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RESEARCH ARTICLE

THERAPEUTIC POTENTIAL OF *Moringa oleifera* ROOT EXTRACT IN ATTENUATION OF BERYLLIUM INDUCED OXIDATIVE STRESS, BIOCHEMICAL ALTERATIONS AND HISTOPATHOLOGICAL LESIONS IN RAT

[1]Narottam Das Agrawal, [1]Kuldeep Agrawal, [2]Saurabh Gupta, [2]Kundan Kumar Chaubey, [1]Sangeeta Shukla, [1,*]Ramesh Mathur

[1]Reproductive Biology and Toxicology Laboratory, School of Studies in Zoology, Jiwaji University, Gwalior- 474011 (M.P.), India

[2]Microbiology Laboratory, Central Institute for Research on Goats, Makhdoom, PO- Farah -281122, Mathura (U.P.), India

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ABSTRACT

Moringa oleifera root extract was screened to encounter the characteristic biochemical and histopathological alterations followed by subchronic exposure to beryllium. Female albino rats were administered beryllium nitrate at doses of 1mg/kg *i.p*. once a day for 35 consecutive days followed by treatment of different doses of *Moringa oleifera* (50,100,150 and 200 mg/kg *p.o*.) for 7 consecutive days. Administration of beryllium nitrate induced oxidative stress resulting in elevation of lipid peroxidation, reduction in reduced glutathione with decrease in the activities of superoxide-dismutase and catalase in liver and kidney. Beryllium nitrate significantly elevated leakage of serum alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), urea, bilirubin and creatinine whereas hemoglobin and blood sugar were decreased. A significant fall was observed in activitities of alkaline phosphatase, adenosine triphosphatase in liver and kidney; glucose-6-phosphatase and glycogen in liver due to beryllium intoxication. Beryllium also disturbed the histoarchitecture of liver and kidney. The different doses of *Moringa oleifera* root extract reversed the alterations of all the variables more towards the control at biochemical as well as histopathological level. It was concluded that dose of 150 mg/kg of *Moringa oleifera* was found to be most effective in attenuating beryllium induced oxidative stress, biochemical and histopathological alterations.

INTRODUCTION

Human beings are continuously exposed to foreign compounds through environmental exposure, consumption of food or during intake of medicines and produce a variety of toxic manifestations due to damage to liver and kidney. Beryllium is one of the toxic metals that posses extreme risks to human health. Workers are directly exposed to beryllium in industries during mining and processing of beryllium alloys (Kolanz, 2001). Due to exceptional material properties: light weight, enhancing metal hardening capacity and high melting and boiling point, beryllium extensively used in the manufacture of instruments and structural components such as aircraft and space shuttle brakes, satellite mirrors, X-ray windows, electrical circuits, computer components and served as a neutron moderator in nuclear reactors and in nuclear weapons (Taylor and Sauer, 2002). Thus the use of beryllium and beryllium containing alloy is increasing day by day. U.S. consumption of beryllium was estimated to be 320 tons in 2010, up from 167 tons in 2009. This increased demand has also led to increasing prices for beryllium that was US$ 230 per pound in 2010, up from US$ 154 in 2009. A number of diseases such as bronchitis, pneumonitis, dermatitis, acute pneumonitis, hepatomegaly, chronic pulmonary granulomatosis, berylliosis and chronic beryllium diseases (CBD) have been reported due to beryllium intoxication. The United States Environmental Protection Agency (USEPA) has added beryllium to the class A carcinogen list (Taylor and Sauer, 2002). It is suggested that beryllium and its compounds provoke oxidative stress and leads to various pathological consequences, apoptosis (Sawyer *et al*., 2005). Increased ROS level (oxidative stress) may play a key role in the immunopathogenesis of CBD and suggest a rationale for antioxidant therapy (Dobis *et al*., 2008). Thus inhibition of beryllium induced oxidative stress can be a better approach in prevention of beryllium related diseases. In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver and kidney function, offer protection to the hepatorenal damage. Therefore, many folk remedies from plant origin are evaluated for their possible antioxidant and antitoxic effect against chemical induced hepato-renal toxicity. Restriction on the use of synthetic anti-oxidants is being imposed because of their carcinogenicity (Mishra *et al*., 2009). Thus; there is an urgent need to develop potent anti-hepatotoxic, anti-nephrotoxic and antioxidant drug especially among natural products against beryllium-induced hepato-renal disorders. *Moringa oleifera* (Moringaceae) is one of the important medicinal herbs, considered as miracle tree (also known as drum stick tree or horseradish tree) as all the parts of the plant are rich in antioxidants and useful for human health. In recent decades, many scientific studies using the extracts of leaves, pods and roots of *Moringa oleifera* were carried out to confirm many potential uses for the treatment of a variety of human ailments as analgesic (Hukkeri *et al*., 2006), heart complains (Nandave *et al*., 2009), eye disease, dyspepsia, enlargement of spleen, tumors (Parvathy and Umamaheswari, 2007), ulcers (Debnath and Guha, 2007), antioxidant (Sultana *et al*., 2009), hepatoprotective (Hamza, 2010) and diuretics (Karadi *et al*., 2008). But there is no scientific evidence showed antitoxic potential of *Moringa oleifera* root extract against beryllium or metal induced systemic toxicity. In the present study, we investigated antioxidant and antitoxic potential of *Moringa oleifera* root extract against beryllium induced oxidative stress, hepatorenal biochemistry and histopathological lesions in rats.

