World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Nephroprotective effect of *Vitex negundo linn*. on cisplatin induced nephrotoxicity in male albino rats

M. Janakiraman*[1], K. Jeyaprakash[2]

*[1]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 - 613 005, Tamilnadu, India

Received: 07-02-2015 / Revised: 13-03-2015 / Accepted: 25-03-2015

ABSTRACT

The present study was carried out to investigate the nephroprotective effect of *Vitex negundo* extract on nephrotoxicity induced by cisplatin in male albino rats. Rats were randomly divided into four groups. Group I was treated as Normal control. Group II was treated with single intraperitonial dose of cisplatin (16mg/kg body weight) on 1st day. Group III was treated with single intraperitonial dose of cisplatin on 1st day which is followed by the oral dose of silymarin (50mg/kg body weight/day) from 2nd day to 15th day. Group IV was treated with single intraperitonial dose of cisplatin on 1st day which is followed by the oral dose of *Vitex negundo* (200mg/kg body weight/day) from 2nd day to 15th day. After 15 days treatment, all rats were sacrificed and collected the blood samples were used for the analysis of biochemical and hematological parameters in all groups and body weights also measured before sacrification of rats. Biochemical results of Vitex negundo treated group showed a decreased in levels of blood urea, uric acid, blood urea nitrogen and creatinine and increased in body weight, albumin and total protein when compared to cisplatin treated groups. Hematological results of Vitex negundo treated group also showed an increased in levels of Hb, RBC, WBC, PCV, MCV and MCHC when compared to cisplatin treated rats. The results of this study were concluded that *Vitex negundo* protected the rats from the deleterious effects of cisplatin.

Key words: *Vitex negundo,* Cisplatin, Silymarin, Nephrotoxicity and Hematology

INTRODUCTION

Nephrotoxicity is the third most common problem of the renal system with an estimated lifetime risk of 2-5% in Asia, 8-15% in Europe and America and around 20% in the Middle East. Some phytochemical studies have reported that certain Indian medicinal plants showed beneficial effects on kidney injury [1]. Nephrotoxic injury is damage to one or both of the kidneys that results from exposure to a toxic material, usually through ingestion. Nephrotoxic injury can lead to acute renal failure, in which the kidneys suddenly lose their ability to function or chronic renal failure, in which kidney function slowly deteriorates. If unchecked, renal failure can result in death. Chronic exposure to drugs, occupational hazards, or environmental toxins can lead to chronic interstitial renal diseases [2]. Cisplatin (cis-diamino dichloroplatinum II: CDDP), is one of the highly effective, frequently used antineoplastic drug or DNA alkylating agent or potent anticancer agent used to treat solid tumors such as testicular, ovarian, cervical, bladder and lung cancers as well as solid tumors resistant to other treatment regimens. Despite its clinical usefulness, cisplatin treatment has been associated with several toxic effects particularly nephrotoxicity. Several mechanisms have been suggested for cisplatin induced renal toxicity i.e. Apoptosis, inflammatory mechanism and generation of reactive oxygen species [3]. Nephrotoxicity is a main clinical problem and occurs in 25-35% of patients receiving a single dose of it [4].

Vitex negundo L. (Tamil- Vella nochi, Hindi- Nirgundi) belongs to the family Lamiaceae. It is an aromatic large shrub. It is commonly bears tri-or penda-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in

***Corresponding Author Address:** M. Janakiraman, Assistant professor and Head, Department of Biochemistry, J.J.College of Arts and Science (Autonomous), Pudukkottai-622 422, Tamilnadu,India; Email: mugavaiprofmj@gmail.com*

branched tomentose cymes [5]. 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 [6]. Traditionally it is used as vermifuge, in headache, catarrh, acute rheumatism, expectorant, fever, sinusitis, to increase memory, as hypolipidaemic, in bodyache[7]. It has antioxidant, anti-inflammatory and immunomodulatory and anticonvulsant activities [8]. As the leaves of *Vitex negundo* L. possess antioxidant and anti-inflammatory properties, this study has been undertaken to evaluate the effect in experimentally induced cisplatin and also find its probable mechanism of action including biochemical and hematopoietic potential in cisplatin induced rats.

MATERIALS AND METHODS

Plant material: The leaves of *Vitex negundo* were collected from Manapparai, near Trichy district, Tamilnadu, India. The botanical identity of the plant material was authenticated by 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 Pvt Ltd, Hyderabad, India) was used to induce nephrotoxicity and Silymarin (Ranbaxy Laboratories Ltd, Punjab, India) 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 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 a 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/day) from 2nd day to 15th day for 14 days after single i.p. dose of cisplatin on day1. Group IV: Rats were treated with oral dose of vitex *negundo* (200 mg/ kg of body weight/day) from 2nd day to 15th day for 14 days after single i.p. dose of cisplatin on day1. After the experimental period, collected blood samples were used for the studies of biochemical and hematological studies in all groups.

Parameters assessed for renal function

Body weight: On days 1 and 16 of the experiment, the rat weights were measured respectively with Mettler weighing balance. The absolute and change in weights in reference to the initial weight per group were calculated [9].

Biochemical Analysis: Prior to termination of the experiment on the 15th day, the rats were fasted overnight. On the 16th day the fasted rats were sacrificed under chloroform anesthesia and blood samples were directly collected from the heart chambers by cardiac puncture. The collected blood samples were allowed to clot and centrifuged to separate serum and this serum was used for analysis of kidney markers like urea, blood urea nitrogen by diacetylmonooxime method [10], uric acid by uricase method [11], creatinine by alkaline picrate method [12], Albumin by bromocresol green method [13] and total protein by lowrys method [14].

Hematological Analysis: Blood samples were also used for the analysis of Hemoglobin (Hb) by Acid haematin method [15], Red blood cell (RBC) count, white blood cell count (WBC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) by haemocytometry method and packed cell volume (PCV) by Win Trobe's tube method [16].

