

Protective effect of *Tribulus terrestris* linn on liver and kidney in cadmium intoxicated rats

G Dhana Lakshmi¹, P Ravi Kumar¹, K Bharavi^{1*}, P Annapurna², B Rajendar¹, Pankaj T Patel¹,
C S V Satish Kumar¹ & G S Rao¹

Department of ¹Pharmacology & Toxicology and ²Veterinary Pathology, NTR College of Veterinary Science,
Gannavaram 521 102, India

Received 18 May 2011; revised 14 December 2011

Administration of cadmium (Cd) significantly increased the peroxidation markers such as malondialdehyde and protein carbonyls along with significant decrease in antioxidant markers such as super oxide dismutase and reduced glutathione in liver and kidney tissues. Cadmium also caused a significant alteration in hepatic and renal functional markers in serum viz. total protein, albumin, alanine transaminase, blood urea nitrogen and creatinine. Prominent pathological changes observed in liver were severe vascular and sinusoidal congestion with diffuse degenerative changes and mononuclear infiltration into peripheral areas, while the kidney showed vascular and glomerular congestion, cloudy swelling of tubular epithelium. Co-administration of ethonolic extract of *T. terrestris* or vitamin E along with Cd significantly reversed the Cd induced changes along with significant reduction in Cd load.

Keywords: Cadmium, Kidney, Liver, Rats, *Tribulus terrestris*

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant in soil, water, air, food and smoke¹. Cadmium is mainly used in the industry for coating steel, glass and plastics (including polyvinyl chloride), and for nickel cadmium battery production². Phosphate fertilizers also show a big cadmium load³. Cadmium contamination of soil and water has raised concern because this metal bioaccumulates in the upper levels of food chain including humans and has a very long biological half-life (10-30 years) in the body and its toxicity is dependent on the route, amount and the duration of exposure⁴. Chronic exposure to low doses of cadmium results in its accumulation in liver and kidney, rendering them primary target organs⁵. Cadmium induces toxicity in various target organs through several mechanisms. However most of its toxic effects are believed to result from a mechanism related to its ability to generate free radicals, at a rate enough to overwhelm the antioxidant defense system of the body⁶.

Oxidative damage induced by free radicals can be prevented by the use of antioxidants such as ascorbic

acid, tocopherols, carotenoids and certain herbs⁷. Several Indian plants are considered as potential sources of antioxidants which can counter the peroxidative stress in biological system due to several stresses, including, heavy metals, pesticides and mycotoxins⁸.

Tribulus terrestris (TT) is a flowering plant (family Zygophyllaceae) native to warm temperature, and tropical regions. It has long been used in the traditional Chinese and Indian systems of medicine, for the treatment of various ailments and is popularly claimed to improve sexual functions in man⁹. TT and its related species have been reported to have antioxidant effects in various forms of experimentally induced oxidative stresses¹⁰⁻¹². In this back drop, the present work is designed to study the protective effect of TT on the liver and kidney of the rats exposed to cadmium toxicity.

Materials and Methods

Chemicals—All the chemicals were of analytical grade and obtained from commercial sources like Qualigens Pvt Ltd., Mumbai and Sisco Research Laboratories, Mumbai.

Animals—Adult Wister albino male rats (40), aged about 60 days with average body weight of 147 g were obtained from M/s Mahaveer Enterprises

*Correspondent author
Mobile: +91 9951302224
E-mail: kbharavi@yahoo.com; bharavibharavi@gmail.com

(Regn. No. 46/1999/CPCSEA), Hyderabad. The experimental design was approved by Institutional Animal Ethics Committee.