Corresponding author: Ramesh Mathur
Reproductive Biology and Toxicology Laboratory, School of Studies in Zoology, Jiwaji University, Gwalior- 474011 (M.P.), India

MATERIALS AND METHODS

Collection and Preparation of *Moringa oleifera* Root Extract

The plant of *Moringa oleifera* was identified by the department of Botany, Jiwaji University, Gwalior (M.P). The fresh roots of *Moringa oleifera* were collected from the campus of Jiwaji University and was cut, washed with fresh water and shade dried. The dry roots were crushed; grinned and fine powder was formed. This fine powder (10% w/v) was soaked in 50% (v/v) ethanol for 7 days with intermittent vigorous shaking; supernatant was collected and filtered with what-man filter paper no 1. Filtrate, was dried at room temperature under reduced pressure. This prepared extract was stored in refrigerator at 4°C for further use.

Toxicant and Chemicals

Beryllium nitrate was purchased from Sigma Aldrich Company (St Louis, MO, USA). All other chemicals used in this study were of pure and analytical grade.

Animals

Adult Female Albino rats of *Wistar* strain (10-12 weeks old; 150 $\pm$10 g body weight) were randomly selected from departmental animal facility. Animals were housed under standard husbandry conditions (25$\pm$2°C temp., 60%-70% relative humidity and 14 h light and 10 h dark). Animals were fed on standard commercially available pellets of standard diet (Pranav Agro Industries Ltd. New Delhi, (India) and drinking water *ad libitum*. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the committee for the purpose of control and supervision of experiments on Animals (CPCSEA) Chennai, India and experimental protocols were approved by Institutional Ethics Committee (CPCSEA/501/01/A) of Jiwaji University.

Toxicant and Therapeutic Agent

Beryllium nitrate (35% w/v) was diluted in triple distilled water making up doses of 1mg/2ml/kg (Nirala *et al*., 2007). Doses of different concentration of *Moringa oleifera* root extract were prepared in triple distilled water with 1% gum acacia as describe in experimental design.

Experimental Design

Forty two rats were divided into 7 groups of six in each. Animals of group 1 were administered $Na(NO_3)$ at doses of 1 mg/kg *i.p*. daily, group 2 served as *Moringa oleifera* per se, group 3-7 were administered $Be(NO_3)_2$ at doses of 1 mg/kg *i.p*. daily for 5 weeks followed by doses of *Moringa oleifera* as mentioned below for one week.

Group 1: $Na(NO_3)$ (1 mg/kg *i.p*.) once a day daily for 5 weeks .
Group 2: *Moringa oleifera* per se (200 mg/kg p.o.) once a day daily for last 2 weeks.
Group 3: $Be(NO_3)_2$ (1 mg/kg *i.p*.) once a day daily for 5 weeks.
Group 4: $Be(NO_3)_2$ (as ingroup3) + *Moringa oleifera* (50 mg/kg *p.o*.) daily for 1 week
Group 5: $Be(NO_3)_2$ (as in group 3) + *Moringa oleifera* (100 mg/kg *p.o*.) daily for 1 week
Group 6: $Be(NO_3)_2$ (as in group 3) + *Moringa oleifera* (150 mg/kg *p.o*.) daily for 1 week
Group 7: $Be(NO_3)_2$ (as in group 3) + *Moringa oleifera* (200 mg/kg *p.o*.) daily for 1 week

After 24 h of final administration, animals were euthanized under light ether anaesthesia withdrawing blood in vials by puncturing retro-orbital venous sinus and serum was isolated. Liver and kidney were immediately excised, blotted free of adhering fluid and processed for biochemical studies and histopathological preparations. Standard techniques were applied to assay following blood and tissue biochemical parameters.

Blood Biochemical Parameters

Hemoglobin was estimated in blood using Sahli's apparatus (Swarup *et al*., 1992). The activities of aspartate amino- transferase (AST) and alanine aminotransferase (ALT) were determined by the method of Reitman and Frankel (Reitman and Frankel, 1957). Blood sugar, bilirubin, urea and creatinine were assessed by kit methods as per instructions provided by the company (E-Merck, India).

