Advanced Pharmaceutical Bulletin, **2013**, 3(2), 309-313
doi: http://dx.doi.org/10.5681/apb.2013.050
http://apb.tbzmed.ac.ir/

Cadmium-Induced Toxicity and the Hepatoprotective Potentials of Aqueous Extract of *Jessiaea Nervosa* Leaf

Ama Udu Ibiam[1], Emmanuel Ike Ugwuja[1,2]*, Christ Ejeogo[3], Okechukwu Ugwu[4]

[1] *Department of Biochemistry, Faculty of Biological Sciences, Ebonyi State University, PMB 053, Abakaliki, Nigeria.*

[2] *Department of Chemical Pathology, Faculty of Clinical Medicine, Ebonyi State University, PMB 053 Abakaliki, Nigeria.*

[3] *Department of Biochemistry, Institute of Management and Technology (IMT), Enugu, Nigeria.*

[4] *Department of Biotechnology, Faculty of Biological Sciences, Ebonyi State University, PMB 053, Abakaliki, Nigeria.*

ARTICLEINFO

Article Type:
Research Article

Article History:
Received: 9 February 2013
Revised: 11 March 2013
Accepted: 12 March 2013
ePublished: 20 August 2013

Keywords:
Jussiaea nervosa
Cadmium toxicity
Biochemical parameters
Hepatoprotection

ABSTRACT

Purpose*:** Hepatoprotective potentials of *Jussiaea nervosa* leaf extract against Cadmium-induced hepatotoxicity were investigated. ***Methods*:** Forty albino rats were randomly assigned into groups A-G with 4 rats in each of the groups A-F. Group A served as control and were given feed only while rats in groups B-F were orally exposed to varying concentrations of cadmium for six weeks. Effects of cadmium were most significant at 12 mg/Kg body weight (BW), and this dose was used for subsequent test involving oral administration of *Jussiaea nervosa* leaf extracts. In this segment, group G (n= 16) was sub-divided into four: G_1-G_4, with each sub-group containing four rats. Rats in sub-group G_1 were given cadmium and feed only and served as positive control. Rats in sub-groups G_2, G_3, and G_4 were given cadmium and 20, 50 and 100g/kg BW of *Jussiaea nervosa* extract, respectively, for six weeks. Blood and liver were analysed using standard laboratory techniques and methods. ***Results: Liver function parameters (ALT, AST, ALP, bilirubin) were significantly ($p<0.05$) elevated in exposed rats in comparison to the controls, except for total protein and albumin, which were significantly decreased. Histopathological assessment reveals renal pathology in exposed rats in sharp contrast with the controls. *Jussiaea nervosa* extract however lowered the values of liver function parameters with 100mg/Kg BW dose producing the highest ameliorative effects. Similarly, the serum albumin and total protein significantly ($p<0.05$) improved with normal liver architecture. ***Conclusion:*** The results show the hepatoprotective potentials of *Jussiaea nervosa* extract against Cd toxicity.

Introduction

Cadmium (Cd) is a naturally occurring metal that is widely distributed throughout the biosphere. It is found in soils, sediments, air and water,[1,2] foods, especially of vegetable origin.[3] In fact about 98% of the ingested Cd comes from terrestrial foods while 1% comes from aquatic foods and another 1% from drinking water.[4] Other natural sources of Cd in the atmosphere include volcanic activity, forest fires and wind-borne transport of soil particles.[5] Industrialization has made Cd almost ubiquitous in the environment,[6] thereby increasing its propensity of exposure to humans. Occupational exposures to cadmium are encountered in the manufacturing of batteries, pigments, plastic stabilizers, production and processing of Cd alloys, recycling of domestic wastes and non-ferrous smelters.[7]

Cigarette smoke is one of the most common sources of Cd exposure in the environment.[8] A study conducted by Ibiam and Uwakwe[9] revealed high content of Cd in different brands of cigarettes smoked in Nigeria, especially, the locally manufactured ones; no wonder smoking in public places has been banned in many developed countries of the World.

