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Protective potential of *Moringa oleifera* Lam. along with curcumin and piperine against beryllium-induced alterations in hepatorenal biochemistry and ultramorphology in rats

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Moringa oleifera Lam. (Moringaceae) is a medicinally important plant, used as traditional medicine all over the world particularly in South Asia and India. Hydroalcoholic (50% v/v) root extract of *M. oleifera* (150 mg/kg, p.o.) with piperine (2.5 mg/kg, p.o), or curcumin (5.0 mg/kg, p.o.) was administered daily for 1 week in Female Wistar albino rats against beryllium toxicity (1.0 mg/kg, *i.p.* daily for 5 weeks). Beryllium altered hepatorenal function and enhanced the leakage of AST, ALT, and LDH, depleted SALP activity, and increased the level of urea, uric acid, creatinine, triglyceride and total cholesterol in the blood. Beryllium altered tissue biochemical parameters by a decrease in SDH, ALPase, ATPase activities, and increased ACPase activity, depleted hemoglobin and ALAD activity with an increase in ALAS activity and serum bilirubin. A significant amount of beryllium deposited in the liver, kidney, spleen, and bones. *M. oleifera* with curcumin showed better antitoxic potential by reversal of hepatorenal function towards normal and restored the activity of SDH, ALPase, ATPase, ACPase, and hemoglobin level normal. *M. oleifera* with curcumin effectively mobilized beryllium from the body and restored ultrastructure of liver and kidney. It was concluded that curcumin enhances the antitoxic potential of *M. oleifera* root extract and reduces beryllium body burden in rats.

Keywords: Beryllium body burden, Biochemical parameters, Electron microscopy, Heme biosynthesis

Beryllium (Be) is a lightweight metal with unique physical and chemical properties *viz*., corrosion resistance, stiffness, high melting, and boiling points, enhancing metal hardening capacity, and high electrical and thermal conductivity[1]. It has gained importance for use in various commercial and defense applications, including aircraft and satellite structures, nuclear applications, precision instruments, high-speed electronic circuits, medical devices, and dental applications[2].

Beryllium is quite toxic to all the life forms, including human and animals. The general population is exposed to naturally occurring beryllium *via* ambient air, drinking water, diet, and tobacco smoking on a daily basis. Beryllium has been observed in drinking water and means (μg/kg) beryllium content was found to be <0.6 in Spain, <0.1 in USA, 0.008 in Germany and 1.24 in Saudi Arabia[3]. Beryllium exposure results in chronic beryllium disease (CBD) and cancer as well as hepatorenal damage[4-6]. Beryllium interferes with various metabolic pathways and alters the normal physiological process[6,7]. About 18% of people who are exposed to beryllium at the workplace may develop CBD depending on a number of risk factors such as genetic susceptibility, duration, the concentration of beryllium exposure, and their smoking habits[8]. Lower levels of exposure pose a risk of beryllium sensitization (BeS) in individuals who are directly or indirectly exposed to beryllium[9]. In 2004, NIOSH estimated that up to 134000 workers in the United States were exposed to beryllium[10]. Current occupational health standards for Be do not provide adequate protection against the development of CBD or sensitization[2]. In a study of Israel, out of 83 dental technicians, 9 were newly identified as having beryllium sensitized (BeS) individuals and were 6 known as having CBD[2]. In an effort to reduce

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incidence of CBD and BeS, the Occupational Safety and Health Administration (OSHA) in 2017 establishes a new permissible exposure limits of 0.2 micrograms of beryllium per cubic meter of air (0.2 µg/m^3) as an 8 h time-weighted average and 2.0 (µg/m^3) as a short-term exposure limit[11].

The exact mechanism of beryllium toxicity is not well defined, however, increased oxidative stress, impaired antioxidant defense systems and consequently, lipid peroxidation is major participants in the development and progression of beryllium-related diseases[6,12]. Thus, inhibition of beryllium-induced oxidative stress and clinical manifestations may be an approach in the prevention of beryllium-related diseases. There is growing interest in the potential use of medicinal plants as an alternative treatment for metal toxicity as plants/ herbs are well studied against a variety of ailments and with fewer side effects.

Moringa oleifera Lam. (Moringaceae) is commonly known as Sahjan, a highly nutrient-rich plant with exceptional medicinal properties, widely used to treat various health problems[13]. All its parts are a rich source of minerals, amino acids, antioxidants, anti-aging, and anti-inflammatory compounds and is employed as medication for a variety of ailments particularly in South Asia and India[14,15]. Its roots possess medicinal properties like analgesic and anti-arthritic[16], antiulcer[17], antioxidant[6], antimicrobial[18] and employed as a cardiac tonic, antiepileptic, used against nervous debility, asthma, enlarged liver and spleen, deep-seated inflammation and diuretic in calculus affection[19].

Piperine is an active principle of *Piper longum* Linn. and *Piper nigrum* Linn. (Piperaceae), known for its several biological activities, including antioxidant and hepatoprotective[20], anti-inflammatory[21], anticonvulsant[22] and bioavailability enhancing activity for some drugs[23]. Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa* Linn. (Zingiberaceae) has a wide spectrum of biological and pharmacological activities including antioxidant[24], anti-inflammatory[25], antitoxic[26], hepatoprotective and anti-carcinogenic[27]. The Present study investigated the antitoxic potential of *M. oleifera* root extract along with piperine and curcumin against beryllium-induced hepatorenal dysfunction in rats.