Preparation of ethanolic extract of Tribulus terrestris (eTT)—The whole plant material at flower and fruit bearing stage was collected from NTR College of Veterinary Science farm and identified by Botanist. Whole plant material was air dried in shade and ground to coarse powder. Powdered plant material (20) was taken in a conical flask and analytical grade ethanol was added in 1:10 ratio. The conical flask was closed air tight with non absorbent cotton and aluminum foil. Then it was subjected to constant stirring over night using an orbital shaker at room temperature. Next day the plant material was filtered through filter paper and the filtrate was subjected to slow evaporation by keeping in a water bath at 60°C until a consistent solid mass was formed. This ethanolic extract was made into suspension with double distilled water before administration.

Experimental design—The rats were divided into four groups of 8 rats each. Group 1 was maintained as control and group 2 rats received CdCl₂ at the rate of 3 mg/ kg body weight SC once a week for 4 weeks. Group 3 rats received CdCl₂ like the rats in group 2 along with eTT at the rate of 5 mg/kg body weight. po, daily for 6 weeks starting from day of cadmium administration. Rats in group 4 were administered with CdCl₂ along with vitamin E at the rate of 75 mg/kg body weight, po daily for 6 weeks. After collecting approximately 1-1.5 mL of blood from retro-bulbar plexuses of the right eye for obtaining serum, all the rats were sacrificed at the end of 6th week by cervical dislocation. Liver and kidney were collected immediately and kept in ice cold phosphate buffer. A portion of the organs was homogenized with tissue homogenizer individually to make 10% of homogenate to assay the tissue antioxidants such as super oxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and tissue peroxidation markers such as thiobarbituric acid reacting substances (TBARS) and protein carbonyls. Liver and kidney functional markers such as total protein, albumin, globulin, alanine transaminase (ALT), blood urea nitrogen (BUN) and creatinine were assayed in serum. Pieces of tissues from livers and kidneys were immediately kept in 10% formalin fixative to study histopathology. The remaining liver and kidney portions were kept in hot air oven for 24 h for further processing to estimate concentration of cadmium.

Biochemical analysis

Estimation of antioxidant markers of liver and kidney—SOD was estimated by the method involving inhibition of superoxide dependant reduction of tetrazolium dye MTT to formazan¹³. Catalase activity was determined by monitoring the decrease in absorbance at 240 nm due to decomposition of H₂O₂¹⁴ and GSH was estimated based on a reaction of reduced glutathione with 5-5' dithiobis-2-nitrobenzoic acid (DTNB)¹⁵.

Estimation of peroxidation markers of liver and kidney—Malondialdehyde, the product of lipid peroxidation was estimated by reaction with thiobarbituric acid as per the method prescribed by Balasubramanian *et al.*¹⁶ Protein carbonyls were estimated based on the reaction of amino carbonyls with 2, 4-dinitrophenyl hydrazine to form hydrazones, which can be detected spectrophotometrically at 372 nm¹⁷. Total protein in liver and kidney tissue homogenate was quantified as per Lowry *et al.*¹⁸.

Estimation of liver and kidney functional markers—Total protein, albumin, ALT, BUN and creatinine of serum were estimated by using the standard kits.

Histological studies—Formalin fixed liver and kidney pieces were processed by paraffin embedding, sections of 4-5 μ thickness were cut and stained by routine Harris haematoxylin and eosin method.

Estimation of cadmium concentration—Dry ashing procedure was used for the mineral analysis in organs. Wet tissue was dried at 100° C for 24 h. Dried sample was transferred to a cool muffle furnace and the temperature was slowly raised to 450°C and ashed overnight. The ash was dissolved in 2 mL HNO₃ plus double distilled water (DDW). After filtering, the digesta was made up to 25 mL with DDW and concentration of Cd was determined with Atomic Absorption Spectrophotometer (NOVA 300)¹⁹.

Statistical analysis—The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) 12th version. Differences between means were tested using Duncan's multiple comparison test and significance was set at $P < 0.05$.

Results

The average body weight gain was significantly reduced in group 2 compared to control. But co-administration of eTT or vitamin E with Cd exposure showed a significant increase in weights compared to Group 2 (Fig. 1).