Tissue Biochemical Parameters

Lipidperoxidation (LPO) was determined by measuring thio-barbituric acid reactive substances (TBARS) (Sharma and Krishnamurthy, 1968), reduced glutathione (GSH) level was determined by dithionitrobenzoic acid (DTNB) (Brehe and Burch, 1976), activity of superoxide-dismutase (Misra and Fridovich, 1972) and catalase (Aebi, 1984) was determined in liver and kidney. Activities of adenosine triphosphatase (ATPase) (Seth and Tangari, 1966) and alkaline phosphatase (Fiske and Subbarow, 1925) were determined in liver and kidney. Activity of glucose-6-phosphatase (G-6-Pase) (Baginski *et al*., 1974) and glycogen (Seifter *et al*., 1950) were determined in liver.

Histopathology

Liver and kidney samples were fixed in Bouin's fixative and processed to obtain 5µm thick paraffin sections and stained with hematoxylin and eosine (H&E) for histological observations.

Statistical Analysis

Results are presented as mean± S.E. of six animals used in each group. Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) taking significant at 5% level of probability followed by Student's t-test taking significant at $P \leq 0.05$ (Snedecor and Cochran, 1994). Percent protection was calculated by the following formula.

% Protection = 1-(D-N/T-N) X 100 [D=Drug, N=Normal, T=Toxicant]

RESULTS

Blood Biochemical Parameters

The results of blood biochemical parameters are presented in Table 1. Administration of beryllium nitrate induced significant leakage of serum ALT and AST ($P \leq 0.05$) as compared to the control group. Oral administration of *Moringa oleifera* extract at different doses (50,100,150 and 200mg/kg) showed significant recoupment in dose dependent manner ($P \leq 0.05$). Significant decrease in hemoglobin in blood whereas increase in serum urea, creatinine and bilirubin was observed after beryllium intoxication. *Moringa oleifera* root extract significantly increased hemoglobin and blood sugar in blood whereas it decreased the elevated levels of urea, creatinine and bilirubin towards control. The 150 and 200mg/kg doses of *Moringa oleifera* extract revealed more significant therapeutic effectiveness ($P \leq 0.05$). Doses of *Moringa oleifera* at 50 and 100mg/kg showed less effective significant changes.

Tissue Biochemical Parameters

A significant increase in the level of TBARS in liver and kidney was observed after 35 days of intoxication when compared with the control group ($P \leq 0.05$). Treatment with different doses of *Moringa oleifera* reversed the level of TBARS significantly towards control by inhibiting lipid peroxidation (LPO) in dose dependant manner ($P \leq 0.05$). Reduced glutathione is presumed to be an important endogenous defense against peroxidative destruction of cellular membranes, significant decline was observed after beryllium administration ($P \leq 0.05$).

Table 1. Effect of *Moringa oleifera* root extract against beryllium induced blood biochemical

Groups	Hemoglobin (g/dl)	ALT (IU/L)	AST (IU/L)	Blood Sugar (mg/dl)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	14.8 ±0.94	45 ±3.15	72 ±4.64	103 ±6.15	0.2 ±0.01	34 ±2.92	0.5 ±0.03
M Per se	15 ±0.93	49 ±2.96	70 ±4.08	101 ±5.22	0.24 ±0.01	36 ±3.58	0.5 ±0.03
Toxicant [Be]	12 ± 0.71$^{\#}$	120 ±7.85$^{\#}$	116 ±7.28$^{\#}$	69 ±4.98$^{\#}$	0.51 ±0.03$^{\#}$	63 ±3.5$^{\#}$	0.8 ±0.05$^{\#}$
Be+ M (50mg/kg)	13.3 ±0.94	103 ±5.92	113 ±6.19	70 ±5.32	0.34 ±0.01*	61 ±3.8	0.7 ±0.05*
% Protection	46.5%	22.7%	6.9%	3.0%	55%	7%	33.3%
Be+ M (100mg/kg)	13.6 ±0.87	89 ±4.97*	90 ±5.32*	71 ±4.76	0.31 ±0.02*	44 ±3.18*	0.7 ±0.05*
% Protection	57.2%	41.4%	59.1%	5.9%	65%	65.6%	33.3%
Be+ M (150mg/kg)	14.0 ±0.73	65 ±3.65*	84 ±5.07*	73 ±4.27	0.30 ±0.01*	43 ±3.62*	0.5 ±0.03*
% Protection	71.5%	73.4%	71.0%	11.8%	68.0%	69.0%	100%
Be+ M (200mg/kg)	13.8 ±0.94	88 ±4.66*	82 ±4.86*	74 ±5.17	0.28 ±0.02*	51 ±3.35*	0.6 ±0.03*
% Protection	64.3%	42.7%	77.3%	14.8%	74.2%	42%	66.6%
F values (at 5% level)	1.14	25.73$^{@}$	9.84$^{@}$	6.50$^{@}$	16.98$^{@}$	10.79$^{@}$	6.21$^{@}$