Materials and Methods

Chemicals

Beryllium nitrate, piperine, and curcumin were purchased from Sigma-Aldrich Company (St. Louis, MO). All the therapeutic agents were stored and refrigerated in desiccators to avoid oxidation and thermal decomposition. All the chemicals used in this study were of pure and analytical grade and procured from standard chemical dealers.

Maintenance of animals

Female *Rattus novergicus* strain Wistar (10-12 weeks old having a body weight of 160 ± 10 g) were randomly selected from departmental animal facility. Animals were housed under standard husbandry conditions (25 ± 2°C temp., 60-70% relative humidity and 14 h light and 10 h dark). Animals were fed on a standard pelleted animal 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), India, and experimental protocols were approved by Institutional Animal Ethics Committee (CPCSEA/501/01/A) of Jiwaji University, Gwalior, India.

Identification of plant material and preparation of the extract

Identification, a collection of roots of *M. oleifera* and preparation of extract was done according to Agrawal *et al*., 2015[6].

Preparation of doses and treatments

Beryllium nitrate (35% w/v; Sigma-Aldrich, St. Louis, MO) was diluted in triple distilled water making up doses of 1 mg/2 mL/kg and administered intraperitoneally[6]. The doses of *M. oleifera* root extract (150 mg/kg/5 mL) were prepared in 1% gum acacia and the doses of piperine (2.5 mg/kg/3 mL) and curcumin (5 mg/kg/3 mL) were prepared in olive oil and administered orally with the help of intragastric rubber catheter. Forty-two adult female rats were divided into seven groups of six animals in each as follows:

Group 1: Normal control

Group 2: $Be(NO_3)_2$ (1.0 mg/kg i.p. daily) for 5 weeks

Group 3: $Be(NO_3)_2$ (as in group 2) + *M. oleifera* (150 mg/kg, p.o.) after beryllium toxicity daily for 1 week

Group 4: $Be(NO_3)_2$ (as in group 2) + piperine (2.5 mg/kg, p.o.) after beryllium toxicity daily for 1 week

Group 5: $Be(NO_3)_2$ (as in group 2) + curcumin (5.0 mg/kg, p.o.) after beryllium toxicity daily for 1 week

Group 6: $Be(NO_3)_2$ (as in group 2) + *M. oleifera* (150 mg/kg, p.o.) + piperine (2.5 mg/kg p.o.) after beryllium toxicity daily for 1 week

Group 7: $Be(NO_3)_2$ (as in group 2) + *M. oleifera* (150 mg/kg, p.o.) + curcumin (5 mg/kg p.o.) after beryllium toxicity daily for 1 week

24 h after final administration, animals were sacrificed under mild ether anesthesia and blood was drawn by puncturing retro-orbital venous sinus to isolate serum. The liver and kidney were immediately excised, blotted free of adhering fluid and processed for biochemical studies. Standard techniques were applied to assay various biochemical parameters.

Biochemical study

Serum transaminases (AST and ALT) were determined according to the procedure of Reitman and Frankel[28]. Serum LDH was measured according to the method of Wroblewski and La Due[29]. Hemoglobin by Swarup *et al*.,[30] and ALAD by Berlin and Schaller[31] were determined in blood, and ALAS was estimated in the liver by Maines[32]. Succinate dehydrogenase (SDH) by Slater and Bonner[33], alkaline phosphatase (ALPase) and acid phosphatase (ACPase) by Fiske and Subbarow[34], adenosine triphosphatase (ATPase) by Seth and Tangari[35] were determined in liver and kidney. SALP, urea, uric acid, creatinine, bilirubin, triglyceride, and total cholesterol were determined by the kit method as per instructions provided by the company (E-Merck, Mumbai, India).

Beryllium estimation

Blood, small pieces of liver, kidney, brain, spleen, bone, and hair were taken, volume of blood and wet tissues weight were recorded. Samples were prepared following wet digestion method using a solution of nitric acid and perchloric acid in the ratio of 4:1. After complete digestion, dry ash was dissolved in a fixed volume of triple distill water. Beryllium concentration was determined with atomic absorption spectrophotometer (VP 90, Thermo Electron Corporation, UK) at wavelength 235 nm[36].

Ultra-morphological examinations

Small pieces (1 mm^3) of liver and kidney were fixed in 3.2% glutaraldehyde (prepared in 0.1 M phosphate buffer) at 4°C for 18 h. This was followed by washing the tissue with phosphate buffer. Post-fixation of the tissue was done with 1% osmium tetraoxide solution. Tissues were dehydrated in acetone series and subsequently embedded in epon resin and were polymerized for 20 h at 70°C. Ultra-thin sections were cut on a Reichert Jung Ultra cut-E Microtome, using glass knives. The sections were placed on uncoated grids and stained with uranyl acetate and lead citrate[37]. They were examined in a JEOL JEM 1200 EX transmission electron microscope at 80 KV[38].

Statistical analysis

Results are presented as mean ± SE 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 $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$[39]. The extent of recovery was calculated as percent protection[6].

Results

Effect of *M. oleifera* with piperine and curcumin on serum biochemical parameters

Tables 1 & 2 show the effect of *M. oleifera* with piperine or curcumin against beryllium- induced serum biochemical parameters. Administration of beryllium nitrate caused significant ($P \leq 0.001^{***}$) increase in activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea, uric acid, bilirubin, creatinine, triglyceride, cholesterol and significantly ($P \leq 0.001^{***}$) decreased the activity of serum alkaline phosphatase. Therapy with *M. oleifera* alone and in combination with piperine or curcumin showed recovery in altered serum biochemical variables whereas combination therapy of *M. oleifera* with curcumin showed more significant recoupment ($P \leq 0.05^{*}$; $P \leq 0.01^{**}$; $P \leq 0.001^{***}$) in these variables towards normal which was confirmed by one-way analysis using ANOVA. The extent of protection by therapeutic agents is expressed in percentage.