Cadmium toxicity in humans and other primates is no longer in doubt. For instance, exposure to Cd produces deleterious effects on the cellular architecture and metabolism in a variety of body tissues,[10] including testis,[11] liver and pancreas,[12] kidneys,[13,14] and bone.[14]

In recent times, the use of traditional medicine is increasingly gaining popularity in both developed and developing Worlds.[15] The therapeutic values of many plants have been identified and exploited for the management of many disease conditions. *Jussiaea nervosa* is one of the herbal plants used for treating various diseases. The plant has been classified by the Association of Scientific Identification, Conservation and Utilization of Medicinal Plants in Nigeria.[16] It belongs to the family of *Onagracea*. Its common local names include *arira mmili* (Igbo), *sha shatau* (Hausa),

***Corresponding author:** Emmanuel Ike Ugwuja, Department of Biochemistry, Faculty of Biological Sciences, Ebonyi State University, PMB 053, Abakaliki, Nigeria. Tel: +2347035122010, Email: ugwuja@yahoo.com

and *ewuru odo* (Yoruba). The chemotherapeutic uses of *J. nervosa* include antidote to poison, alcoholic intoxication and in the treatment of diarrhoea, dysentery as well as vomiting.[16] To the best of our knowledge, there is no report on the phytochemical constituents of *Jussiaea nervosa* and its protective effects against metal intoxication in animals. Considering the ease with which humans are exposed to Cd, both at home (foods, drinking water, cigarette), work places and the abundance of *Jussiaea nervosa* in our environment, it is pertinent to scientifically evaluate whether the consumption of the extract can protect against Cd toxicity. Therefore, this study evaluated the hepatoprotective potentials of the leaf extract of *Jussiaea nervosa* on Cd-intoxicated rats.

Materials and Methods

Collection and Preparation of Plant Material

The leaves of *Jussiaea nervosa* was collected from a farm in a swampy area of phase 6, Trans-Ekulu, Enugu East Local Government Area of Enugu State Nigeria. The plant was identified and authenticated by Prof. J.C. Okafor, Consultant Agro forester and Taxonomist, University of Nigeria, Nsukka.

The leaves were washed and sun dried for 3 days. The dried leaves were homogenized using a manual grinder. The resultant powder was soaked in distilled water and boiled on slow heat for about two hours. The preparation was then suction-filtered. Soaking and filtration was repeated until all the soluble compounds had been extracted. This was adjudged by loss of colour of the filtrate. The extract was concentrated to dryness at 60 °C using electric oven. It was carefully evaporated to dryness on water bath at 40 °C.[17] The extract was kept in a refrigerator until used.

Animals

Male Wister albino rats (n=40), weighing 145-165 g purchased from animal house of the Department of Pharmacy, University of Nigeria, Nsukka were kept in standard cages at 25 °C and 12h light/dark condition in the animal room of the Department of Biochemistry, Ebonyi State University, Abakaliki. The animals were fed on commercial rats' feeds and were given water *ad libitum* for a period of two week to allow them acclimatize. All the rats received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

Experimental Design

The rats were randomly divided into seven groups (A, B, C, D, E, F and G). There were four (4) rats in each of the groups A-F. Rats in group A were given feed only and served as negative control while rats in groups B, C, D, E and F were orally exposed to varying concentrations (1, 2, 4, 8, 12mg/Kg body weight, respectively) of cadmium (cadmium chloride) for six weeks. Effects of cadmium were most significant at 12 mg/Kg body weight. This informed the use of cadmium at 12 mg/Kg body weight for the subsequent test, which involved oral administration of *Jussiaea nervosa* leaf extract. In this segment, group G (n=16 rats) was sub-divided into four: G_1, G_2, G_3 and G_4, with each sub-group containing four (4) rats.

Rats in sub-group G_1 were given cadmium and feed only and served as positive control. However, rats in sub-groups G_2 , G_3 and G_4 were in addition to cadmium, administered 20, 50 and 100g/kg body weight of *Jussiaea nervosa* leaf extract, respectively. The experiment lasted for six (6) weeks. Both the control and experimental animals were allowed free access to water. At the end of the experiment, the rats were sacrificed. Blood samples were collected for biochemical analysis while the livers were excised for histopathological examinations.

Phytochemical, Biochemical and Histochemical Analysis

The phytochemical analyses of *Jussiaea Nervosa* leaf extractwas done using A.O.A.C[18] and A.O.A.C,[19] while the levels of Alanine transaminase (ALT), Aspartate transminases (AST), alkaline phosphatase (ALP), serum bilirubin and albumin were determined using test kits (Randox Laboratories, UK) in accordance with manufacturer's instructions.