Variables: Abbreviations: Be=Beryllium nitrate, M=*Moringa oleifera*, ALT=Alanine transaminase; AST= Aspartate transaminase; ANOVA$^{@}$=Significant; Values are mean ±S.E. N=6, $^{\#}$P≤0.05 *vs*. control group, *P≤ 0.05 *vs*. beryllium.

Table 2. Effect of *Moringa oleifera* root extract against beryllium induced oxidative stress

Groups	Lipid Peroxidation (n moles TBARS/mg Protein)		Reduced Glutathione (µmole/gram)		Superoxide dismutase (U/mg Protein)		Catalase (U/mg Protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	0.28 ±0.02	0.45 ±0.02	7.6 ±0.41	6.6 ±0.41	70 ±3.25	60 ±3.86	59 ±3.18	56 ±2.59
M Per se	0.30 ±0.02	0.49 ±0.02	7.2 ±0.58	6.4 ±0.36	68 ±4.10	62 ±3.96	60 ±4.31	58 ±3.31
Toxicant [Be]	0.61 ±0.04	0.90 ±0.05	4.2 ±0.26	4.9 ±0.27	34 ±2.04	35 ±2.92	41 ±2.47	36 ±3.42
Be+ M (50mg/kg)	0.60 ±0.04	0.80 ±0.05	5.5 ±0.28	5.7 ±0.47	36 ±1.93	37 ±2.06	43 ±2.68	38 ±3.94
% Protection	3.1%	22.3%	38.3%	47.1%	6.0%	8.0%	12.0%	10.0%
Be+ M (100mg/kg)	0.52 ±0.03	0.75 ±0.04	5.6 ±0.31	5.7 ±0.47	42 ±2.58	39 ±2.62	44 ±3.53	40 ±2.69
% Protection	27.3%	33.4%	41.2%	47.1%	23.0%	16.0%	16.7%	20.0%
Be+ M (150mg/kg)	0.40 ±0.02	0.60 ±0.03	6.4 ±0.43	6.2 ±0.49	52 ±2.73	45 ±2.43	48 ±3.13	47 ±2.69
% Protection	64.0%	66.6%	64.8%	76.5%	50.0%	40.0%	38.8%	55.0%
Be+ M (200mg/kg)	0.46 ±0.02	0.70 ±0.03	6.0 ±0.53	6.3 ±0.36	54 ±2.12	48 ±3.38	49 ±3.50	48 ±2.90
% Protection	45.5%	44.5%	53.0%	83.0%	56.0%	52.0%	44.5%	60.0%
F values (at 5% level)	11.9$^{@}$	12.8$^{@}$	8.4$^{@}$	2.0	29.4$^{@}$	9.8$^{@}$	4.3$^{@}$	5.9$^{@}$

Abbreviations: Be=Beryllium nitrate, M=*Moringa oleifera*, ANOVA$^{@}$=Significant; Values are mean ±S.E. N=6, $^{\#}$P≤0.05 *vs*. control group, *P≤ 0.05 *vs*. beryllium.

Table 3. Effect of *Moringa oleifera* root extract against beryllium induced tissue biochemical variables

Groups	ATPase (mg Pi/100g/min)		ALPase (mg Pi/100g/hr)		G-6-Pase (µmole Pi/min/g)	Glycogen (mg/100g)
	Liver	Kidney	Liver	Kidney	Liver	Liver
Control	2000 ±106	1557 ±81	54.0 ±3.82	2340 ±128	6.9 ±0.55	2570 ±145
M Per se	1990 ±115	1540 ±115	55.0 ±4.08	2370 ±139	6.5 ±0.40	2530 ±150
Toxicant [Be]	1170 ±62	1221 ±69	22.0 ±1.69	1150 ±73	4.55 ±0.30	1434 ±95
Be+ M (50mg/kg)	1357 ±80	1285 ±73	33.0 ±2.33	1178 ±93	4.7 ±0.25	1753 ±139
%Protection	22.6%	20.0%	34.4%	3.0%	7.0 %	28.1%
Be+ M (100mg/kg)	1440 ±75	1314 ±73	38.0 ±2.88	1727 ±120	5.9 ±0.32	1966 ±109
%Protection	32.6%	28.0%	50.0%	49.0%	57.5%	46.9%
Be+ M (150mg/kg)	1560 ±82	1485 ±81	41.0 ±3.04	1898 ±134	6.1 ±0.35	2107 ±115
%Protection	47.0%	78.6%	59.4%	62.9%	66.0%	59.3%
Be+ M (200mg/kg)	1565 ±95	1482 ±85	46.0 ±3.13	1930 ±101	6.3 ±0.48	2140 ±125
%Protection	47.6%	77.7%	75%	65.6%	74.5%	62.2%
F values (at 5%level)	10.8$^{@}$	2.9$^{@}$	15.5$^{@}$	17.6$^{@}$	5.5$^{@}$	9.7$^{@}$

