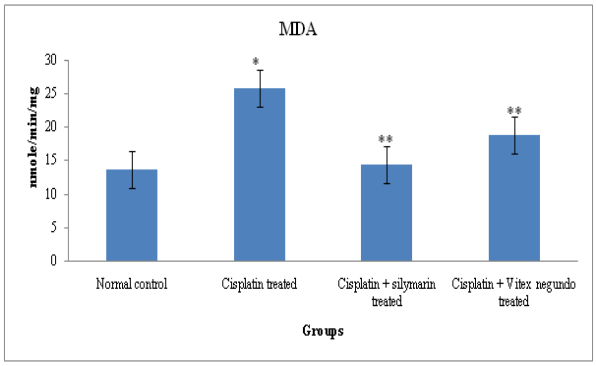


Fig 1: Effect of *Vitex negundo* on Renal Malondialdehyde (MDA) Level in Cisplatin induced Nephrotoxic Rats

Statistically significant of *P < 0.05 compared to Normal control group and **P < 0.05 compared to cisplatin treated group.

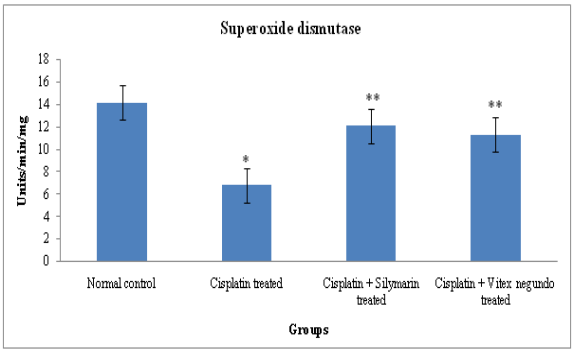
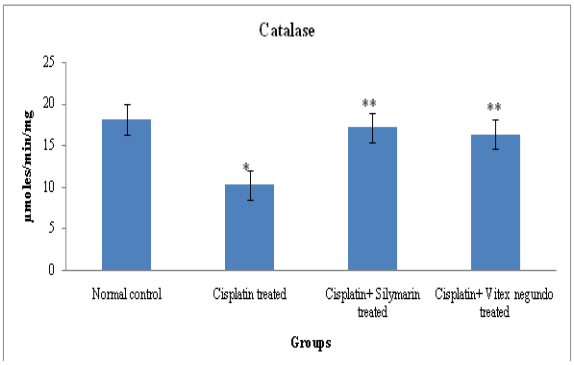
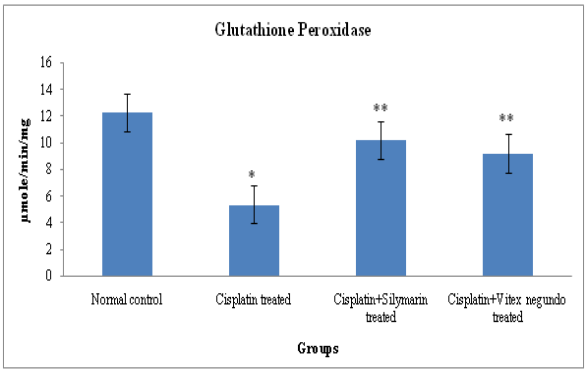


Fig 2: Effect of *Vitex negundo* on Renal Enzymatic Antioxidants in Cisplatin induced Nephrotoxic Rats
(A).Superoxide dismutase (SOD) level in cisplatin induced nephrotoxic rats. Statistically significant of *P < 0.05 compared to Normal control group and **P < 0.05 compared to cisplatin treated group.



(B). Catalase (CAT) level in cisplatin induced nephrotoxic rats

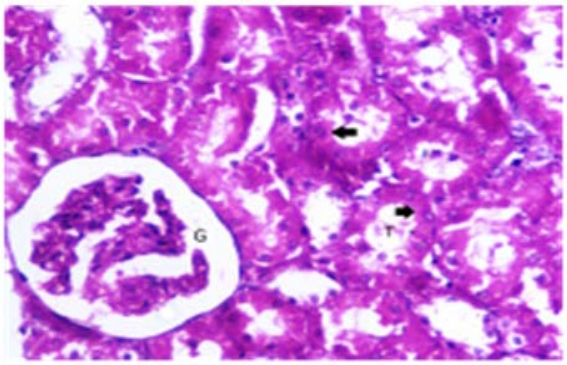
Statistically significant of *P < 0.05 compared to Normal control group and **P < 0.05 compared to cisplatin treated group