The level of antioxidants in liver and kidney such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were significantly reduced while the level of peroxidation markers such as thiobarbituric acid reacting substances (TBARS) and protein carbonyls were significantly increased in Cd treated rats compared to control rats. Administration of eTT and vitamin E significantly reversed the above values (Table 1).

The functional markers of liver in serum such as total protein and albumin were significantly decreased while the ALT levels were significantly increased following Cd administration. Kidney functional markers such as serum creatinine and BUN were also significantly increased compared to those of control group. The above altered functional markers were

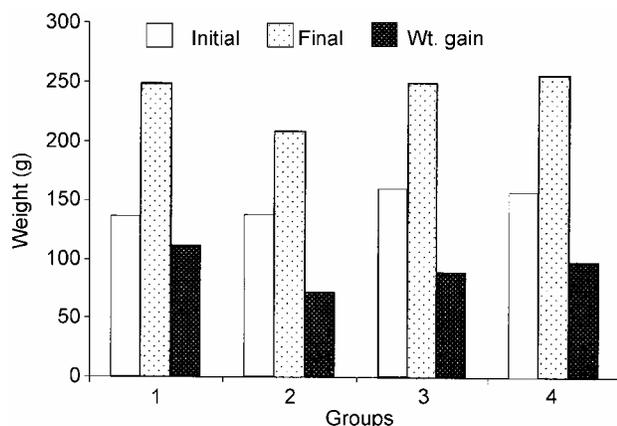


Fig. 1—Effect of ethanolic extract of *T. terrestris* on body weight gain in cadmium intoxicated rats

significantly reversed with eTT or vitamin E administration (Table 2).

Cadmium treated group liver showed severe vascular and sinusoidal congestion with diffuse degenerative changes as evidenced by cloudy swelling. In addition periportal areas showed infiltration of mononuclear cells in large numbers (Fig. 2). In Group 3 that was given eTT along with Cd, the liver sections revealed very mild cloudy swelling of hepatocytes (Fig. 2) and in group 4, treatment with Vitamin E along with Cd, revealed near normal architecture with very mild degenerative changes in hepatocytes in few cases (Fig. 2). Microscopic examination of kidney sections from group 2 revealed severe vascular and glomerular congestion. Degenerative changes were also observed in proximal tubular epithelium showing cloudy swelling (Fig. 2). In few cases the kidney tubular lumen contained eosinophilic hyaline casts. The lesions in group 3 were mild degenerative changes in the proximal tubular epithelium (Fig. 2) and in group 4 the kidney sections exhibited near normal architecture with only mild cloudy swelling in proximal tubular epithelium (Fig. 2).

Significantly higher levels of cadmium were found in liver and kidney of cadmium treated group compared to control, where as following supplementation with eTT extract and vitamin E in groups 3 and 4 respectively, a significant reduction in Cd level was observed in these organs when compared to those in group 2.

Table 1—Effect of ethanolic extract of *T. terrestris* on antioxidant and peroxidation markers of liver and kidney tissues of cadmium intoxicated rats (values are mean±SE from 8 animals in each group)

Group	Tissue antioxidant markers						Peroxidation markers			
	SOD (U/mg protein)		CAT (mM H ₂ O ₂ utilized.min.mg of protein)		GSH (µm/mg of protein)		TBARS (M of MDA/g of protein)		Protein carbonyls (nM/mg of protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
1	21.34± 0.17 ^c	21.51± 0.18 ^c	65.50± 0.41 ^c	12.04± 0.15 ^c	54.54± 0.35 ^c	31.74± 0.42 ^c	63.75± 0.21 ^c	36.16± 0.84 ^c	2.65± 0.05 ^c	6.49± 0.1 ^c
2	10.00± 0.25 ^a	10.53± 0.25 ^a	33.0± 0.30 ^a	4.85± 0.15 ^a	27.67± 0.58 ^a	17.35± 0.41 ^a	95.70± 0.21 ^a	110.84± 0.4 ^a	8.89± 0.10 ^a	10.57± 0.06 ^a
3	20.90± 0.19 ^b	20.83± 0.25 ^b	64.35± 0.65 ^b	11.37± 0.11 ^b	53.28± 0.33 ^b	31.03± 0.39 ^b	64.15± 0.21 ^b	36.84± 0.06 ^c	3.11± 0.09 ^b	6.92± 0.08 ^c
4	20.74± 0.17 ^b	20.52± 0.22 ^b	64.46± 0.69 ^b	11.78± 0.14 ^b	53.97± 0.31 ^b	31.11± 0.38 ^b	63.85± 0.21 ^c	36.17± 0.65 ^c	2.95± 0.11 ^c	6.66± 0.11 ^c