Abbreviations: Be=Beryllium nitrate, M=*Moringa oleifera*, ATPase =Adenosine triphosphatase; ALPase = Alkaline Phosphatase; G-6-Pase = Glucose-6-phosphatase; ANOVA$^{@}$=Significant; Values are mean ±S.E. N=6, $^{\#}$P≤0.05 *vs*. control group, *P≤ 0.05 *vs*. beryllium.

Post treatment of *Moringa oleifera* was very effective in restoring the glutathione content (Table 2). All doses of *Moringa oleifera* improved the GSH level in liver and kidney; however, therapy at150mg/kg was very effective. Administration of beryllium nitrate decrease the activity the major antioxidant enzymes such as superoxide-dismutase and catalase (Table 2) significantly. Treatment with different doses of *Moringa oleifera* root extract restore the activity of these enzymatic variables however significant recovery was seen in higher doses which was confirmed by one way analysis of variance. In present study, significant decline in the activities of alkaline phosphatase, adenosine triphosphatase in liver and kidney, Glucose-6-phosphatase (G-6-Pase) and glycogen in liver (Table 3) in beryllium administered animals were observed, which were significantly reversed towards control with higher doses of *Moringa oleifera* extract ($P \leq 0.05$). Since higher doses (150 mg and 200 mg/kg b.w) of *Moringa oleifera* root extract showed significant result in recovery of various biochemical variables but there was no significant difference in recovery of these two higher doses. Thus therapy with *Moringa oleifera* root extract at 150 mg/kg is considered to be more effective in the treatment of beryllium induced hepatorenal toxicity.

Histopathology

Liver of control rat showed well formed chord arrangement of hepatocytes, having conspicuous prominent nucleus and normal appearance of central vein. In beryllium treated group, liver showed structural loss and disintegration of hepatocytes, massive hepatic necrosis, hyperchromatia of nuclei and vacuolation in hepatocytes. Treatment with 50 mg/kg dose of *Moringa oleifera* could not improve considerably as hepatocyte showed hypertrophy and cytoplasmic vacuolation. With treatment of 100 mg/kg of *Moringa oleifera* hypertrophy of hepatocytes were considerably reduced; however, some nuclei showed hypertrophy and hyperchromatia, more

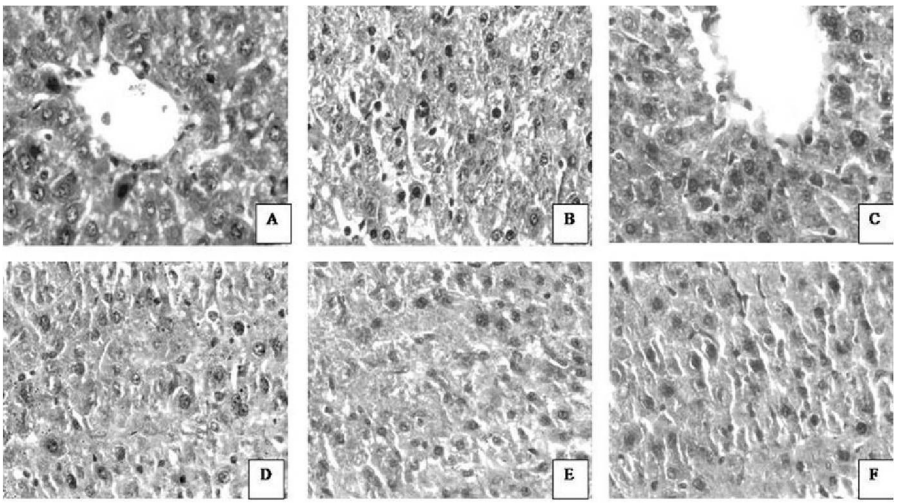

Figure 1. A: Liver section of control group showed well arrangement of hepatocytes with intact nuclei, absence of cytoplasmic vacuoles. B: Liver section of beryllium administration induced hepatic cell necrosis, hyperchromatia of nuclei, and disturbed chord arrangement of hepatocytes with more cytoplasmic vacuoles. C: Liver section of *Moringa* with 50mg dose could not improve as hepatocytes showed hypertrophy and cytoplasmic vacuolation. D: Liver section of *Moringa* with 100mg showed reduction in hyperchromatia of nuclei however some part showed hypertrophy. E: Liver section of *Moringa* with 150mg showed better chord arrangement, hexagonal appearance of hepatocytes with reduction in hyperchromatia of nuclei. F: Liver section of *Moringa* with 200mg showed maintained chord arrangement of hepatocytes with absence of cytoplasmic vacuolation. All slides were seen at 400x original magnification.