Effect of *M. oleifera* with piperine and curcumin on heme biosynthesis

Table 2 explains the effect of *M. oleifera* with piperine or curcumin on beryllium-induced disturbance in heme biosynthesis. Beryllium significantly depleted hemoglobin ($P \leq 0.05^{*}$) and significantly altered enzymatic activities of the heme biosynthetic pathway *i.e*. δ-aminolevulinic acid dehydratase (ALAD), δ-aminolevulinic acid synthetase (ALAS). The activity of ALAD was significantly ($P \leq 0.001^{***}$) decreased in blood,

Table 1 — Effect of *M. oleifera* with piperine and curcumin against beryllium induced alterations in serum biochemical parameters

Groups	ALT (IU/L)	AST (IU/L)	SALP (mg Pi/h/100mL)	LDH (pyruvate/min/mg)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	47 ±2.44	70 ±4.65	190 ±11.5	43.5 ±2.49	38 ±2.29	1.0±0.08	0.3 ±0.02
Be *perse*	113 ±6.87***	120 ±6.22***	110 ±6.95***	140±8.16***	68 ±4.20***	2.8±0.21***	0.6 ±0.05***
Be +MO	89 ±5.22*	102 ±5.84*	132 ±8.05	90.4±4.89 ***	55 ±2.88*	2.0±0.16*	0.5 ±0.02
% Protection	36.3%	36.0%	27.5%	51.3%	43.3%	44.4%	33.3%
Be +Pip	96±5.58	102±6.13	130 ±8.16	80±5.32 ***	54±2.84*	2.1±0.13*	0.5±0.02
% Protection	25.7%	36.0%	25.0%	62.1%	46.6%	38.8%	33.3%
Be+ Cur	95±5.77	100±5.77	127 ±7.81	78±4.39 ***	50±3.20**	1.9±0.12**	0.5±0.02
% Protection	27.2%	40.0%	21.2%	64.2%	60.0%	50.0%	33.3%
Be +MO +Pip	72 ±4.07***	90 ±5.77**	158 ±8.66**	60.8±5.33***	48 ±2.43**	1.6±0.10***	0.45 ±0.02*
% Protection	62.1%	60.0%	60.0%	82.1%	66.6%	66.6%	50.0%
Be+ MO +Cur	67 ±3.95***	83 ±4.59***	161 ±10.64**	55.5±3.67***	40 ±2.88***	1.3±0.06***	0.4 ±0.02**
% Protection	69.6%	74.0%	63.7%	87.5%	93.3%	83.3%	66.6%
ANOVA (F Values)	19.7$^{@}$	8.8$^{@}$	9.09$^{@}$	35.2$^{@}$	11.1$^{@}$	18.4$^{@}$	8.1$^{@}$

[Values are mean ±SEN=6, P < 0.05*; P <0.01**; P <0.001****vs*. control group., P < 0.05*; P <0.01**; P <0.001*** *vs*. Be administered group. ANOVA@ =Significant. Be=Beryllium nitrate, MO=*M. oleifera*, Pip=Piperine, Cur = Curcumin. ALT=Alanine transaminase, AST=Aspartate transaminase, SALP= Serum alkaline phosphatase, LDH= Lactate dehydrogenase]

Table 2 — Effect of *M. oleifera* with piperine and curcumin against beryllium induced alterations in heme biosynthesis and serum lipid profile

Groups	Hemoglobin (g/dL)	ALAD (nM/min/l RBCs)	ALAS (nM/min/g liver)	Bilirubin (mg/dL)	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)
Control	15.6 ±0.84	9.6 ±0.62	7.2 ±0.36	0.37 ±0.02	31 ±2.88	46 ±3.06
Be *perse*	12.8 ±0.79*	4.8 ±0.40***	10.9 ±0.81**	0.77 ±0.04***	69 ±5.33***	75 ±3.95***
Be+ MO	14.9 ±0.93	6.0 ±0.46	9.1±0.73	0.50 ±0.02***	55 ±2.88*	69 ±4.60
% Protection	75.0%	25.0%	48.6%	67.5%	36.8%	20.6%
Be +Pip	13.9 ±0.75	7.0 ±0.57*	8.7 ±0.50 *	0.7 ±0.04	58±3.90	70±4.87
% Protection	39.2%	45.8%	59.4%	17.5%	28.9%	17.2%
Be+ Cur	14.3 ±0.83	7.2 ±0.52**	8.8 ±0.54	0.72 ±0.04	57±3.78	68±3.55
% Protection	53.5%	50.0%	56.7%	12.5%	31.5%	24.1%
Be+ MO +Pip	15.0 ±1.15	7.5 ±0.50**	7.6 ±0.41**	0.47 ±0.02***	50 ±2.88*	63 ±3.31*
% Protection	78.5%	56.2%	89.1%	75.0%	50.0%	41.3%
Be+ MO +Cur	15.4 ±1.02	8.2 ±0.52***	7.4 ±0.41**	0.40 ±0.02***	45 ±2.88**	55 ±2.88**
% Protection	92.8%	70.8%	94.5%	92.5%	63.1%	68.9%
ANOVA (F Values)	1.11	8.6$^{@}$	5.1$^{@}$	21.6$^{@}$	10.8$^{@}$	6.4$^{@}$