Values are mean±SEM (n=8) One way ANOVA (SPSS)
Means with different superscripts differ significantly (P<0.05).

Table 2—Effect of *Tribulus terrestris* on liver and kidney functional markers and cadmium level of cadmium intoxicated rats. (values are mean±SE from 8 animals in each group for tissue functional markers and 6 animals for Cd concentration)

Group	*Tissue functional markers					#Cadmium concentration (µg/g wet tissue)	
	Liver			Kidney		Liver	Kidney
	Total protein (g/dl)	Albumin (g/dl)	ALT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)		
1	3.8±0.02 ^c	1.46±0.02 ^c	18.9±0.10 ^c	7.86±0.06 ^c	0.146±0.006 ^c	0.07±0.066 ^c	0.02±0.004 ^c
2	2.02±0.07 ^a	1.01±0.04 ^a	61.16±0.11 ^a	12.66±0.14 ^a	1.54±0.03 ^a	0.13±0.011 ^a	0.38±0.002 ^a
3	3.57±0.06 ^b	1.40±0.02 ^b	32.66±0.20 ^b	7.97±0.04 ^b	0.15±0.008 ^b	0.08±0.005 ^c	0.15±0.01 ^b
4	3.75±0.03 ^c	1.45±0.03 ^c	19.68±0.10 ^b	7.87±0.05 ^b	.14±0.05 ^c	0.09±0.007 ^c	0.11±0.013 ^b

Values are mean±SEM (*n=8; #n=6) One way ANOVA (SPSS) Means with different superscripts differ significantly (P<0.05).

Discussion

Cadmium is one of the ubiquitous environmental pollutant and 7th most hazardous substance²⁰ and is also a carcinogen²¹. In the body Cd produces toxic effects by a mechanism related to its ability to generate free radicals at a range high enough to overwhelm the natural antioxidant defense system of the body⁶ and results in a condition known as oxidative stress.

In the present study Cd toxicity with particular reference to liver and kidney was assessed in terms of tissue antioxidant markers, peroxidation markers, functional markers and extent of Cd accumulation in liver and kidney.

Accumulation of Cd in liver and kidney in Cd administered rats was reported previously by Jihan *et al*²². In the present experiment, Cd accumulation in the liver and kidney of group 2 rats had significantly reduced the antioxidant markers viz. SOD, CAT and GSH, while the peroxidation markers viz. TABRS and protein carbonyls were found significantly increased, when compared to those in control group. The results of the present study are in agreement with earlier findings of reduction in the antioxidant markers with simultaneous increase in peroxidation markers in rats under Cd influence^{23,24}. The tissue peroxidation induced by Cd lead to liver and kidney dysfunction which was reflected as alteration in various functional markers in serum. These alterations consisted of significant decrease in total protein and albumin with significant increase in ALT levels indicating hepatotoxicity and significant increase in serum levels of BUN and creatinine levels suggesting nephrotoxicity.

Cadmium induced tissue damage was also revealed by severe pathological changes in liver and kidney of

Cd treated rats. The liver exhibited severe vascular and sinusoidal congestion with diffuse degenerative changes and mononuclear infiltration was noticed in peripheral areas. Kidney sections revealed severe vascular and glomerular congestion, cloudy swelling of tubular epithelium. Severe histological changes in liver and kidney of Cd exposed rats were also reported by El-Sokkary *et al*²⁵. Thus the results indicated that Cd at the administered dose in the study produced severe oxidative damage in liver and kidney.