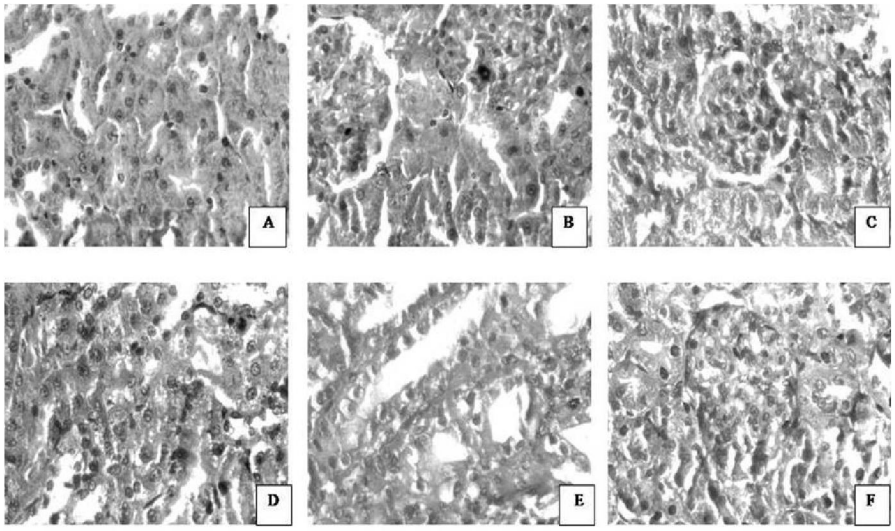

Figure 2. A: Kidney section of control rat showed well formed uriniferous tubules with both basal and apical nuclei along with normal lumen. B: Kidney section with beryllium treated group showed wide space between glomerulus and capsule wall due to constriction of glomerulus. Uriniferous tubules showed hypertrophy. Tubular obstruction was seen in most of the tubules with epithelial hypertrophy. C: Kidney section of *Moringa* 50 mg showed reduction in hypertrophy of the epithelial cells of uriniferous tubules, better structure of glomeruli, epithelial cells with hypertrophy. D: Kidney section of *Moringa* 100 mg showed wide lumen of uriniferous tubule with some hypertrophied epithelial cells. E: Kidney section of *Moringa* 150 mg showed normal structure of uriniferous tubule with wide lumen. F: Kidney section of *Moringa* 200 mg showed compact glomeruli with normal uriniferous tubule while some other parts showed hypertrophied epithelial cells. All slides were seen at 400x original magnification.

cananiculli was observed. In 150 mg/kg *Moringa oleifera* treatment, chord arrangement of hepatocytes was organized in some part of lobules; hepatocytes assumed hexagonal appearance, the cananiculli were distinct, hyperchromatia of the nucleus was also reduced considerably. With 200 mg/kg of *Moringa oleifera* treatment, chord arrangement of hepatocytes was maintained with the proliferation of cananiculli. There was no cytoplasmic vacuolation and rich glycogen distribution around the nuclei was observed (Figure 1A-F). Kidney of control group showed well formed Bowman's capsule with normal glomeruli and uriniferous tubules with both basal and apical nuclei along with normal lumen. Beryllium administration showed wide space between glomerulus and capsule wall due to constriction of glomeruli, uriniferous tubules with hypertrophy and apical nuclei. Treatment with of 50 mg/kg of *Moringa oleifera*, hypertrophy of the epithelial cells of uriniferous tubules was reduced to some extent; however, nuclei were apical and wide lumen. With 100 mg/kg *Moringa oleifera* treatment, the lumen was wide; however all the uriniferous tubule were not normal as some has still hypertrophied epithelial cells. With 150 mg/kg of *Moringa oleifera*, the uriniferous tubule showed more or less normal structure with normal lumen, glomeruli also showed normal structure. Treatment with 200 mg/kg of *Moringa oleifera* glomeruli compact the uriniferous tubules, near the glomeruli some hypertrophy with epical nuclei was observed while at other places the uriniferous tubule were normal (Figure 2 A-F).