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 on Body weight: Table 1 and Fig.1 showed the effect of *Vitex negundo* on average body weight of different groups over the duration of the study. The average body weight of the animals (group II) which received cisplatin significantly decreased when compared to the normal control animals (group I). When *Vitex negundo* leaves extract (Group IV) was given, the body weight significantly increased when compared to cisplatin treated animals (group II). Similarly, silymarin treated group (III) also have nearly same effect of *Vitex negundo* against cisplatin induced nephrotoxicity.

Effect on Biochemical parameters: Table 2 and Fig 2 showed the effect of *Vitex negundo* leaves extract on kidney markers in serum in all groups of animals over the duration of the study. Blood urea, creatinine, Uric acid and blood urea nitrogen level significantly increased in cisplatin treated group (II) when compared with normal control group (I). However, oral administration of *Vitex negundo* group (IV) significantly ($p<0.05$) decreased Blood urea, creatinine, uric acid and blood urea nitrogen when compared with cisplatin treated group (II) whereas Albumin and total protein level decreased in cisplatin treated group (II) when compared with normal group (I). It is also recovered by oral administration of *Vitex negundo* group (IV) significantly ($p<0.05$) increased albumin and total protein level when compared with cisplatin treated group (II). Similarly, silymarin treated group (III) also have nearly same effect of *Vitex negundo* against cisplatin induced nephrotoxicity.

Effect on Hematological Parameters: Table 3 and Fig.3 showed the effect of *Vitex negundo* leaves extract on hematological parameters in blood of all groups of animals over the duration of the study. Hb, RBC,WBC, PCV, MCV and MCHC level decreased in cisplatin treated group (II) when compared with normal control group (I).However, oral administration of *Vitex negundo* group (IV) significantly ($p<0.05$) increased the levels of Hb, RBC,WBC, PCV, MCV and MCHC in the serum when compared with cisplatin treated group (II). Similarly, silymarin treated group (III) also have nearly same effect of *Vitex negundo* against cisplatin induced nephrotoxicity.

DISCUSSION

In the present study, our results observed that significant reduction in Body weight of the rats treated with cisplatin. This result on body weight indicated about the effect of cisplatin may be attributed to the injured renal tubules and subsequently loss of ability of tubular cells to reabsorb water, leading to dehydration and loss of body weight or due to catabolic effects of cisplatin [17]. Moreover, the reduction in body weight of the animals in present study correlates with the decreased food intake during experimental period. Due to the anti-inflammatory activity of *vitex negundo*, this weight loss was attenuated against cisplatin induced rat was also observed in our study[18].

Biochemical results obtained in the present study was indicated the marked elevation in the level of blood urea nitrogen (BUN), creatinine, uric acid, urea and significant reduction in the level of albumin and total protein was also observed in our study.In kidneys urea is filtered out of blood by glomeruli and is partially being reabsorbed with water. Due to cisplatin effect on kidney of experimental rats, the urea clearance rate of glomeruli was reduced which leads to elevated levels of urea in serum [19]. BUN reflects the amount of nitrogen present in the body called urea and it is used to determine extra nitrogenous wastes, filtered by kidneys. Elevated level of urea in serum of rats treated with cisplatin also leads to increase the level of blood urea nitrogen [20]. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass. The creatinine clearance test is used to monitor the progression of renal disease. Our study also observed that rate of creatinine clearance by the glomeruli and tubules was reduced in the rats treated with cisplatin which leads to elevate the level of creatinine in the cisplatin treated rats [21]. Uric acid is the relatively water-insoluble end product of purine nucleotide metabolism. Normally uric acid excreted by the kidney, but in renal disease caused by cisplatin treatment causes the reduced excretion of uric acid which leads to elevated level of uric acid in serum [22]. Albumin is the major plasma protein which acts non-specifically as a transport protein for numerous substances including free fatty acids, certain ions (e.g. $Ca^{2+,}$ Zn^{2+}), bilirubin and many drugs. Due to renal tubular damages was caused by cisplatin treatment causes the excretion of albumin in urine which leads to reduced level of albumin in serum [23]. The total protein composed the total amount of two classes of proteins found in the fluid portion of blood. These are albumin and globulin. Total Proteins are important parts of all cells and tissues. Albumin helps prevent fluid from leaking out of blood vessels. Globulins are an important part of our immune system. Due to loss of albumin in urine which also leads to reduced level of total protein in serum [24]. All these biochemical effects caused by cisplatin was significantly

reduced and recovered by the oral administration of vitex negundo and silymarin was also observed in this study [25].

Hematopoietic system is one of the most sensitive systems to evaluate the hazards effects of poisons and drugs in humans and animals [26].The myelosupression and anemia are the most common problem encountered in cancer chemotherapy [27]. From this stydy, our results also observed that decreased RBC, WBC, Hb, PCV, MCV and MCHC in cisplatin treated rats due to myelosupressive effects of cisplatin [28]. Erythropoietin secreted from kidney stimulates proliferation and differentiation of erythroid precursors in haemopoietic tissues [29]. Thus, lack of erythropoietin due to cisplatin induced kidney damage would have been the attributing factor for decreased RBC. The free radical such as reactive oxygen species (ROS) generation by cisplatin which through binding to RBC membrane might cause oxidative damage resulting in decreased Hb levels [30]. Furthermore, decreased RBC and Hb by cisplatin may be the attributing factor for decreased level of PCV, MCV and MCHC in the present study. In addition to this, acute leukopenia is one of the side-effects of cisplatin which leads to reduced level of WBC in the cisplatin treated rats [31]. However, oral treatment with *Vitex negundo* significantly increased in the level of Hb, RBC, WBC, PCV, MCV and MCHC value also recorded against cisplatin induced nephrotoxicity respectively. Results of this study showed that vitex negundo extract could contains active biological principle(s) like flavonoids, alkaloids and polyphenolic compounds recovering the biochemical and hematotoxic effect of cisplatin with subsequent enhancement of hematopoiesis. Thus, the nephroprotection of *Vitex negundo* extract could be improving the body weight, biochemical and hematological status in rats exposed to dose of cisplatin.