Statistical Analysis

All the tested parameters were subjected to statistical analysis. Statistical analysis was done by One-way Analysis of Variance (ANOVA) and means were compared by Dunnettes comparison.[20]

Results and Discussion

The results of the proximate composition of *Jussiaea nervosa* leaf extract are presented in Table 1. Carbohydrate recorded the highest value of 50.90±0. 96 % while protein was the lowest with a value of 3.60±0.58%. The plant is edible and the proximate analysis is important because it provides information of its nutritional worth and other beneficial effects on humans.[21]

Table 1. Proximate composition of *Jussiaea nervosa* aqueous extract.

Parameter	Mean ± S.D (%)
Moisture	10.00 ± 0.00
Ash	15.5 ± 0.86
Fat	5.5 ± 0.00
Protein	3.6 ± 0.58
Fibre	15.5 ± 1.54
Carbohydrate	50.9 ± 0.96
Data represent means ± SD of three measurements.	

The results of the phytochemicals analysis as presented in Table 2 showed the presence of saponins, flavonoids, alkaloids and tannins. Steroids glycosides and phenols were absent. Comparatively, flavonoids had the highest value (30.33+0.64%) while tannins were the least with a mean value of 1.24+0.10%. These values are

consistent with phytochemical analysis reports on some other medicinal plants found in Nigeria and West African sub region.[17]

Table 2. Phytochemical Analysis of *Jussiaea nervosa* aqueous leaf extract.

Parameter	Concentration (mg/l)
Steriod	ND
Glycosides	ND
Saponins	1.85 ± 0.50
Flavonoids	30.33 ± 0.64
Phenols	ND
Alkaloid	11.20 ± 0.99
Tannins	1.24 ± 0.10

Table 3 shows the effects of Cd exposure on some liver functions biomarkers. Exposure to cadmium was observed to cause impairment of hepatocyte functions in dose-dependent manner, with higher doses having more severe effects. For instance, the values of serum ALT, AST, ALP, total bilirubin and conjugated bilirubin increased significantly ($p<0.05$), while total protein and albumin decreased significantly ($p<0.05$). The values of ALT, AST, ALP, total bilirubin and conjugated bilirubin were observed to increase by up to 327, 160, 286, 154 and 208%, respectively, at Cd dose of 12 mg/Kg body weight. Total protein and albumin levels decreased by 67 and 61 %, respectively at the same cadmium dosage.

Table 3. Effects of cadmium exposure on some liver function biomarkers in albino rats.

Experimental groups	Proteins (g/dl)		Liver enzymes (U/L)			Bilirubin (µmol/l)	
	Total	Albumin	AST	ALT	ALP	Total	Conjugated
A	6.0±0.5^{a}	3.6±0.2^{a}	18.7±0.2^{a}	38.4±0.3^{a}	52.1±0.7^{a}	9.7±0.9^{a}	2.4±0.7^{a}
B	4.6±0.4^{b}	2.9±0.3^{b}	37.8±0.3^{b}	56.5±0.4^{b}	77.3±0.3^{b}	21.4±0.4^{b}	4.8±0.2^{b}
C	3.6.±0.1^{b}	2.4±0.1^{b}	45.0±0.0^{b}	58.8±0.2^{b}	82.4±1.2^{b}	22.8±0.5^{b}	5.70±0.2^{b}
D	3.3±0.1^{b}	2.0±0.1^{c}	47.1±0.4^{b}	68.0±0.1^{b}	99.1±0.1^{b}	23.2±0.3^{b}	5.9±0.6^{b}
E	2.9±0.1^{c}	1.6±0.1^{d}	63.0±0.1^{c}	94.5±0.4^{c}	198.4±0.7^{c}	23.8±0.6^{b}	6.7±1.1^{c}
F	2.0±0.9^{d}	1.4±0.1^{d}	79.8±1.0^{c}	100.1±1.2^{c}	209.2±1.1^{c}	24.5±0.6^{b}	7.4±0.8^{c}

Values represent means ±S.D. Values with different superscript in the same column are significantly different ($p<0.05$).