Tribulus terrestris, a traditional aphrodisiac herb, contains 'protodioscin' which significantly increases testosterone and dihydrotestosterone in primates, rabbits and rats and was reported useful in mild to moderate cases of erectile dysfunctions²⁶. In addition TT also exhibited antioxidant properties¹¹. In group 3 rats, simultaneous administration of eTT along with Cd significantly reduced the Cd burden in liver and kidney and also caused restoration of antioxidant, peroxidation and functional markers when compared to Cd alone treated group 2. The favourable recovery in these biochemical profiles is further indicated by the less severe histological changes in liver and kidney.

Vitamin E has been found to have antioxidant and cytoprotective role²⁷ in Cd induced oxidative stress and lipid peroxidation²⁸. In group 4, administration of vitamin E along with Cd, showed a good protective effect by restoration of biochemical profiles in liver and kidney tissue and the hepatic and renal functional indicators in serum. This was also well correlated with near normal histological architecture and Cd concentration in liver and kidney.

In conclusion it can be interpreted that ethanolic extract of TT possesses antioxidant property that was

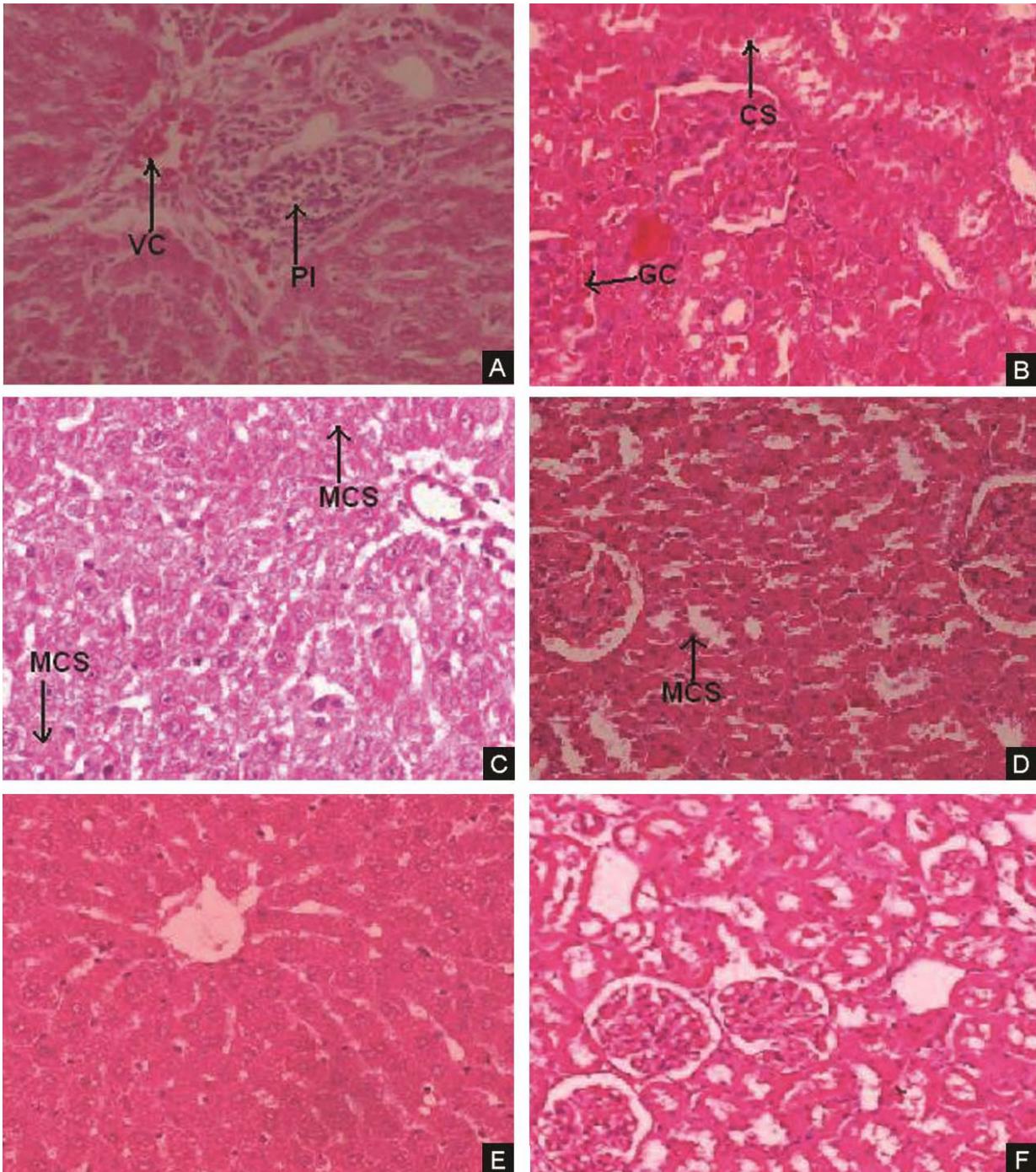


Fig. 2—Light microscopic study of (A) cadmium treated group rat liver showing vascular congestion and periportal infiltration with mononuclear cells. (B) cadmium treated group rat kidney showing glomerular congestion and cloudy swelling of tubular epithelium. (C) Cd and TT extract treated rats liver showing mild cloudy swelling. (D) Cd and TT extract treated rats kidney showing mild cloudy swelling of tubular epithelium. (E) Cd and vitamin E treated rats liver showing near normal architecture with very mild cloudy swelling. (F) Cd and vitamin E treated rats kidney showing near normal architecture with very mild cloudy swelling of tubular epithelium. VC: vascular congestion; PI: periportal infiltration with mononuclear cells; GC: glomerular congestion; CS cloudy swelling of tubular epithelium; MCS: mild cloudy swelling. Figs A-E: H&E, 400 \times ; Fig. F: H&E, 100 \times

able to protect the liver and kidney from cadmium insult.