CONCLUSION

The results of the present study was indicated that *Vitex negundo* extract attenuates renal injury in rats following cisplatin treatment, possibly by inhibiting inflammation and enhancing or maintaining the biochemical and hematopoietic potentials. These findings suggest that the probable efficacy of extract as a novel nephroprotective agent which might be due to the presence of flavonoids and phenolic compounds in *Vitex negundo*. Therefore, renal protective action of vitex negundo may be beneficial for patients undergoing chemotherapy of cisplatin.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

ACKNOWLEDGEMENTS

The authors are very grateful to Prof. 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 express their sincere thanks to Dr.G.V.S.Murthy, Scientist F, Botanical Survey of India, Coimbatore, Tamilnadu, India for authentication of plant for this research work.

Table 1: Effect on Average body weight changes in the different experimental groups

Groups	Treatment	Average body weight(g)	
		Day 1	Day 16
I	Normal control(Normal saline 2ml/kg b.w) for 15 days	179.4±2.25	193.9±2.06
II	Treated with cisplatin (16mg/ kg b.w i.p) single dose	166.4±2.79	117.0±2.27*
III	Treated with silymarin (50mg /kg b.w) in cisplatin induced rats for 15 days	205.9±3.51	210.9±3.46**
IV	Treated with *Vitex negundo*(200mg/kg b.w) in cisplatin induced rats for 15 days	195.1±1.34	209.1±1.34**

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).

Table 2: Effect on Serum Kidney Markers in the different experimental groups

Groups	Albumin (g/dl)	Total Protein (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Blood Urea Nitrogen (mg/dl)	Uric acid (mg/dl)
I	3.62±0.02	7.14±0.65	0.56±0.01	30.31±0.01	14.15±0.02	4.38±0.04
II	1.37±0.01*	4.24±0.02*	1.30±0.04*	83.21±0.10*	38.83±0.04*	7.23±0.08*
III	2.91±0.12**	6.91±0.03**	0.61±0.03**	45.52±0.02**	21.24±0.01**	4.94±0.06**
IV	2.50±0.14**	6.20±0.01**	0.72±0.01**	48.23±0.01**	22.51±0.02**	5.04±0.03**

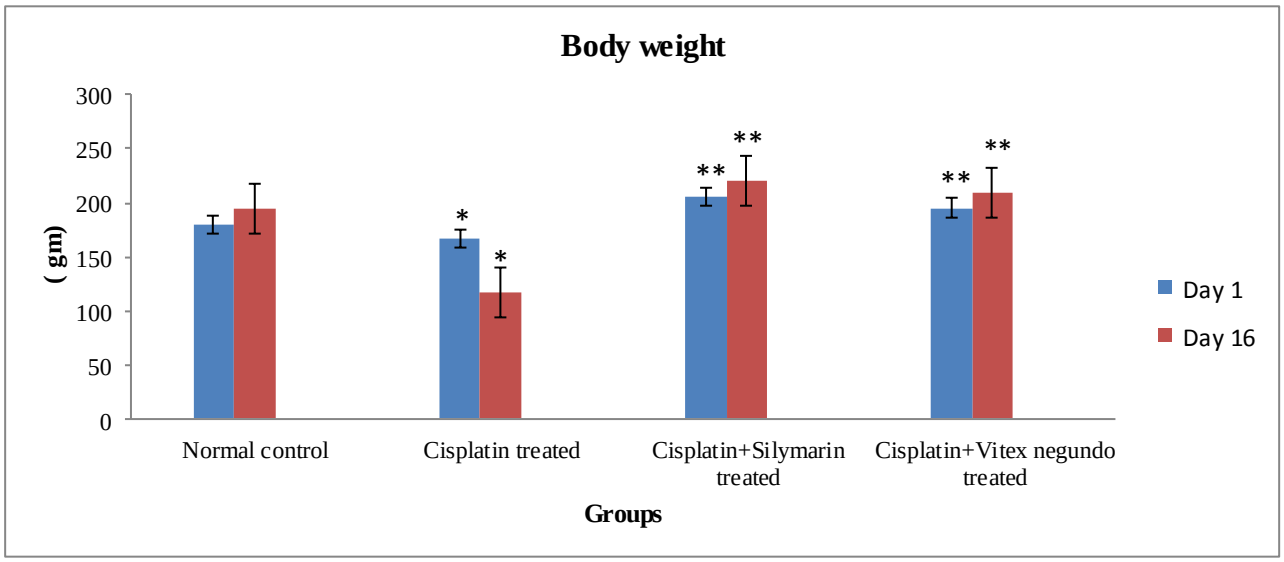
All values were expressed as mean±SD(n=6). Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group(II).

Table 3: Effect on Hematological Parameters in the different experimental groups

Groups	Hb (g/dl)	RBC (million/cu.mm)	WBC (cells/cu.mm)	PCV (%)	MCV (fL/cell)	MCHC (g/dl)
I	13.12±0.07	5.61±0.04	5.65±1.45	42.21±0.06	75.24±0.03	31.08±0.02
II	7.12±0.05*	3.11±0.01*	3.56±1.30*	23.39±0.04*	75.20±0.07*	30.44±0.06*
III	12.88±0.03**	5.51±0.08**	5.24±1.20**	41.45±0.07**	75.23±0.04**	31.07±0.05**
IV	12.17±0.05**	5.21±0.05**	5.02±0.57**	39.20±0.03**	75.22±0.05**	31.05±0.03**