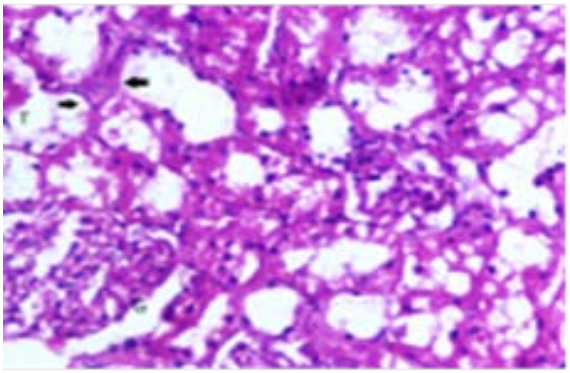
(C).Glutathione peroxidase (GPx) level in cisplatin induced nephrotoxic rats

Statistically significant of *P < 0.05 compared to Normal control group and **P < 0.05 compared to cisplatin treated group

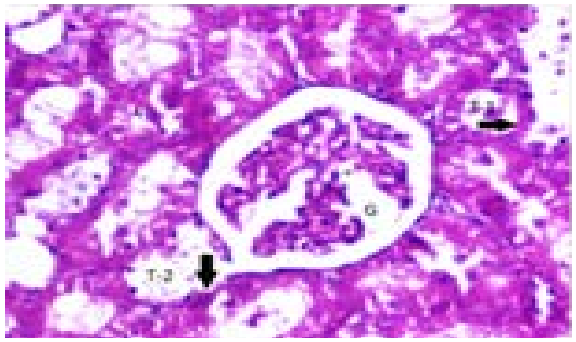
Fig 3: Effect of *Vitex Negundo* on Histopathological Examination in Cisplatin induced nephrotoxic rats.



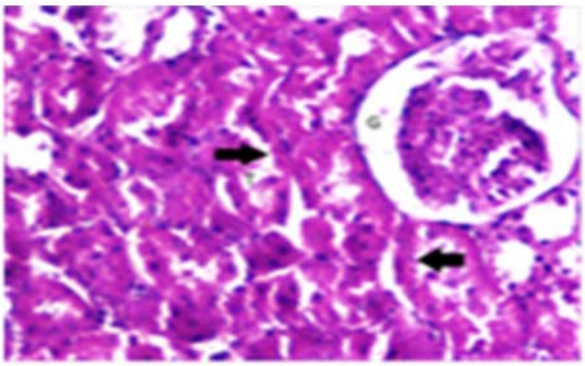
(A). Section of normal rat kidney (Group I) showing normal organization of tubular epithelial cells (**T**) and glomeruli (**G**).



(B). Section of rat kidney treated with Cisplatin (Group II) Showing severe necrosis of tubular epithelial cells (**T**) and glomeruli (**G**).



(C). Section of rat kidney treated with Silymarin (Group III) showing regenerative changes in tubular epithelial cells (**T**) and glomeruli (**G**).



(D).Section of rat kidney treated with *Vitex negundo* (Group IV) showing complete regenerative changes in tubular epithelial cells (**T**) and glomeruli (**G**).

DISCUSSION

In the present study, we attempted to investigate the effect of methanolic extract of *vitex negundo* leaves on cisplatin induced nephrotoxicity in rats. Due to accumulation of cisplatin in proximal and distal nephrons, Reactive oxygen species (ROS) were elevated and Free radicals like Superoxide anion,Hydrogen peroxide and Hydroxyl radical were also increased and decreases the antioxidant enzyme production in cisplatin induced rats.[22]

Superoxide is the primary ROS produced in the course of oxygen metabolism which is highly reactive, cytotoxic ROS. Superoxide is converted to a far less reactive product, hydrogen peroxide by a family of metalloenzymes known as SOD which constitute a front line of defense against ROS-mediated injury. [23] Oxidative stress is the major cause for the development of chronic renal failure. The cisplatin-induced animals show a decrease in tissue SOD level, which may be due to the depletion of copper and zinc in the kidney which are essential for the activity of enzymes. In the present study, the treatment with *Vitex negundo* and silymarin significantly increased SOD level in kidney tissue compared to cisplatin-induced group.