[Values are mean ± SEN=6, P < 0.05*; P <0.01**; P <0.001****vs*. control group., P < 0.05*; P <0.01**; P<0.001*** *vs*. Be administered group. ANOVA$^{@}$ =Significant. Be=Beryllium nitrate, MO=*M. oleifera*, Pip=Piperine, Cur = Curcumin. ALAD=δ-Aminolevulinic acid dehydratase, ALAS= δ-Aminilevulinic acid synthetase]

whereas activity of ALAS was significantly (P ≤0.01**) increased in the liver. Therapy with *M. oleifera* did not show significant recovery in activities of ALAD and ALAS, whereas combination of *M. oleifera* with piperine or curcumin showed significant effect in renewal of hemoglobin and activities of ALAD and ALAS towards normal, however *M. oleifera* with curcumin was found to be better in alteration of these two enzymes towards normal and restored hemoglobin in blood, which was confirmed statistically.

Effect of *M. oleifera* with piperine and curcumin on tissue biochemistry

Beryllium intoxication significantly increased (P ≤0.05*) the activity of acid phosphatase and decreased the activities of SDH, alkaline phosphatase and adenosine triphosphatase significantly (P ≤0.001***)

in liver and kidney (Table 3). Therapy with *M. oleifera* significantly restored the activity of SDH in liver and kidney and alkaline phosphatase in liver only. Combination therapy of *M. oleifera* with piperine or curcumin significantly restored the activities of SDH, alkaline phosphatase, acid phosphatase and adenosine triphosphatase in liver and kidney towards normal, however, combination therapy of *M. oleifera* with curcumin showed better effect in restoration of activities of SDH, alkaline phosphatase, acid phosphatase and adenosine triphosphatase towards normal which was confirmed by one way analysis of variance.

Effect of *M. oleifera* with piperine and curcumin on beryllium mobilization

Beryllium concentration was determined in various organs of rats (liver, kidney, spleen, brain, bone, hair, and blood) by atomic absorption spectroscopy (Table 4). It was observed that liver and spleen were most affected organs, occupied maximum amount of beryllium whereas bone and kidney posses lesser amount after liver and spleen, however, brain, hair and blood occupied the very little amount of beryllium ($P \leq 0.05^{*}$). Therapy with *M. oleifera* reduced beryllium body burden but a combination of *M. oleifera* with piperine or curcumin showed a significant effect in the reduction of beryllium body burden. Combination therapy of *M. oleifera* with curcumin showed a more pronounced effect in reduction of beryllium body burden from all the organs and blood which was confirmed by one-way analysis of variance using ANOVA.

Effect of *M.oleifera* with piperine and curcumin on beryllium-induced alteration in ultamorphology of the liver and kidney

Electron micrograph of liver of control rat showed well-formed nucleus, smooth surface, and homogenous chromatin. Nucleoli were clear and large structure. Mitochondria were large and elongated. The smooth endoplasmic reticulum distributed between the nuclear wall and mitochondria while the rough endoplasmic reticulum was distributed from nuclear wall to the cell membrane. Glycogen granules were distributed in the cytoplasm more towards the nucleus (Fig. 1A). In beryllium treated rats, the cytoplasm showed the presence of vacuoles. Glycogen granules were sparsely seen in the cytoplasm. Nuclear pores were not as abundant as in the control. Some unspecific structures were also seen with large patches. Elongated Kupffer cells were also observed on the lining of the cananiculi (Fig. 1B). Combination therapy of *M. oleifera* with piperine showed the nucleus to be round with uniformly distributed chromatin. Mitochondria were many but showed deformation. Both smooth and rough endoplasmic reticulum was dilated. Large packages of old zymogen granules were observed and identified by

Table 3 — Effect of *M. oleifera* with piperine and curcumin against beryllium induced alterations in tissue biochemical parameters

Groups	Succinate Dehydrogenase ($K_3Fe(CN)_6$ reduced/min/mg protein)		Adenosine triphosphatase (mg Pi/min/100 g)		Alkaline Phosphatase (mg Pi/h/100 g)		Acid Phosphatase (mg Pi/h/100 g)	
	Liver	Kidney	Liver	Liver	Liver	Kidney	Liver	Kidney
Control	48.0±3.30	40±2.88	1985 ±121	190 ±11.5	70 ±4.65	2061 ±115	190 ±11.5	198 ±11.6
Be *perse*	22.5±2.06***	21±1.86***	1135 ±93***	250 ±12.8**	28 ±2.14***	1315 ±93***	250 ±12.8**	260 ±13.3**
Be+ MO	32±2.82*	30±2.62*	1300 ±67	226 ±12.8	49 ±3.19***	1534 ±93	226 ±12.8	222 ±14.9
% Protection	37.2%	47.3%	19.4%	40.0%	50.0%	29.3%	40.0%	61.2%
Be +Pip	29±2.39	28±2.58	1340 ±73	230 ±12.8	34 ±2.68	1450 ±81	230 ±12.8	235 ±12.2
% Protection	25.4%	36.8%	24.1%	33.3%	14.2%	18.0%	33.3%	40.3%
Be + Cur	31.5±3.19*	31±2.85*	1449 ±90*	228 ±14.2	38 ±2.68*	1495 ±115	228 ±14.2	230 ±11.9
% Protection	35.2%	52.6%	36.9%	36.6%	23.8%	24.1%	36.6%	48.3%
Be+ MO+Pip	40.4±3.12***	34.3±2.57**	1471 ±93*	205 ±11.0*	54±4.16***	1625±81*	205 ±11.0*	213 ±11.3*
% Protection	70.1%	70.0%	39.5%	75.0%	61.9%	41.5%	75.0%	75.8%
Be+ MO+ ur	42±2.92***	36±2.78**	1607 ±93**	200 ±10.0*	64 ±4.35***	1711 ±93*	200 ±10.0*	208 ±12.7*
% Protection	76.4%	78.9%	55.5%	83.3%	85.7%	53.0%	83.3%	83.8%
F Values (at 5% level)	9.3$^{@}$	5.4$^{@}$	8.7$^{@}$	2.8$^{@}$	19.7$^{@}$	6.0$^{@}$	2.8$^{@}$	2.6$^{@}$