References

- 1 Jarup L, Berglund M & Elinder C G, Health effects of cadmium exposure: A review of its literature and a risk estimation, *Scand Work Environ Health*, 24 (1998) 1.
- 2 Tsalev D L & Zaprianov Z K, Atomic Absorption Spectrometry in Occupational and Environmental Health Practice, (CRC Press, Boca Raton, FL,USA) 1993, 105.
- 3 Godt J, The toxicity of cadmium and resulting hazards for human health, *J Occup Med Toxicol*, 1 (2006) 22.
- 4 Satarug S, Baker J R, Urbenjapol S, Haswell-Elkins M, Reilly P E, Williams D J & Moore M R, A global perspective on Cadmium pollution and toxicity in non-occupationally exposed population, *Toxicol Lett*, 137 (2003) 65.
- 5 Smalinskiene A, Ryselis S, Abdrakhmanov O, Kregzdyte R, Sadauskiene I & Ivanov L, Investigation of cadmium concentration after acute intoxication, *Biologija*, 2(1) (2004) 113.
- 6 Bagchi D, Bagchi M, Hassoun E A & Stohs S J, Cadmium induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague–Dawley rats, *Biol Trace Elem Res*, 52(2) (1996) 143.
- 7 Kamashi K, Gopala Reddy A, Reddy K S & Reddy V R, Evaluation of zinc against salinomycin toxicity in broilers, *Indian J Physiol Pharmacol*, 48 (2004) 89.
- 8 Farrukh A, Iqbal A & Zafar M. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants, *Turk J. Biol*, 30 (2006) 177.
- 9 Gauthaman K, Adaikan P G & Prasad R N V, Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in castrated rats, *Life Sci*, 71(12) (2002) 1385.
- 10 Kavitha A V & Jagadeesan G, Role of *Tribulus terrestris* (Linn.) (Zygophyllaceae) against mercuric chloride induced nephrotoxicity in mice, *Mus musculus* (Linn.), *J Environ Biol*, 27(2) (2006) 397.
- 11 Amin A, Lotfy M, Shafiullah M & Adeghate E, The protective effect of *Tribulus terrestris* in diabetes, *Ann N Y Acad Sci*, 1084 (2006) 391.
- 12 Kadry H, Abou Basha L, El Gindi O & Temraz A, Antioxidant activity of aerial parts of *Tribulus alatus* in rats, *Pak J Pharm Sci*, 23(1) (2010) 59.
- 13 Madesh M & Bala Subramanian K A, Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide, *Indian J Biochem Biophys*, 35 (1998) 184.
- 14 Calliborne A L, Assay of catalase in: *Hand book of oxygen radical research*, edited by Greenwald RA (CRC Press, Boca Raton, FL,USA) 1985.
- 15 Moron M S, Depierre J W & Mannervik B. Levels of glutathione, glutathione reductase and glutathione S transferase in rat lung and liver, *Biochemical et. Biophysica Acta*, 582 (1979) 67.
- 16 Balasubramanian K A, Manohar M & Mathan V I. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. *Biochimica et Biophysica Acta*, 962 (1988) 51.
- 17 Levine R L, Garland D, Oliver C N, Amici A, Climent I, Lenz A G, Ahn BW, Shaltiel S & Stadtman ER, Determination of carbonyl content in oxidatively modified proteins, *Methods Enzymol*, 186 (1990) 464.
- 18 Lowry O H, Rosenbrough M J, Farr A L & Rawdall R A, Protein measurement with the Folin phenol reagent, *J. Biol. Chem*, 193 (1951) 265.
- 19 Uyanik F, Even M, Atasever A, Tuncoku G & Kolsuz A H, Changes in some Biochemical Parameters and organs of broilers exposed to cadmium and effect of zinc on cadmium induced alterations, *Isr J Vet Med*, 56 (2001) 128.
- 20 ATSDR, The 2007 CERCLA priority list of hazardous substances, Agency for toxic substances and disease registry, U.S. Department of Health and Human Services, Atlanta, GA, (2007).
- 21 IARC, *Cadmium and Cadmium compounds*. (International Agency for Research on Cancer Monographs, Lyon, France) 1993.
- 22 Jihen el H, Imed M, Fatima H & Abdelhamid K, Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation, *Food Chem Toxicol*, 46(11) (2008) 3522.
- 23 Jeyaprakash K & Chinnaswamy P, Effect of spirulina and Liv-52 on cadmium induced toxicity in albino rats, *Indian J Expl Biol*, 43(9) (2005) 773.
- 24 Renugadevi J & Prabu SM, Naringenin protects against cadmium-induced oxidative renal dysfunction in rat, *Toxicology*, 256(1-2) (2009) 128.
- 25 EL–Sokkary G H, Nafady A A & Shabash E H, Melatonin administration ameliorates cadmium-induced oxidative stress and morphological changes in the liver of rat, *Ecotoxicology and Environmental Safety*, 73(3) (2010) 456.
- 26 Gauthaman K and Ganesan A P, The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction--an evaluation using primates, rabbit and rat, *Phytomedicine*, 15(1-2) (2008) 44.
- 27 Beytut E, Yuce A, kamiloglu N N & Aksakal M, Role of dietary vitamin E in Cadmium induced oxidative damage in rabbit's blood, liver and kidney, *Int J Vitam Nutr Res*, 73(5) (2003) 351.
- 28 Warren S, Samir Patel & Carolyn M Kapron, The effect of vitamin E exposure on cadmium toxicity in mouse embryo cells in vitro, *Toxicology*, 142(2) (1999) 119.