Catalase (CAT) is a common enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen. [24] CAT is highly effective in inhibiting various ROS-mediated injuries and could protect the kidney from cisplatin-induced nephrotoxicity. [25] The cisplatin-induced animals show a decrease in tissue CAT level compared to cisplatin-induced group. But the treatment with *vitex negundo* and silymarin significantly increased the kidney tissue CAT level that shows its anti-oxidant activity during nephrotoxicity.

Glutathione peroxidase (GPx) is the most important antioxidant enzyme in humans which is highly expressed in the kidney, involved in scavenging and inactivating hydrogen and lipid peroxides, providing protection to the body against oxidative stress and also removes peroxides and peroxynitrite that can cause renal damage.[26]The treatment with *Vitex negundo* and silymarin significantly increased GPx level in kidney tissue which reveals their anti-oxidant efficacy against oxidative stress induced by cisplatin.

Lipid peroxidation (LPO) is generated naturally in small amounts in the body mainly by the effect of several ROS i.e., hydroxyl radical and hydrogen peroxide. An increase in the concentration of end products of LPO is the evidence for the involvement of free radicals in human disease. [27] Oxidative stress can damage proteins and DNA that are more significant targets of injury than lipids and LPO which often occur late in the injury process.[28] It is reported that cisplatin-mediated renal tissue injury increased kidney tissue LPO level due to release of free radicals, which is directly interrelated with an increase in LPO level during nephrotoxic condition. The treatment with *Vitex negundo* significantly reduced the kidney LPO level and counteracted the formation of free radicals induced by cisplatin-mediated nephrotoxicity that displayed its protective role in the prevention of renal damage. The standard drug silymarin also exhibited similar effect during nephrotoxicity. In this study, cisplatin induced animals showed increased LPO level in kidney comparable to the measurement of previous reports. [29]

From this study, our results also clearly showed that marked dilation of proximal convoluted tubules with sloughing off almost entire epithelium due to desquamation of tubular epithelium was evident. Cellular debris in the tubular lumen and increased tissue in the interstium is also an indication of cisplatin-induced renal necrosis. [30] The treatment with *vitex negundo* protects the renal tubular necrosis and glomerulus from the effect of cisplatin treated group animals.

CONCLUSION

The present study revealed that the methanolic extract of *Vitex negundo* leaves possess potential antioxidant activity in experimental model system. The antioxidant property may be due to the presence of phenols, flavonoids like Quercetin and other phytochemical molecules that are present in the crude extract. Further investigations on the mechanism of action of *Vitex negundo* are required and may have a considerable impact on future clinical treatments of patients with renal failure.

ACKNOWLEDGEMENT

The authors are very grateful to Dr.G.Archunan, Professor and Head, Department of Animal Science, Bharathidasan University, Tiruchi, Tamilnadu, India for providing necessary facilities and support to carry out the research work. The authors also thankful to Dr.T M Subba Rao MD, Special Diagnostics Centre, Coimbatore, Tamilnadu, India for his support to carry out histopathological study.

REFERENCES

1. Johnson L.R.Essential medical physiology, third edition Elsevier academic press; 2003. Page 351.
2. Hoste E. A et al. Acute kidney injury: epidemiology and diagnostic criteria. Curr Opin Crit Care 2006; 12:531-7.
3. Kohli H. S *et al*. Treatment-related acute renal failure in the elderly: a hospital-based prospective study. Nephrol Dial Transplant 2000; 15:212-7.
4. Valerie A et al. Acute kidney injury associated with reuse of traditional medicines. Nature Reviews Nephrology 2008; 4:664-671.
5. Thadhani R et al**.** Acute renal failure. N Engl J Med 1996; 334:1448- 60.
6. Schrier RW *et al*. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. J Clin Invest 2004; 114:5–14.
7. Merouani A, Shpall EJ, Jones RB, et al**.** Renal function in high dose chemotherapy and autologous hematopoietic cell support treatment for breast cancer. Kidney Int 1996; 50: 1026–31.