[Values are mean ±SE N=6, $P \leq 0.05^{*}$; $P \leq 0.01^{**}$; $P \leq 0.001^{***}$ *vs.* control group., $P \leq 0.05^{*}$; $P \leq 0.01^{**}$; $P \leq 0.001^{***}$ *vs.* Be administered group. ANOVA$^{@}$ =Significant. Be=Beryllium nitrate, MO=*M. oleifera*, Pip=Piperine, Cur = Curcumin]

Table 4 — Distribution of beryllium in vital organs of rat and its mobilization by *M. oleifera* with piperine and curcumin

Group	Liver (µg/100 mg)	Kidney (µg/100 mg)	Brain (µg/100 mg)	Spleen (µg/100 mg)	Bone (µg/100 mg)	Hair (µg/100 mg)	Blood (µg/100 mL)
Control	0.05 ± 0.00	0.03 ± 0.002	0.02 ± 0.001	0.01 ±0.001	0.02 ±0.011	0.02 ±0.001	0.05 ± 0.004
Be *perse*	32.0 ±3.14***	4.2 ±0.21***	0.52 ±0.028***	21.5 ±1.53***	6.0 ±0.57***	0.09 ±0.005***	0.45 ±0.02***
Be+ MO	22.5 ±2.03*	2.8 ±0.20***	0.26 ±0.022***	13.3 ±0.68***	4.5 ±0.30*	0.06 ±0.003**	0.27 ±0.02***
% Reduction	29.6%	33.3%	52.0%	38.4%	23.7%	34.2%	40.0%
Be +Pip	24.8 ±2.18	3.0 ±0.25**	0.31 ±0.021***	15.7 ±0.87**	4.6 ±0.27	0.07 ±0.005*	0.32 ±0.027**
% Reduction	22.5%	28.5%	42.0%	27.1%	23.4%	25.7%	28.8%
Be + Cur	24.2 ±2.28	2.9 ±0.28**	0.28 ±0.018***	14.8 ±0.94**	4.3 ±0.26*	0.07 ±0.005*	0.3 ±0.025**
% Reduction	24.3%	30.9%	48.0%	31.3%	28.4%	28.5%	33.3%
Be+ MO+ Pip	20.6 ±1.81*	2.3 ±0.20***	0.22 ±0.02***	10.8 ±0.61***	3.9 ±0.27**	0.05 ±0.003***	0.23 ±0.021***
% Reduction	35.6%	45.2%	60.0%	50.1%	35.1%	45.7%	48.8%
Be+ MO+ Cur	20.3 ±1.69**	1.8 ±0.11***	0.19 ±0.01***	10.3 ±0.51***	3.3 ±0.28**	0.04 ±0.002***	0.25 ±0.017***
% Reduction	36.5%	57.1%	66.0%	52.4%	45.1%	60.0%	51.1%
ANOVA (F Values)	23.2$^{@}$	41.6$^{@}$	58.6$^{@}$	58.6$^{@}$	34.1$^{@}$	3.0$^{@}$	36.9$^{@}$

[Values are mean ±S.E. N=6, $P < 0.05^{*}$; $P < 0.01^{**}$; $P < 0.001^{***}$ *vs.* control group, $P < 0.05^{*}$; $P < 0.01^{**}$; $P < 0.001^{***}$ *vs.* Be administered group. ANOVA$^{@}$ =Significant. Be=Beryllium nitrate, MO=*M. oleifera*, Pip=Piperine, Cur = Curcumin]

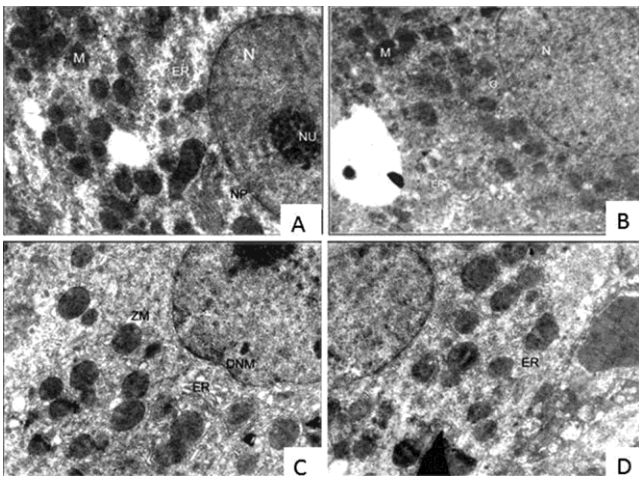

Fig. 1 — Electronmicrograph of liver A – Control liver showing well formed nucleus (N) with smooth surface and homogenous chromatin and clear nuclei (Nu), large and elongated mitochondria (M), glycogen granules are distributed in the cytoplasm more towards the nucleus (2200 ×). B – Beryllium treated liver showing vacuolation in the cytoplasm and decrease in number of nuclear pores as compared to control liver (2200 X). C – Therapy with MO+ Pip showing deformed nuclear membrane (DNM). D– Therapy with MO+ Cur showing well formed nuclear membrane with uniformly distributed chromatin, better formed mitochondria and moderate size of packages of zymogen granules (2200 ×)

characteristic electron dense appearance. Newly packed zymogen granules were also observed (Fig. 1C). Combination therapy of *M. oleifera* with curcumin showed better-formed mitochondria with dilated endoplasmic reticulum. Glycogen granules were frequently observed. The number of old and new zymogen granules were more as compared to the beryllium perse treatment (Fig. 1D).