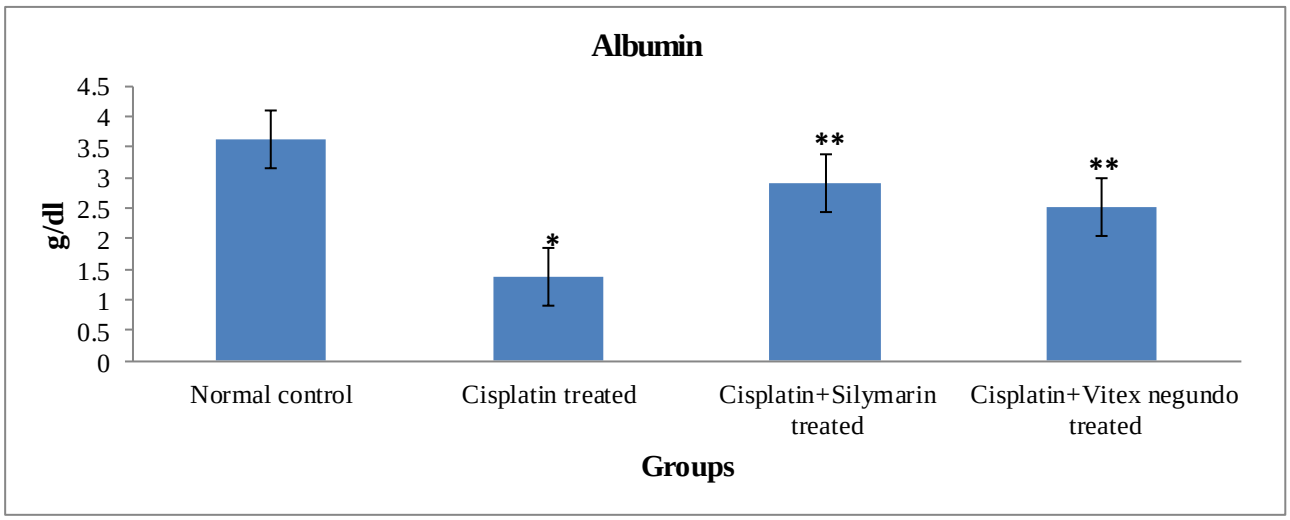
All values were expressed as mean±SD (n=6).statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II). Hb = Hemoglobin, PCV = Packed cell volume, WBC= White blood cells, RBC=Red blood cells, MCV=Mean corpuscular volume, MCHC= Mean corpuscular hemoglobin concentration.

Fig.1: Effect on Average body weight (gm.) changes in the different experimental groups.

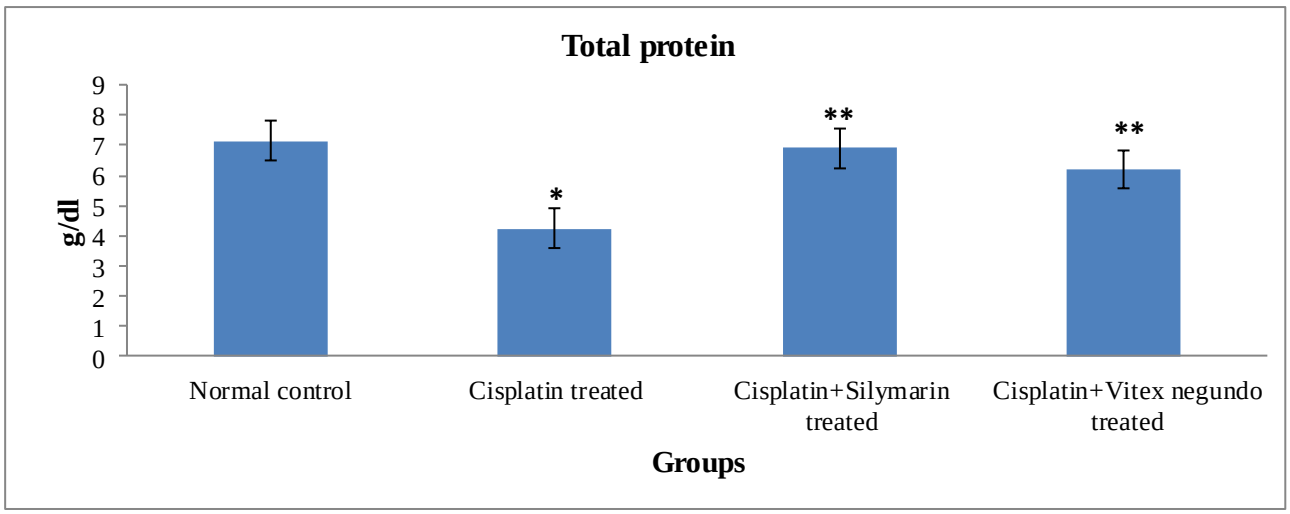


All values were expressed as mean±SD (n=6).Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).

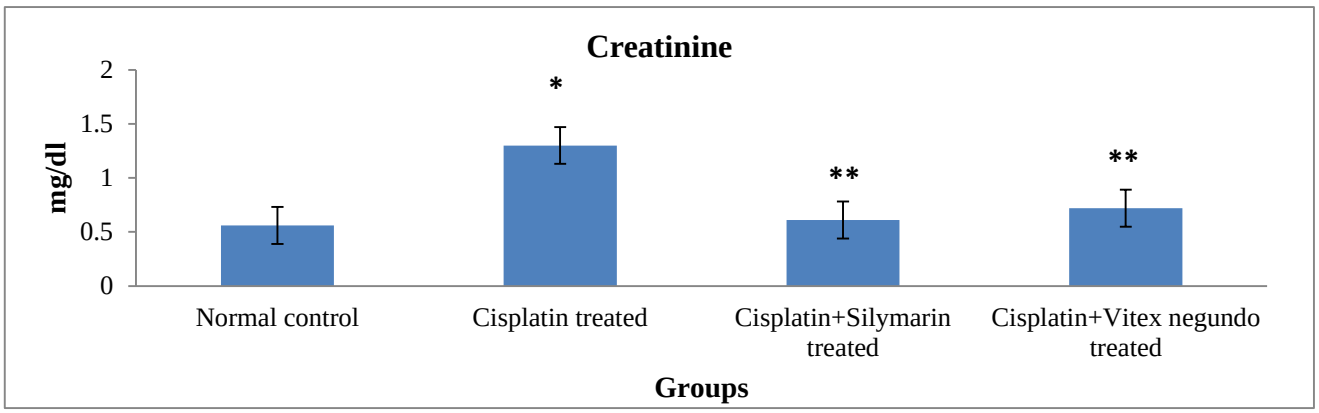
Fig 2: Effect on Serum kidney markers in the different experimental groups