8. Samarghandian S, Afshari JT, Davoodi S. Honey induces apoptosis in renal cell carcinoma. Phcog Mag 2011; **7:**46–52.
9. Shirwaikar A, Verma R, Lobo R. Phytotherapy - Safety aspects. Natural Product Radiance 2009; 8**:**55–63.
10. Shanmugam KR et al. Protective effect of ginger against alcohol-induced renal damage and antioxidant enzymes in male albino rats. Indian J Exp Biol 2010; 48:143–9.
11. Chidrawar VR, Ushir YV, Sudarshan S, Patel KN, Patel NJ, Vadalia KR. Possible Role of Natural Nephroprotective; *Hemidesmus indicus* in Congestive Heart Failure. Phcog Res 2009; 1:367–74.
12. Juri Bora Borgohain & Lakhinandan Borgohain, Bonsaak Aru Iyar Byobohar, Banalata, Dibrugarh, 1st ed; 2010.
13. Mukundam Borah, Shagufa Ahmed, Swarnamoni Das. A comparative study of the antibacterial activity of the ethanolic extracts of *Vitex negundo* L., Fragaria vesca L., Terminalia arjuna and Citrus maxima. Asian J Pharm Biol Res 2012; 2(3): 183-87.
14. Vishal R Tandon. Medicinal uses and biological activities of *Vitex negundo*. Natural Product Radiance 2005; 4 (3): 162-65.
15. Tiwari OP et al. Antioxidant properties of different fractions of *Vitex negundo*. Food Chem 2007; 100: 1170-76.
16. Al-Hashem, F. Camel's milk protects against aluminium chloride-induced toxicity in the liver and kidney of white albino rats. Am. J. Biochem. Biotechnol 2009: 5: 98-109.
17. Heath RL, Backer L. Photo peroxidation in isolated chloroplast stoichiometry of fatty acids peroxidation. Arch Biochem Biophy 1998; 125: 189-98.
18. Kakkar P et al.A modified spectrophotometric assay of superoxide dismutase, Ind.J.Biochem.Biophys 1984; 21:130-2.
19. Sinha.colorimetric assay of catalase. Anal.Biochem 1972; 47: 389-394.
20. Rotruck J.T et al.selenium: Biochemical role as a component of glutathione peroxidase.Science 1973; 179: 588-590.
21. Bartelink H et al.The combined use of radiotherapy and chemotherapy in the treatment of solid tumours.EurJ Cancer 2002; 38: 216-222.
22. Durak I, Ozbek H *et al*. Cisplatin induces acute renal failure by impairing antioxidant system in guinea pigs: effects of antioxidant supplementation on the cisplatin nephrotoxicity. Drug Chem Toxicol 2002; 25:1–8.
23. Vaziri ND, Dicus M, Ho ND, Boroujerdi-Rad L, Sindhu RK: Oxidative stress and deregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. Kidney Int 2003; 63:179–185.
24. Chirino YI, Hernández-Pando R, Pedraza-Chaverri J: Peroxynitrite decomposition catalyst ameliorates renal damage and protein nitration in cisplatin-induced nephrotoxicity in rats. BMC Pharmacol 2004; 4:20.
25. Ma SF, Nishikawa M, Hyoudou K, Takahashi R, Ikemura M, Kobayashi Y, Yamashita F, Hashida M: Combining cisplatin with cationized catalase decreases nephrotoxicity while improving antitumor activity. Kidney Int 2007; 72: 1474–1482.
26. De Haan JB, Stefanovic N, Nikolic-Paterson D, Scurr LL et al:Kidney expression of glutathione peroxidase-1 is not protective against streptozotocin induced diabetic nephropathy.Am J Physiol Renal Physiol 2005;289:544-551.
27. Halliwell B et al. Lipid peroxidation: its mechanism,measurement and significance. Am J Clin Nutr 1993; 57: 715S–724S.
28. Sener MT, Sener E, Tok A, Polat B, Cinar I, Polat H, ed al, Biochemical and histologic study of lethal cisplatin nephrotoxicity prevention by mirtazapine. Pharmacol Rep 2012; 64: 594–602.
29. Tanaka H *et al*.Histopathological study of human cisplatin nephropathy,Toxicol Pathol 1986;14:247-57.
30. Kawai Y, Nakao T *et al*. Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. J Pharmacol Sci 2006; 100:65–72.

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