DISCUSSION

Present study reports the antioxidant and antitoxic potential of *Moringa oleifera* root extract against beryllium induced alterations in various serum, biochemical parameters and histopathology in liver and kidney. A significant fall in hemoglobin was observed in present study, might be due to diminishment in the synthesis of heme and glob in proteins, which consequently decreased the hemoglobin content of the erythrocytes. Decrease in hemoglobin is due to suppression of the

activity of δ-amino levulinic acid synthatase (ALAS) and δ-amino levulinic acid dehydratase (ALAD) (Sakaguchi *et al*., 1997). It is assumed that *Moringa oleifera* root extract play an important role in heme synthesis by increasing the levels of ALAS and ALAD to maintain the level of hemoglobin in erythrocytes. This may be potent antioxidant action of phytochemicals present in *Moringa oleifera* root extract. *Moringa oleifera* is a panacea. It contains alkaloids, flavonoids, anthocyanins, proanthocyanidins and cinnamates and is highly reputed in folklore and traditional system of medicine as a remedy for variety of ailments. Roots of *Moringa oleifera* are rich in gallic tannins, catechol tennins, steroids and triterpenoids, flavanoids, saponins, anthraquinones, alkaloids and reducing sugars (Kasolo *et al*., 2011) and acts as antioxidants. Exposure to beryllium significantly decreased blood sugar level in blood which is due to disturbances in carbohydrate metabolism. Decrease in blood sugar may also due to decrease in the activity of enzymes involved in gluconeogenesis by which glucose is regularly supplied and maintained at normal level in blood. Hypoglycemia from a toxic dosage of beryllium salt has been reported due to inactivation of phosphoglucomutase, hexokinase and many other key enzymes involved in carbohydrate metabolism (Nirala *et al*., 2008). The major reason for hypoglycemia appears to be due to a decline in hepatic glycogen. The significant fall in hepatic glycogen was observed in this study may also be due to disturbances in carbohydrate metabolic pathway. Treatment with different doses of *Moringa oleifera* do not alter beryllium induced decrease in blood sugar level, this may be due to potent hypoglycemic effect of *Moringa oleifera* root extract, however; it maintained the glycogen level in dose dependent manner.

The decrease in blood sugar due to beryllium toxicity provides the signal for glycogenolysis thus the level of hepatic glycogen decreases. Glucose-6-phosphate formed as end product of glycogen breakdown, cannot pass the membrane of hepatocytes until it is converted to glucose by the action of Glucose-6-phosphatase (Agrawal *et al*., 2013). G-6-Pase is a SER membrane enzyme involved in glycogenolysis. Beryllium administration decreased enzymatic activity; it may be due to binding of beryllium ions to its phosphate group. The degradation of SER membrane, which is evident in ultra structural study of the liver and kidney of beryllium treated animals (Nirala *et al*., 2007), also depicts the reduction of related enzymes. Treatment with hydro-alcoholic extract of *Moringa oleifera* helps in maintaining the integrity of cellular membrane, restored glucose-6-phosphatase activity in dose dependent manner. However, *Moringa oleifera* at 150 mg/kg maintain the glycogen level towards normal more significantly by storing the activity of G-6-Pase and other enzymes involved in carbohydrate metabolism. Beryllium damage to liver cells raises the leakage of ALT and AST in blood circulation. Our findings with beryllium nitrate elevated these enzymes significantly ($P < 0.05$) indicating severe hepatic cell necrosis. Treatment with *Moringa oleifera* may provide antioxidant which combines with toxic beryllium ions or reactive metabolites to inactivate them.

Thus therapy with *Moringa oleifera* prevented cellular injury and organ dysfunction prominently and subsequently inhibited rapid leakage of these enzymes into blood circulation. Hyperbilirubinaemia which indicates the severity of necrosis (Singh *et al*., 2005) raised significantly ($P < 0.05$) with beryllium intoxication. Curative treatment with *Moringa oleifera* restore all the enzymes studied and bilirubin in a dose dependent manner showing its potential to maintain the normal functional status of the liver. Beryllium nitrate disturbed normal kidney function by alteration of normal histoarchitecture of kidney and decreased the glomerular filteration rate, thus level of urea and creatinine increased in blood. Treatment with *Moringa oleifera* root extract significantly decreased beryllium induced increase in urea and creatinine by increasing glomerular filteration rate and normal kidney function as well as improvement in histoarchitecture of kidney. Metal toxicity is attributed to generate reactive oxygen species (ROS), which causes peroxidation of membrane lipids (Hermes-Lima and Zenteno-Savin, 2002) and induces a plethora of alterations in structure and function of cellular membranes. Increased TBARS after beryllium administration indicated enhanced LPO due to failure of the antioxidant defense mechanism. Glutathione (GSH) is an endogenous antioxidant, attains milimolar levels inside cells, which make it one of the most highly concentrated intracellular antioxidants. Intracellular GSH status appears to be sensitive indicator of cell's overall health, and its ability to resist toxic challenge. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Slater *et al*., 1995; Duke *et al*., 1996). Be induced oxidative stress though its ability to deplete endogenous thiol antioxidants and increase ROS levels (Dobis *et al*., 2008). In present study beryllium nitrate depletes the cellular Glutathione and induced the oxidative stress. *Moringa oleifera* rich in antioxidants which nutrilise the beryllium generated free radicals and protect the endogenous antioxidants ultimately inhibiting the lipid peroxidation of biological membranes. Thus, therapy with *Moringa oleifera* root extract prevents lipid peroxidation and restoring GSH level in dose dependent manner.