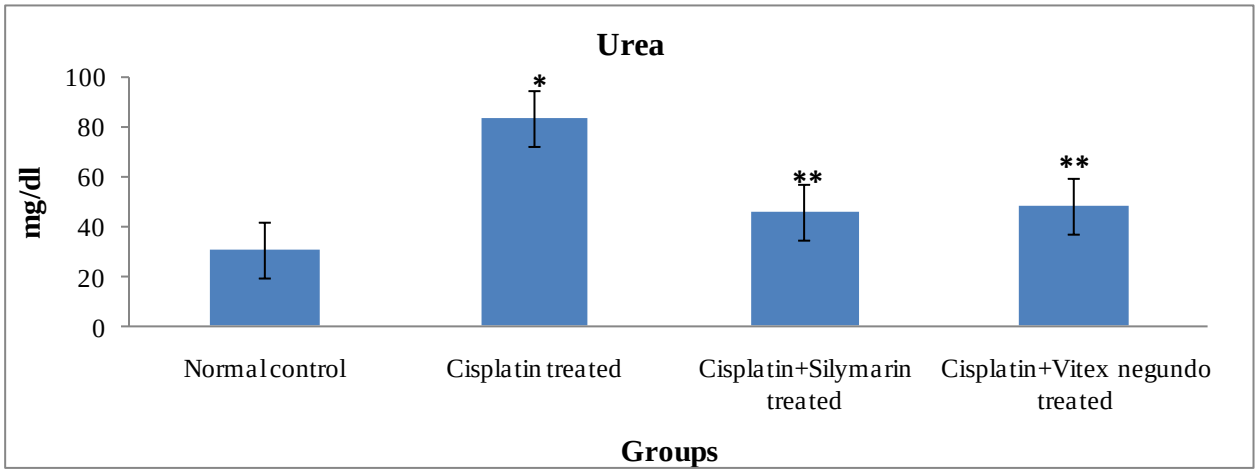
(A).Albumin level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



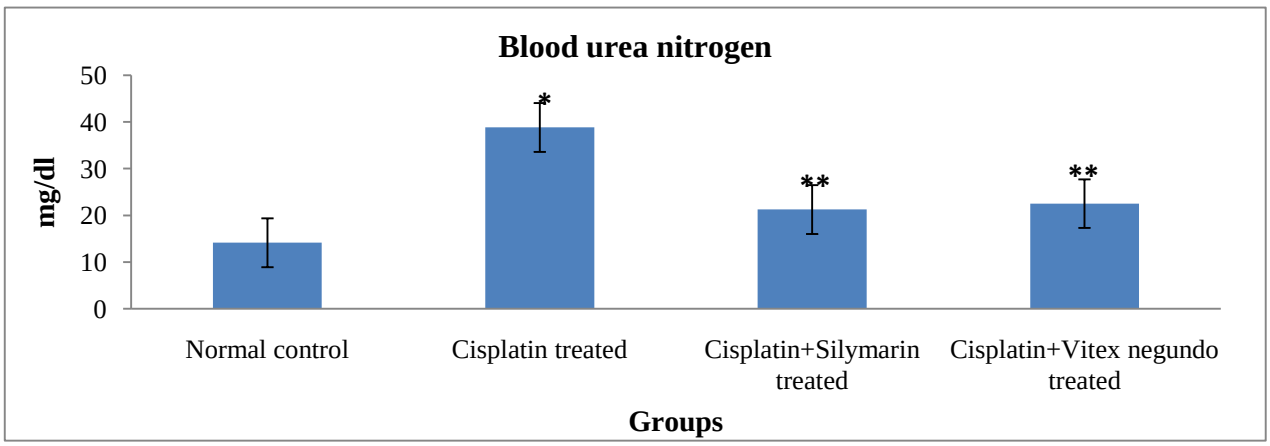
(B).Total protein level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



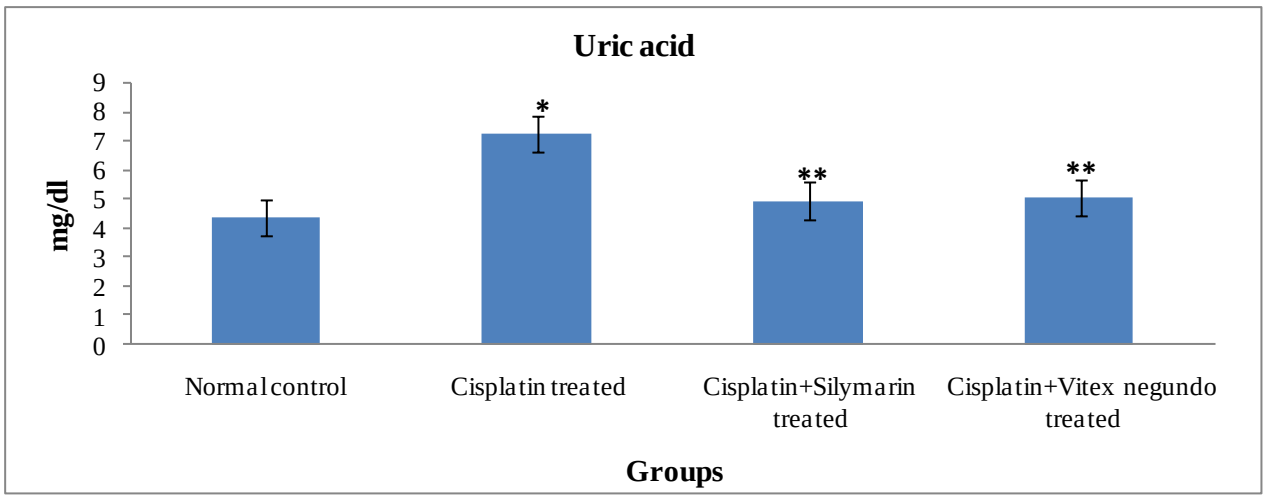
(C).Creatinine level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



(D).Urea level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).