These results were corroborated by the results of the histopathological assessment of the liver which revealed extensive cells turnover, fibrosis, infiltration of inflammatory cells, extensive distortion of the hepatocytes, hepatitis and hepatic necrosis (Figure 1), which are in sharp contrast with the results from the control rats (Figure 2). These are indicative of the liver inflammation due to exposure of the rats to Cd. Cd is a known toxic metal and may have elicited some injuries to the hepatocytes.[22]

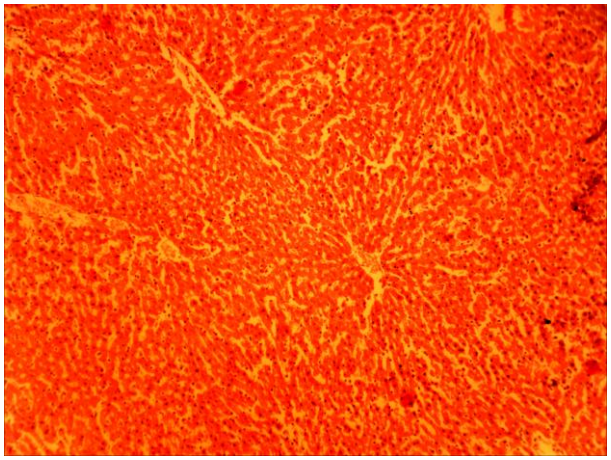

Figure 1. Micrograph of a section of the liver of normal albino rat (control). Section shows normal liver architecture with preserved hepatic vessels.

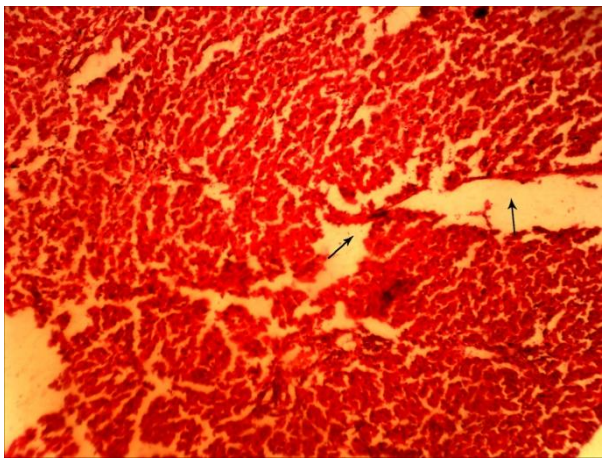

Figure 2. Micrograph of a section of the liver of albino rat exposed to 12 mg of Cd//Kg body weight. Arrows show extensive necrosis with inflammatory cells infiltrating into hepatocytes resulting in hepatisis.

Increased bilirubin levels may have been caused by breakdown of erythrocytes and other haem containing proteins, myoglobin and cytochromes. The haem (from porphrin) of the haemoglobin molecule is separated from the globin and haem is converted mainly in the spleen to bilverdin which is reduced to bilirubin.[23] This is also in tandem with the observed decrease in haemoglobin and packed cell volume levels due to exposure to Cd as previously reported.[24] Another possible explanation is that Cd exposure is known to

induce generation of reactive oxygen species (ROS) in organisms[25] which have the potential to elicit oxidative damage to cells, tissues and organs and even death.

For rats which were exposed to 12 mg/kg Cd and co-treated with various concentrations of *Jussiaea nervosa* leaf extract, the results were different: The values of AST, ALT, ALP, bilirubin and conjugated bilirubin were significantly ($P<0.05$) lower than those found in rats that were exposed to Cd only (Table 4) and comparable to levels found in unexposed rats (negative control). The serum albumin and total protein levels significantly ($p<0.05$) improved in animals co-treated with *Jussaiea nervosa* leaf extract. For instance, ALT, AST, ALP, total bilirubin and conjugated bilirubin which were elevated in the untreated rats almost returned to the values found in the unexposed (negative control) rats on administration of aqueous extract of *J. nervosa* leaves at higher doses with only 97, 83, 245, 62, and 72 % elevation, respectively, and thus causing 233, 94, 23, 147, and 191% improvement, respectively, in the treated rats. Similarly, the values of total protein and albumin, which decreased by 67 and 61%, respectively, in the untreated rats, only decreased by 3 and 17%, respectively, in the *Jussiaea nervosa* treated rats, and thus causing 1811 and 267% improvement, respectively.