Superoxide-dismutase (SOD) and catalase (CAT) are major antioxidant enzymes, play an important role as protective agents against free radical formation in tissues. SOD converts superoxide (O^{2-}) to hydrogen peroxide (H_2O_2) and is a major defense system for aerobic cells in combating the toxic effects of superoxide radical (Mishra *et al*., 2009). H_2O_2 formed by SOD that is eventually scavenged by peroxisomal catalase or glutathione peroxidase (Ilhan *et al*., 2004), hence diminishing the toxic effects caused by these radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes (Sozmen *et al*., 2001). The superoxide anion has been known to inactivate catalase, which are involved in detoxification of hydrogen peroxide. Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase. These antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular organization and enzymatic action. Beryllium may bind at the enzymatic active site as well as induced oxidative stress by excess production of reactive oxygen species and inhibited the enzymatic action. *Moringa oleifera* at different doses showed the alteration of activities of SOD and catalase may be due to antioxidant potential of root extract of *Moringa oleifera*. Since 150 mg/kg showed better protection than other doses; thus *Moringa oleifera* root extract at 150mg/kg was found to be most effective in attenuation of beryllium induced oxidative stress.

The damage of the cellular membrane due to lipid peroxidation also leads to decrease in the activity of membrane bound enzymes. Cellular membrane damage might be responsible for ionic imbalance, mitochondrial damage and stimulation of lysosomes (Reeves *et al*., 1986) and liberation of hydrolytic enzymes, which consequently causes injury to surrounding tissue. The ALPase and ATPase are membrane bound enzymes and any alteration in membrane lipid leads to change in membrane fluidity, which in turn alters cellular functions mediated by these enzymes. Remarkable depletion in the activities of these enzymes was found after beryllium toxicity. Beryllium-phosphate complex could be formed through attachment of beryllium ions to the enzymes via phosphate group or more likely because of displacement of Mg^{+2} by Be^{+2}.The Be^{+2} is a potential target for ATP binding and that Be^{+2} out competes Mg^{+2} for ATP and ADP binding. This suggests a possible mechanism for in-vivo beryllium toxicity through interference with enzyme activity involving ATP **(**Boukhalfa *et al*., 2004**).** *Moringa oleifera* root extract is source of gallic tannins, acts as antioxidants and compete with beryllium as well as beryllium induced ROS thus preventing membrane damage, ultimately maintaining the activities of these two enzymes. Different doses of *Moringa oleifera* root extract maintained the activity of ALPase and ATPase in liver and kidney in dose dependent manner however maximum recovery were found at higher doses of root extract of *Moringa oleifera*. Histopathological studies under light microscope confirm therapeutic efficacy of *Moringa oleifera* root extract against beryllium induced liver damage as evident by the reversal of chord arrangement of hepatocytes, decrease in hypertrophy and well formed cananiculi filled with fluid. Well formed glomeruli, decrease in

hypertrophy as well as wide lumen of uriniferous tubule in kidney were observed in treatment with *Moringa oleifera* in dose dependent manner. More recovery was found to be observed at doses of 150mg/kg and 200 mg/kg of *Moringa oleifera*. Since there is no more difference in both of these doses thus 150 mg/kg dose can be considered better in the attenuation of beryllium induced toxicity and can be suggestive for the treatment of beryllium induced systemic toxicity.

Conclusion

The possible mechanism of hepatic and renal protective action of aqueous doses of hydro-alcoholic *Moringa oleifera* root extract may be due to its antioxidant activity as indicated by protection against beryllium increased lipid peroxidation and depletion of reduced glutathione together with increase in the antioxidant potential by maintaining the normal status of antioxidant enzymes. Rest of the biochemical and pharmacological parameters studied indicate the status of structural and functional integrity of the cells and provide further support to the suggestive mechanism of action. Since *Moringa oleifera* does not reveal any gross behavioral changes or mortality even at doses of 200 mg/kg *p. o.* in rats and therefore it can be considered relatively safe. Further studies are in progress to identify active principle(s) responsible for protective action against metal induced toxicity and to find out synergy among different compounds present in root extract of *Moringa oleifera*.

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