Kidney of control rats showed podocyte gave out pedicles on the base of the basement membrane of the capillary wall. At the base of the basement membrane, the endothelium showed more or less flattened cells. This whole structure formed filter membrane. In endothelium as well as between pedicles some pores (fenestrations) were observed which were closed by a thin diaphragm. In nuclei in uriniferous tubules was found as oval vesicular structures with many nuclear pores, chromatin distributed in nucleoplasm in the form of large patches and closely associated with the nuclear membrane. Mitochondria were many, mostly elongated. Endoplasmic reticulum was distributed between the mitochondria extending from nuclear membrane to cell membrane. The rough endoplasmic reticulum was more predominant than the smooth endoplasmic reticulum (Fig. 2A & 2B). In beryllium treated rats, endothelium of filter membrane was found to be disrupted and fenestrations between the pedicles were more as compared to kidney of control rats. Uriniferous tubules showed deformed nuclei as nuclei had lost their smooth nuclear membrane and attained irregular appearance. The chromatin also showed heavy deposition on the inner side of the

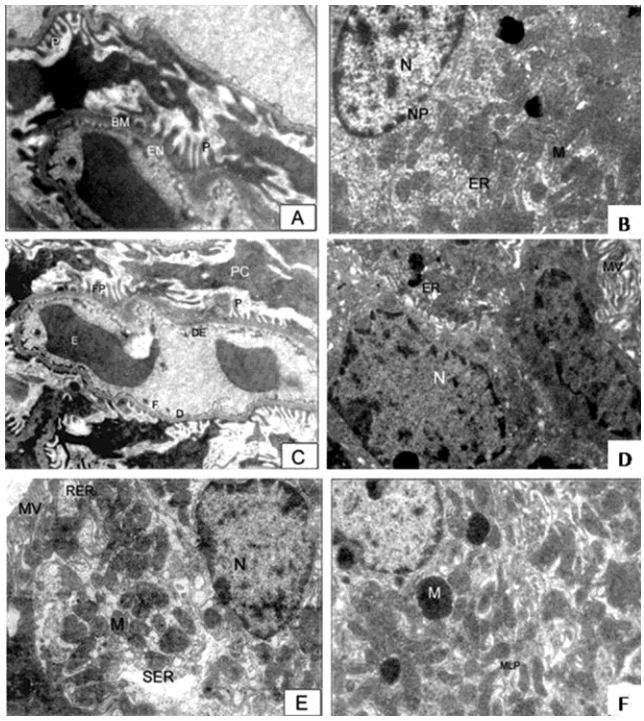

Fig. 2 — Electromicrograph of kidney- (A & B) In control Kidney podocyte (PC) gave out pedicles (P) on the base of basement membrane of capillary wall. Filter membrane better formed with intact endothelium. Uriniferous Tubule showing oval shape of nucleus with many nuclear pores. Mitochondria are many and mostly elongated. RER is more prominent than SER (3500 ×, 2200 ×); (C & D) Beryllium treated kidney showing disruption of endothelium (DE) of filter membrane with more number of fenestrations, deformed nuclei in uriniferous tubule as nuclei has lost their smooth nuclear membrane, chromatin deposition on the inner side of nuclear membrane, more vacuoles in the cytoplasm (2200 ×); (E) Therapy with MO+Pip showing more SER than RER. Mitochondria are associated with zymogen (ZM) granules (2200 ×); (F) Therapy with MO+ Cur showing better formed nuclei (N), multiple lateral processes extending up to plasma membrane, small vesicles (V) formed by the microvilli (MV), highly pigmented nuclei in endothelial cells (2200 ×)

nuclear membrane. The number of vacuoles was increased in the cytoplasm. The mitochondria were plenty organized in all directions around the nuclei (Fig. 2C & 2D). Combination therapy of *M. oleifera* with piperine showed, mostly smooth endoplasmic reticulum was observed between the mitochondria; however, dilated vesicles of rough endoplasmic reticulum studded with the ribosome, were also present. The rough endoplasmic reticulum was less while smooth endoplasmic reticulum was more. In proximal convoluted tubules, the cells had microvilli in most of the ad-luminal surface. They formed moderate size vesicles in the cytoplasm. Mitochondria were elongated and associated with zymogen granules (Fig. 2E). Combination therapy of *M. oleifera* with curcumin showed profuse microvilli extending into the cytoplasm forming small vesicles. Nuclei were better formed, some lysosomes were also seen. The multiple lateral processes extended up to the plasma membrane. The vesicles formed by the microvilli were small. The overall structure of the kidney was better formed as compare to beryllium perse treatment (Fig. 2F).