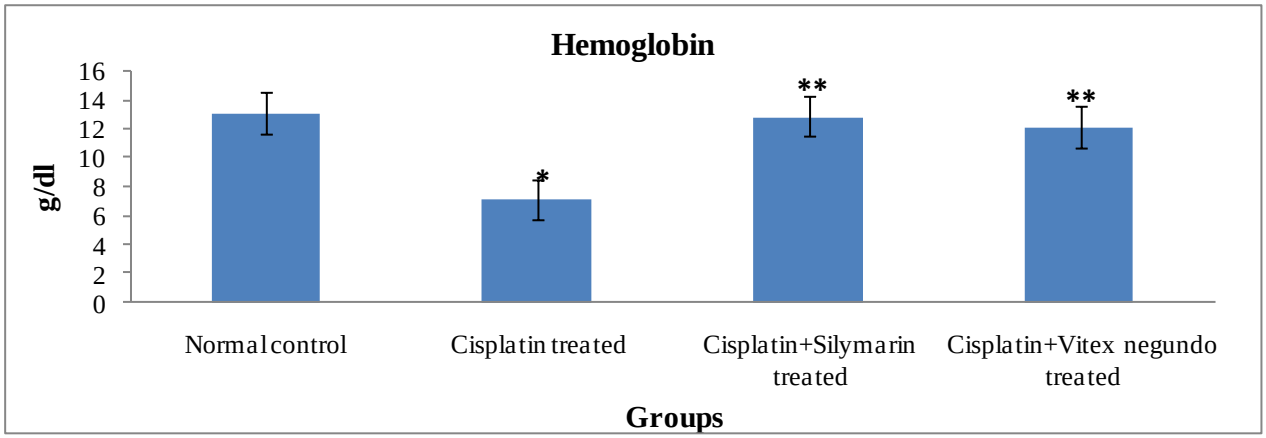


(E).Blood Urea nitrogen level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).

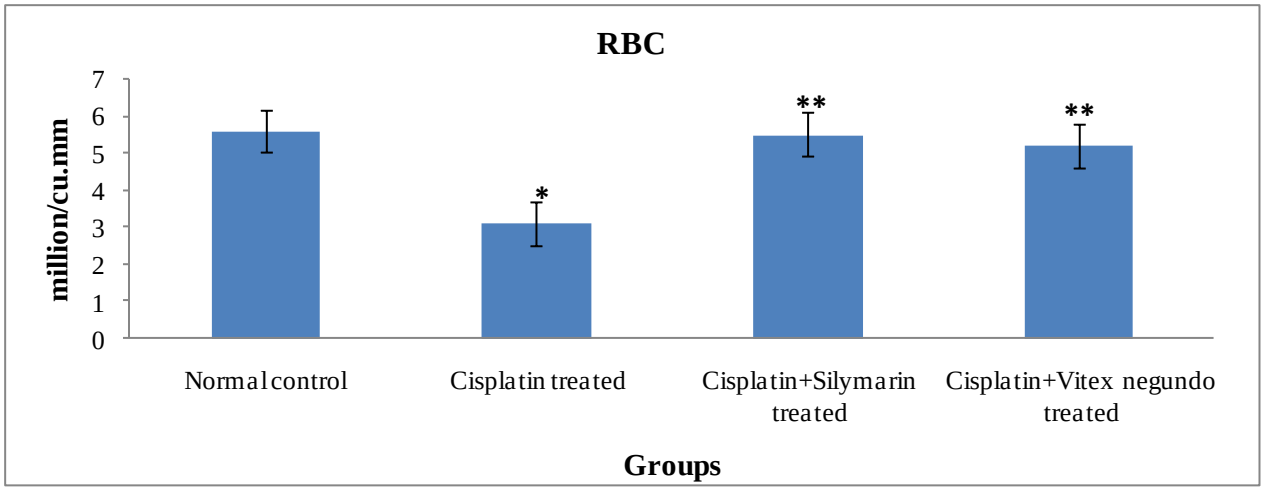


(F): Uric acid level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).

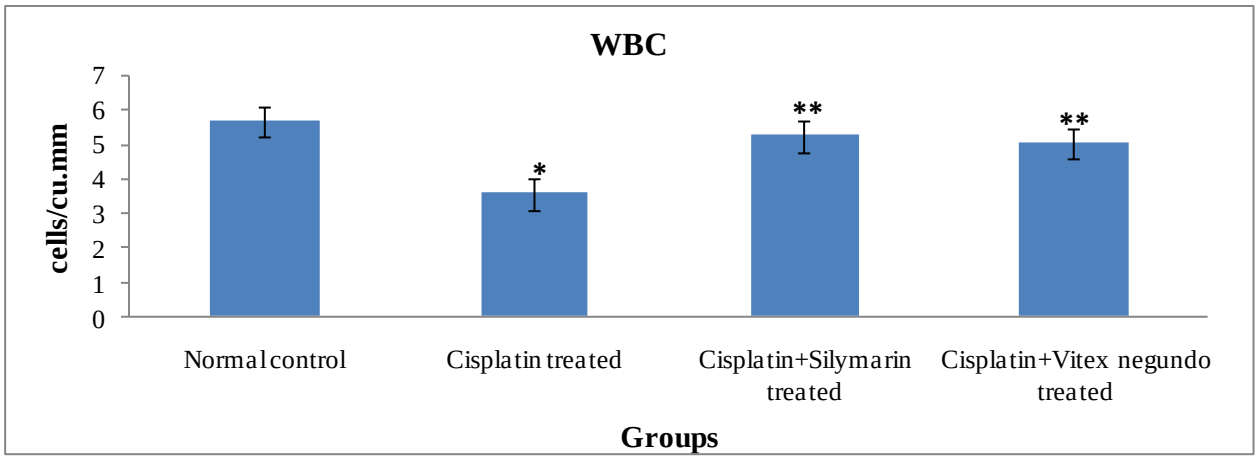
Fig 3: Effect on hematological parameters in the different experimental groups.