Table 4. Effects of *Jussiaea nervosa* extract on liver biochemical parameters of Cd- exposed rats

Experimental groups	Proteins (g/dl)		Liver enzymes (U/L)			Bilirubin (µml/l)	
	Total	Albumin	AST	ALT	ALP	Total	Conjugated
A	6.0 ± 0.48^{a}	3.6 ± 0.2^{a}	18.7 ± 0.2^{a}	38.4 ± 0.3^{a}	52.1 ± 0.7^{a}	9.7 ± 0.9^{a}	2.4 ± 0.7^{a}
G_1	2.0 ± 0.87^{b}	1.4 ± 0.1^{b}	78.8 ± 1.0^{b}	100.2 ± 1.2^{b}	209.2 ± 1.1^{b}	24.5 ± 0.6^{b}	7.4 ± 0.9^{b}
G_2	3.2 ± 0.11^{b}	2.4 ± 0.2^{b}	60.4 ± 0.9^{b}	$92.8\pm0.3_{b}$	207.9 ± 4.1^{b}	20.5 ± 0.9^{b}	6.1 ± 1.0^{b}
G_3	4.8 ± 0.01^{c}	2.6 ± 0.1^{a}	52.4 ± 1.2^{b}	90.4 ± 0.5^{b}	189.9 ± 5.4^{b}	18.8 ± 1.1^{b}	5.4 ± 0.6^{b}
G_4	5.8 ± 0.17^{a}	3.0 ± 0.4^{a}	36.5 ± 0.4^{c}	70.3 ± 0.1^{c}	180.1 ± 0.6^{b}	15.7 ± 1.2^{c}	4.1 ± 0.9^{c}

Values represent means ± S.D. Values with different superscript in the same column are statistically different ($p<0.05$).

The observed results are corroborated by histopathological examination of the liver (Figure 3) which showed minimal damage to the liver architecture and even normal integrity of the liver. The liver architecture was well preserved with moderate central fatty changes. The observed results could be attributed to the protective effects of *Jussiaea nervosa* leaf extract, which contains various bioactive chemical with established known therapeutic/antioxidant properties. The presence of the active principles could have caused chelation of the metals and prevented the adverse effects seen in animals exposed to Cd only.

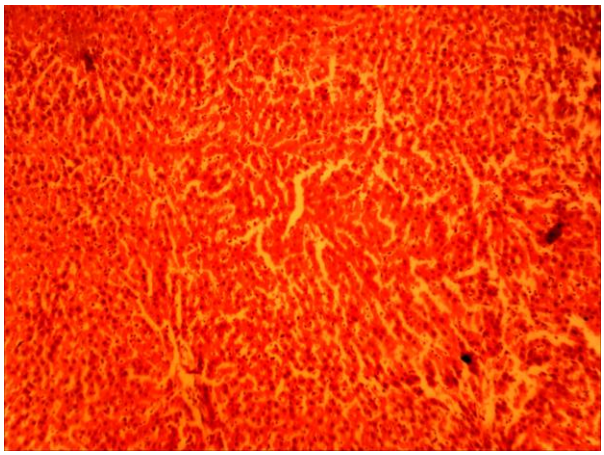

Figure 3. Micrograph of a section of the liver of albino rat exposed to 12 mg/Kg of Cd and treated with aqueous extract of *Jussiaea nervosa* leaf. Section shows normal integrity of liver architecture when compared with control, indicative of the extract protection.

It is therefore reasonable to state that *Jussiaea nervosa* leaf extract contain some phytochemicals which could protect against Cd intoxication, though the mechanism of its action is yet to be established. We can however speculate that the antioxidant properties of the phytochemicals present in *Jussiaea nervosa* leaf extract could have exerted its actions by scavenging ROS produced due to Cd exposure, and therefore, prevented the attendant damage on the liver and macromolecules.[26] It is also possible that some bioactive principles in *Jussiaea nervosa* leaf extract may have induced synthesis of small sulphur rich polypeptides termed phytochelatin which formed complexes with Cd^{2+} and neutralized its toxic effects.[27]

The present results suggest that *Jussiaea nervosa* leaf extract possess hepatoprotective properties against Cd-induced toxicity. Considering the ubiquity of Cd in the environment and the ease with which humans, especially children are exposed; it is recommended that *Jussiaea nervosa* leaf be included in the daily menu to ameliorate Cd toxicity.

Conflict of Interest

There is no conflict of interest to be reported.

References

1. WHO. Environmental Health Criteria. Geneva: World Health Organisation; 1992.
2. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanism of cadmium carcinogenesis. Toxicology 2003;192(2-3):95-117.