Discussion

In the present study, hydroalcoholic root extract of *Moringa oleifera* Lam. with piperine or curcumin were investigated against beryllium-induced alterations in hepatorenal biochemistry, ultamorphology and beryllium body burden in rats. In the previous study, *M. oleifera* with curcumin has been found to be effective antioxidant against beryllium-induced oxidative stress and histopathological alterations in rats[6]. This study reports better antitoxic and hepatorenal protective potential of *M. oleifera* root extract with curcumin against beryllium-induced alterations in hepatorenal biochemistry and ultra morphological alterations in rats.

Beryllium administration through different routes enters into blood circulation, binds with plasma globulin and forms stable beryllium protein complex. Thus, large amounts of beryllium get transported to various organs and causes damage[7]. The liver is one of the principal target organs of toxicological action of beryllium[40] and other toxic compounds[55]. Be induces oxidative stress through its ability to deplete endogenous thiol antioxidants and enhances production of ROS, depletes major antioxidants enzymes, which ultimately causes lipid peroxidation and leads to membrane damage[6]. Oxidative stress is a common mechanism contributing to the initiation and progression of hepatic damage in a variety of liver disorders[56]. Exposure to beryllium nitrate elevated leakage of serum transaminases (AST and ALT) and LDH into blood circulation, increased its level higher than normal and impaired liver functions. Roots of *M. oleifera* are a rich source of gallic tannins, catechol tennins, steroids and triterpenoids, flavanoids, saponins, anthraquinones, alkaloids and reducing sugars[41], which make it a good antioxidant. Treatment with *M. oleifera* neutralize free radicals, prevented cellular injury and organ dysfunction prominently and subsequently inhibited rapid leakage of these enzymes into blood circulation. Curcumin has also

been seen to raise the levels of the endogenous antioxidant glutathione (GSH) by increasing the expression of mRNA of the GSH biosynthetic genes[42]. GSH neutralizes free radicals and protects biological membranes of vital organs of rats[56]. DPPH assay indicated that hydro-alcoholic root extract of *M. oleifera* possesses better free radical scavenging activity than pure alcoholic extract[43]. In the present study, curcumin could act in combination with *M. oleifera*, maintained cellular integrity more effectively and restored LDH, AST, and ALT towards control.

Membrane-bound enzyme, SALP was decreased after beryllium toxicity due to binding of beryllium to its active sites. Curcumin acts as a chelating agent which may bind with various metal ions through its enol group and inhibited the toxic effect of metallic ions. The two molecules of curcumin bind one metal ion in 2:1 ratio[44-46]. *M. oleifera* rich in phenolic compounds and curcumin itself has chelating property, may bind with soluble beryllium that consequently inhibited binding of beryllium to SALP thus restored SALP activity.

Beryllium disturbed normal kidney function and increased serum level of urea, uric acid, and creatinine. Exposure to beryllium-induced membrane damage due to oxidative insult, altered kidney function and resulted in the accumulation of urea, and creatinine in the blood. In histological sections, the narrow lumen of uriniferous tubules in beryllium toxicity obstructed the flow of filtrate[6] and contributed to higher level of urea, uric acid and creatinine in blood. In ultra-morphological sections, degenerated endothelium of filter membrane and increased number of fenestrations also contributed in deviation of normal kidney function. *M. oleifera* root extract along with piperine and curcumin prevented membrane damage and helped in maintaining renal function towards normal. Combination of *M. oleifera* and curcumin maintained the lumen of uriniferous tubules almost normal[6] and prevented damage of endothelium of filter membrane with a decrease in a number of fenestrations, thus level of urea, uric acid and creatinine were restored towards normal.

Exposure to beryllium disturbed lipid metabolism resulted from elevation of cholesterol and triglyceride in serum. Beryllium caused severe lipid peroxidation and increased supply of non-essential fatty acids, which in turn increased triglycerides and total cholesterol in serum. Linoleate, a polyunsaturated fatty acid that can be oxidized to form a fatty acid radical has been observed to be harnessed by curcumin to inhibit lipid peroxidation. Curcumin, performing as a chain-breaking antioxidant, activated an intramolecular Diels-Alder reaction culminating in lipid radical neutralization[47]. Different parts of *M. oleifera* vary in polyphenolic contents and rich in antioxidants other than polyphenols like b-carotene, vitamin A, and vitamin E and showed radical scavenging capacity[48]. Thus, therapy of *M. oleifera* with curcumin effectively decreased lipid peroxidation, prevented the production of free fatty acids and maintained a level of triglycerides and total cholesterol up to normal.

Beryllium depleted blood hemoglobin significantly due to decline in the synthesis of heme and globin protein. Hyperbilirubinaemia indicated the severity of necrosis[49] rises in beryllium toxicity is due to more destruction of RBCs which may also decrease hemoglobin in the blood. Metal toxicity due to lead, arsenic, and cadmium are reported to deplete Hb level by inhibiting key regulatory enzymes of heme biosynthesis[50]. Beryllium suppressed the activity of δ-amino levulinic acid dehydratase (ALAD) in blood and increased the activity of δ-amino levulinic acid synthatase (ALAS) in the liver. These two enzymes act as key regulatory enzymes in heme biosynthesis. ALAS is the first regulatory enzyme undergoes feedback regulation by the end product heme whereas ALAD is the second regulatory enzyme, requires zinc as divalent metal ion as a cofactor for its activity. Be^{+2} is divalent metal ion and quite toxic, it may bind with enzymatic (ALAD) active site and inhibited its action thus, the level of heme is not maintained. Decreased heme content due to beryllium toxicity undergo feed positive regulation for ALAS activation but due to inhibition of ALAD, the amount of heme/hemoglobin in blood is not achieved up to normal. *M. oleifera* root extract is a rich source of antioxidants (polyphenols and procyanidins), piperine and curcumin act as antioxidants, may also bind with beryllium metal ion; help in excretion from the body and protect the vital organs of rat from the harmful effect of beryllium. Treatment with *M. oleifera* alone did not show significant improvement. However, combination therapy of *M. oleifera* with piperine increased the level of hemoglobin by maintaining the activity of ALAS and ALAD towards normal. Piperine has bioavailability enhancing effect for some drugs[23] thus in combination with *M. oleifera* root extract might