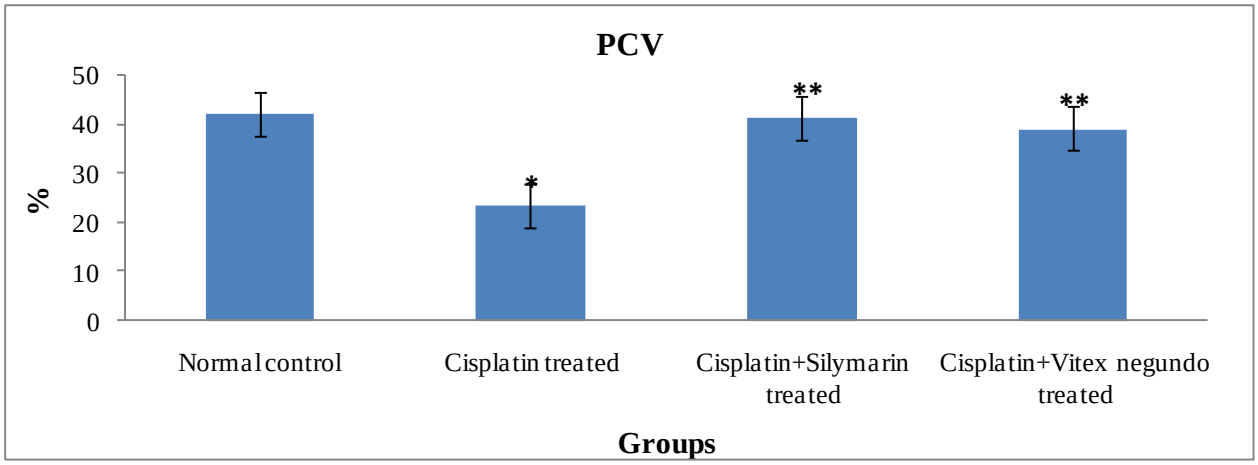
(A).Hemoglobin level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group(II).



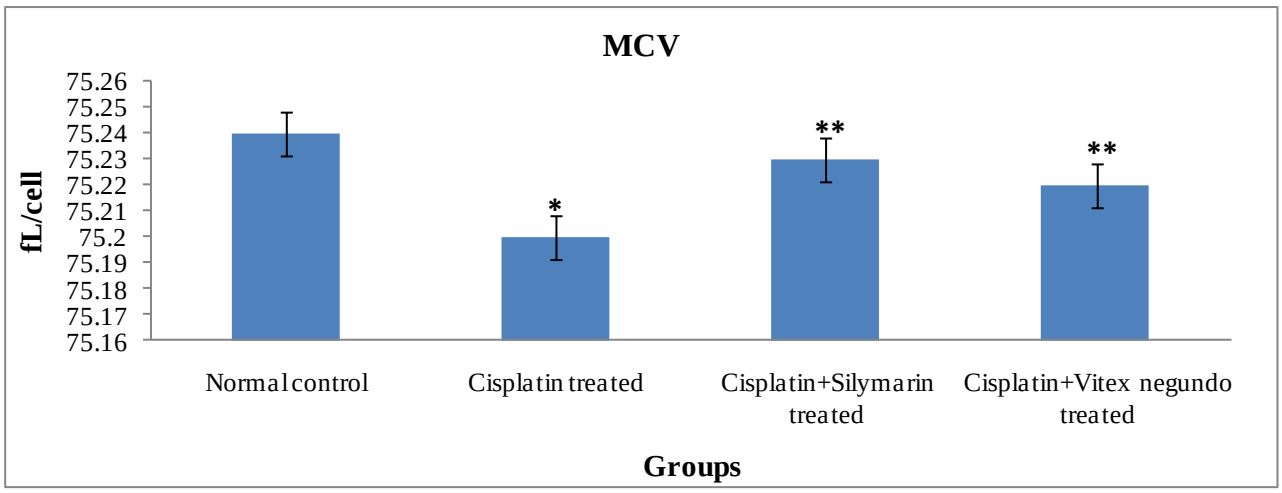
(B).Red Blood Cell level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



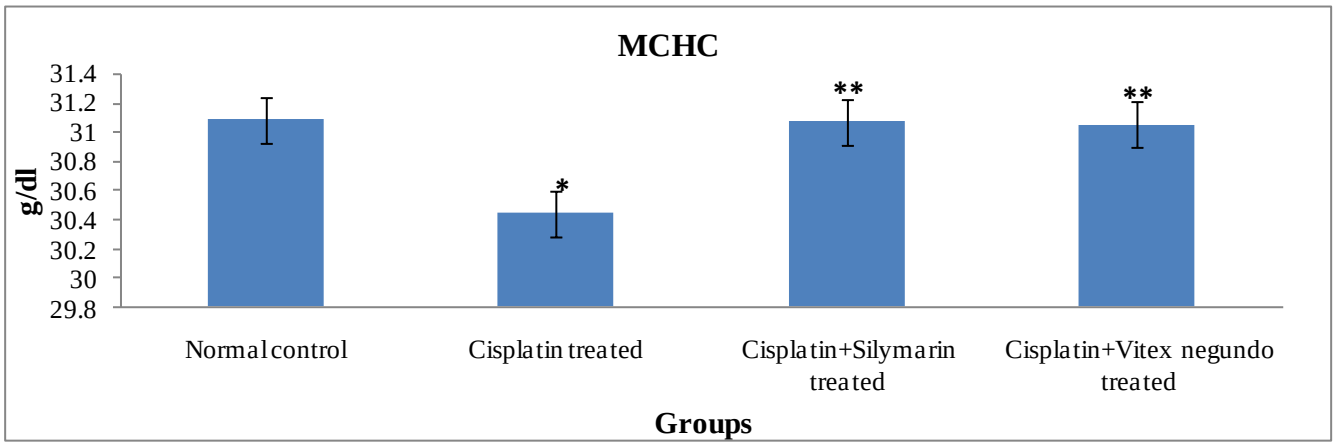
(C): White Blood Cell level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



(D).Packed Cell Volume level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



(E).Mean Corpuscular Volume (MCV) level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).