3. Kiestin PG. Lactational transfer of cadmium in rodents, central nervous system: Effects in the offspring. *Agric Sci Vet* 2003;150:10-1.
4. Van-Assche FJ. A stepwise model to quantify the relative contribution of different environment sources to human cadmium exposure. *Toxicol*ogy1998;98:21-2.
5. Irwin RJ, Van-Mouwerik M, Stevend L, Seese MD, Rasham W. Environmental Contaminates Encyclopedia. 1st ed. Fort Collins, Colorado: National Park Service Water Resources Division; 2003.
6. Margeli A, Theocharis S, Skaltsas S, Skopelitou A, Mykoniatis M. Effect of cadmium pretreatment on liver regeneration after partial hepatectomy in rats. *Environ Health Perspect* 1994;102(Suppl 3):273-6.
7. Cook ME, and Morrow H. Anthropogenic sources of cadmium in Canada. *J Pharmacol* 1992; 60:20-5.
8. Bem EM, Piotrowski JK, Turzynska E. Cadmium, zinc, and copper levels in the kidneys and liver of the inhabitants of north-eastern Poland. *Pol J Occup Med Environ Health* 1993;6(2):133-41.
9. Uwakwe AA, Ibiam UA. Levels of cadmium in different brands of cigerettes sold in Abakaliki metropolis of Nigeria. *Afr J Biochem Res* 2009;3(8):317-20.
10. Al-Motabagani MAH. Effect of cadmium on the morphology of Adrenal Gland in Mice. *J Anat Soc India* 2002;51(2)*:*212-6.
11. Martynowicz H, Skoczyńska A, Karczmarek-Wdowiak B, Andrzejak R. Effect of cadmium on testis function. *Med Pr* 2005;56(2):167-74.
12. Rastogi RB, Singhal RL. Effect of chronic cadmium treatment on rat adrenal catecholamines. *Endocr Res Commun* 1975;2(1):87-94.
13. Axelsson B, Piscator M. Renal damage after prolonged exposure to cadmium. An experimental study. *Arch Environ Health* 1966;12(3):360-73.
14. Jarup L, Alfven T. Low level cadmium exposure, renal and bone effects--the OSCAR study. *Biometals* 2004;17(5):505-9.
15. Jia W, Zhang H. Challenges and opportunities in the Chinese Herbal Drug Industry. 2nd ed. New Delhi: Eastern Publishers; 2005.
16. Sadiq D, Ezi-Ashi T, Onuaguluchi G. Checklist of medicinal plants of Nigeria and their uses. 1st ed. Enugu-Nigeria: Asicumptipn; 2003.
17. Sofowora A. The State of Medicinal Plants 1st ed. Enugu-Nigeria: University of Ibadan;1997.
18. AOAC. Official Methods of Analysis. 15th ed. Washington DC, USA: Association of Official Analytical Chemists; 1990.
19. AOAC. Official Methods of Analysis. 16th ed. Washington DC, USA: Association of Official Analytical Chemists; 1999.
20. William GC, Snedecor GW. Statistical Methods, 8th ed. New York: Lowa State University Press; 1994.
21. Pandey M, Abidi AB, Singh S, Sing RP. Nutritional Evaluation of Leafy Vegetable Paratha. *J Hum Ecol* 2006;19(2):155-6.
22. Suzuki Y, Morita I, Yamane Y, Murota S. Cadmium stimulates prostaglandin E2 production and bone resorption in cultured fetal mouse calvaria. *Biochem Biophys Res Commun* 1989;158(2):508-13.
23. Doumas BT, Perry BW, Sasse EA, Straumfjord JV, Jr. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. *Clin Chem* 1973;19(9):984-93.
24. Ibiam UA, Ugwuj EI, Ejeogo C, Aja PM, Afiukwa C, Oji OU, et al. Hemoprotective and nephroprotective potentials of aqueous extract of *Jussiaea nervosa* leaf in cadmium exposed albino rats. *IOSR J Pharm Biol Sci* 2012;4(1):48-53.
25. Price DJ, Joshi JG. Ferritin. Binding of beryllium and other divalent metal ions. J Biol Chem 1983;258(18):10873-80.
26. Valko M, Izakoric M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem* 2004;266(1-2):37-56.
27. Ming Ho Yu. Environmental Toxicology, 1st ed. Washington DC, USA: Lewis Publishers;2000.