enhance the therapeutic efficacy of *M. oleifera* up to some extent. Curcumin acts as antioxidant and might also possess metal binding capacity[45,46] act in combination with *M. oleifera* and maintain the cellular integrity, restored ALAD and ALAS activities, thus hemoglobin and bilirubin level is maintained towards normal this may be due to the better combination effect of curcumin with *M. oleifera* root extract.

SDH is a mitochondrial enzyme, tightly bound to the inner mitochondrial membrane. It is an oxidative enzyme involved in Kreb's cycle and exhibits an important function in energy-yielding process. Pesticides, metals and metallic compounds alter the mitochondrial structure and decrease the SDH activity in many animals[51]. Beryllium disturbed the lipid bilayer and enhanced the lipid peroxidation of biological membrane and decreased SDH activity. *M. oleifera* is a rich source of antioxidants neutralizes the free radicals and curcumin itself acts as antioxidant, prevented the beryllium-induced oxidative stress and maintain the histoarchitecture of liver and kidney[6]. In the present study, *M. oleifera* with curcumin restored the activity of SDH more effectively towards normal by neutralizing the free radicals and decreased the toxic effect of beryllium which may be due to the better antioxidant effect of curcumin along with *M. oleifera* root extract.

Cellular membrane damage might be responsible for ionic imbalance, mitochondrial damage, and stimulation of lysosomes and liberation of hydrolytic enzymes, which consequently causes injury to surrounding tissues. Beryllium administration increased the activity of acid phosphatase (ACPase) in liver and kidney suggests enhanced tissue catabolism and cellular autophagy leading to tissue damage[52]. Ultrastructural observations also corroborated this fact with cytoplasmic vacuolation due to degeneration of cellular organelles after beryllium toxicity. Combined administration of *M. oleifera* and curcumin showed conspicuous hepatorenal protection due to a reduction in the release of lysosomal enzymes. 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. The be^{+2} is a potential target for ATP binding and that be^{+2} out competes Mg^{+2} for ATP and ADP binding. *M. oleifera* is also a rich source of minerals which provides magnesium ion that further competes with beryllium and helps in maintaining the normal function of these enzymes in both the organs. The root bark of *M. oleifera* is a rich source of procyanidins, are known to contribute significantly to the antioxidant properties of several foodstuffs. Their hydrogen donating abilities and their propensity for nitration make these compounds powerful scavengers of reactive oxygen and nitrogen species. Scientific investigations have indeed shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C[46]. Curcumin is well-known antioxidant and could augment antioxidant potential of *M. oleifera* root extract, showed a better antitoxic effect against beryllium-induced hepatorenal dysfunction.

In our study, liver and spleen occupied maximum amount of beryllium is also in agreement with the view of Witschi & Aldridge[40], however, kidney, blood, brain, bone and hair contained less beryllium. Curcumin possess α, β-unsaturated β-diketone moiety, has been proven to be an excellent chelating agent for most of the metal ion[44,45]. Curcumin exhibits keto-enol tautomerism in an aqueous solution that forms complex with the various metal ions. The various study reported that curcumin act as chelating agent for various metal ions and forms curcumin metal complexes this property of curcumin is due to the presence of -OH group. Enol form of curcumin contributes chelation property of curcumin[46]. It has been found that due to its lipophilic nature, curcumin can cross the blood-brain barrier and chelate metal ions that are toxic to the neurons. It has also been observed that the incidence of Alzheimer's disease is significantly reduced among people that are known to regularly consume turmeric in their diet[53]. Curcumin forms stable complexes with all the metals involved in Alzheimer's disease[54,]. Treatment with *M. oleifera* root extract mobilize beryllium from tissues up to some extent, however, curcumin with *M. oleifera* showed a better effect in removal of beryllium from vital organs of rats as compared to *M. oleifera* with piperine which is calculated by percent reduction. However, both the combination therapy showed a significant effect in the reduction of beryllium from the body. Thus, combination therapy acted upon better in excretion of beryllium from vital organs of rats.

Conclusion

In this study, we demonstrated that beryllium-induced alterations in hepatorenal biochemical variables could be reversed towards normalization by hydro-alcoholic root extract of *Moringa oleifera* Lam. with curcumin. *M. oleifera* root extract with curcumin maintained integrity of biological membrane and showed a better hepatorenal protective effect against beryllium toxicity. The exact mechanism of detoxification by *M. oleifera* root extract and with curcumin remains still unclear; however, phytochemicals present in root extract of *M. oleifera* could contribute in all the detoxification scenario. The better antioxidant and chelating action of curcumin may also contribute its finding. Combination therapy of *M. oleifera* and curcumin was found to be better as a comparison to *M. oleifera* with piperine as calculated by percent protection which might be due to the better combination effect of curcumin along with *M. oleifera* root extract. Thus, it is concluded that combination therapy may serve as a better option in the treatment of beryllium-induced hepatorenal toxicity.

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