(F). Mean Corpuscular Hemoglobin Concentration (MCHC) level in cisplatin induced nephrotoxic rats. Statistically significant of *p < 0.05 compared to Normal control group (I) and **p < 0.05 compared to cisplatin treated group (II).

REFERENCES

[1]. Bhattacharjee S.K. In: Hand Book of Medicinal Plants, Pointer Publishers: Jaipur, 1998.
[2]. Uma Shankar S, Arun Kumar. Nephroprotective evaluation of Rubus ellipticus fruits extracts against cisplatin and gentamicin induced renal-toxicity in rats. Journal of Pharmacy Research 2011; 4 (1): 285-287.
[3]. Sreedevi A et al. Effect of Vernonia cinerea aerial parts against Cisplatin induced Nephrotoxicity in Rats. J Pharmacology online 2011; 2: 548-555.
[4]. Lee KW et al. Sildenafil attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity. Toxicology 2009; 257:137-143.
[5]. Juri Bora Borgohain & Lakhinandan Borgohain. Moringa_oleifera. Bonsaak Aru Iyar Byobohar.1st ed,Banalata Publisher: India, 2010. pp.26-27
[6]. Mukundam Borah et al. 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 Phar Biol Res 2012; 2(3): 183-187.
[7]. Vishal R Tandon. Medicinal uses and biological activities of *Vitex negundo*. Nat. Prod. Radiance 2005; 4 (3): 162-65.
[8]. Tiwari OP, Tripathi YB. Antioxidant properties of different fractions of *Vitex negundo*. Food Chem 2007; 100: 1170-76.
[9]. Khandkhar A et al. Activation of lysosomal enzymes responsible for paracetamol induced nephrotoxicity.J Pharm Pharmacol 1996; 48:437-40.
[10].Marsh WH et al.Non protein nitrogen, urea, creatine and creatinine In:Varley H,Practical Clinical Biochemistry, Vol.1. William Heinemann: London, 1980; pp.456-458.
[11].Ito A. The exercise prescription for hyperuricemia. Nippon Rinsho 2000; 58: 431-436.
[12].Brod J, Siorta J H. Non protein nitrogen, urea, creatine and creatinine In: Varley H, Practical Clinical Biochemistry, Vol. 1. William Heinemann: London, 1980: pp.478-480.
[13].Doumasa BT et al. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta 1971; 31(1):87-96.
[14].Lowry O H et al. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193: 265-270
[15].Sahli,T. In text book of chemical pathology, Ed. Seward, Eimiller, Williams and Williams & Co., Baltimore, 1962: pp.35-71
[16].Mukherjee Kanual L. In text book of Medical Laboratory Technology.Vol.1, Tata Mc Graw Hill Publishing Company Limited: New Delhi, 1988.
[17].Mora L.deo et al. The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. Pharmacol Res 2003; 47: 517-522.
[18].Yunos, N.M. et al. 'Cytotoxicity Evaluations on Vitex negundo Anti-inflammatory Extracts'. Malaysian Journal of Science 2005; 24: 213-217.
[19].Satyavati.D et al.Nephroprotective effect of ethanolic extract of flowers of michaelia champaca against cisplatin induced nephropathy in rats.World Journal of Pharmacy and Pharmaceutical Sciences 2013;6:6352-6365.
[20].Mohana lakshmi.S et al.Protective effect of abutilon indicum against cisplatin induced nephrotoxicity in rats. Inov J Life Sci 2013; 2:35-39.
[21].Sreedevi adikay et al.Effect of decoction of root bark of berberis aristata against cisplatin induced nephrotoxicity in rats.Int J Pharmacy Pharm Sci 2010; 3:154-155.
[22].Subai debnath et al.Nephroprotective evaluation of papaya fruit in cisplatin induced nephrotoxicity.J Pharm Sci Tech 2010;2:241-246
[23].Qumre alam et al.Nephroprotective effect of fruits of solanum xanthocarpum against cisplatin induced nephropathy in rats.International Journal of Advances in Pharmacy, Biology and Chemistry 2013; 2:325-329.
[24].Sreedhevi sasikumar et al.Nephroprotective effect of boerhaavia diffusa against cisplatin induced nephropathy in male wistar albino rats.A Journal of Science and Technology 2014; 3:522-527.
[25].Chawla AS et al.Chemical investigation and anti-inflammatory activity of vitex negundo.J Natural Products 1992; 55: 163-167.
[26].B.Lijuv et al. Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric Curcuma longa L. Food Chem.Toxicol 2013; 53:52– 61.
[27].Price VE, Greenfield RE.Anemia in cancer. Adv Cancer Res 1958; 5:199-200.
[28].Preethi KC, Kuttan R. Hepato and Reno protective action of *Calendula officinales* L. flower extract. Indian J Exp Biol 2009; 47:163-168.
[29].Swayer ST.The erythropoietin receptor and signal transduction. Ann. N.Y. Acad. Sci 1994; 7189:185-190.
[30].Satoh M et al. A novel free radical scavenger, Edarabone Protects against cisplatin induced acute renal damage *in vitro* and *in vivo*. J.Pharm.Exp.Ther 2003; 305:1183-1190.
[31].Hartmann LC et al. Granulocyte colony stimulating factor in severe chemotherapy-induced a febrile neutropenia.N Engl J Med 1997; 336: 1776–80.