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Original Research Article

Phytochemicals and protective effects of *Moringa oleifera* seed extract on CCl_4 induced hepatotoxicity and hemotoxicity in rats

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ABSTRACT

Background: *Moringa oleifera is* high valued plant and used in many countries around the world. The seed of *Moringa oleifera* (MO) is an important part and has a remarkable medicinal, nutritional and socio-economic values, this study, therefore, was designed to clarify the protective effect of *Moringa oleifera* hydroethanolic seed extract (MOSE) against carbon tetrachloride (CCl_4) induced hepatoxicity and hemotoxicity in rats.
Methods: A total of one hundred and five male rats were randomly divided into 7 groups of 15 rats each. The hydroethanolic seed extract (30%) was administered orally for one month at 250 and 500mg/kg body weight. Samples were collected after day1,15 and 30 post administration.
Results: Phytochemical, biochemical, hematological and hisopathological examinations were utilized to investigate hepatoprotective activity of MOSE. The results obtained demonstrated that, phytochemicals such as alkaloids, glycosides, anthraquinones, tannins, flavonoids, gum, resin, saponins, terponoids, protein and fats were detected in the seeds. Treatment with the MOSE caused a significant ($P<0.05$) decrease in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, triglyceride and lipid peroxidation (MDA), while total protein and albumin level significantly ($P<0.05$) increased compared to CCl_4 group. Also, treatment with the MOSE showed a significant ($P<0.05$) increase Hb content and RBCs, whereas WBCs and lymphocyte count significantly ($P<0.05$) decreased throughout the period of administration when compared to the rats in CCl_4 group. The results obtained were comparable to silymarin. Histopathological examination of liver tissues confirmed the biochemical data.
Conclusions: It could be concluded that, CCl_4 induced hepatotoxicity and hemotoxicity is ameliorated by MOSE especially in high dose of (500mg/kg). This effect is attributed to free radical scavenging activity and potent antioxidant activity of its components (Flavonoid, tannin, alkaloid and saponin).

Keywords: CCl_4, Hepatotoxicity, Hemotoxicity, *Moringa oleifera*, Phytochemical

INTRODUCTION

Liver disease is considered huge public health problem on an international scale. Despite newly developed drugs have been used to treat liver disorders, these drugs have often side effects. Therefore, advanced research studies have been performed to search the safe and potent remedies without side effects to treat liver disorders. Natural remedies from medicinal plants are consider the most desired and fascinating area as alternative treatment for hepatotoxicity.[1] Hepatoprotective effects of plants are associated with phytocompounds rich in natural antioxidants as flavonoids, alkaloids, tocopherol, carotenoids, vitamin A, C, E and other phenolic compounds.[2]

Moringa oleifera (Family: Moringaceae) is a valuable tropical and subtrobical plant. It is widely distributed in

many countries of Africa, Arabia, India, South Asia, Latin America and Himalayas. The plant is referred to number of names such as miracle tree, ben oil tree, horse radish tree and drumstick tree. *Moringa* are a good source of protein, amino acids, vitamins (A, B1, B2, B3, C, E), minerals (calcium, iron, phosphorus, magnesium), phytochemical compound (alkaloids, glycosides, sterols, flavonoids, saponin, tannins and various phenolics.[3]

All the parts of this plant have medicinal and therapeutic uses.[4] The seeds of *Moringa oleifera* when likened to other parts of the plant, it was found that it is high value part and has an incredible range of pharmacological and therapeutic uses with high nutritional value. The seeds contain 38-40% oil (ben oil) can be used for cooking, in soaps and perfumes.[5] Seeds are extensively used for liver, renal, hematological, cardiovascular diseases, treating inflammation and used as antidiabetic.[6]

Moringa seeds having efficacy in purification by flocculation of contaminants in drinking water.[7] A significant amount of thiamin, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta -carotene and alpha -tocopherol were detected in seeds.[8]

In folk medicine, MO has been used for management of various liver disorders. This study, therefore, was designed to demonstrate hepatic and hematological effect of MOSE against CCl_4 toxicity through liver function test, MDA evaluation, hematological and histopathological examination and this investigation will further support the claim of the protective property of this plant and will clarify the potency of this plant in treatment of liver disease by a comparison with silymarin.

METHODS

Moringa oleifera

The seeds of *Moringa oleifera* were purchased from Haraz - Company of agricultural seeds, spices and medicinal plants, Cairo, Cairo Governorate, Egypt. The seeds were collected in March 2017 and authenticated by Department of Pharmacognosy, Collage of Pharmacy.

Chemicals

Phytochemical analysis (absolute alcohol 99.9%, ethyle alcohol 96%, ethyle alcohol 95%, ethyle alcohol 70%, methanol, acetone, ammonium hudroxide, nin hydrin, glacial acetic acid 99%, hydrochloric acid 30%, sulphuric acid, ferric chloride solution 98%, α naphthol 10%, mercuric chloride, benzene (pyridine 99%), chloroform, copper sulphate). All above chemicals obtained from (Loba Chemie) PVT/LTD-MumBai-India. *In vivo* studies (carbon tetrachloride was obtained from Alamia Company, Banha, Qalubia Governorate, Egypt and silymarin (Hepaticum)® was obtained from Medical Union Pharmaceuticals Company).

Laboratory animals

Male wister rats weighing 150-200gm were obtained from animal house, Faculty of Veterinary Medicine, Benha University, Egypt. They were fed standard pellet diet and given access water. Rats were kept at a constant environmental and nutritional condition for 15 days for acclimatization before the beginning of the experiment.

Preparation of hydro-ethanolic extract of Moringa oleifera seeds

Moringa oleifera seeds were refluxed with bi-distilled water, shade dried at room temperature. Extracts were prepared according to modified method of Harborne.[9] A dark brown mass was obtained. It re-constituted by dissolving in measured amount hydro ethanol (30%). The extract was stored in refrigerator below 10°C. For *in vivo* studies, a stock solution was made so that each rat receives the calculated dose in 1 mL solvent.

Percentage yield was determined using the formula:

$$\text{Yield \%} = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100$$

Phytochemical analysis

Phytochemical screening of *Moringa oleifera* seeds extract were analyzed by the following procedures to test the presence of different phytochemical groups as alkaloids, glycosides, anthraquinones, cardiac glycosides, saponins, tannins, phlobatannins, flavonoids, resins, gums, terpenoids, proteins and oils.[9-15]

Effect of MOSE on liver function

One hundred and five rats were divided into 7 groups of 15 rats each.

Group 1: Served as negative control group

Fifteen rats received 1ml of hydroethanolic solution (vehicle) orally per day for one month.

Group 2: Served as positive control group

Fifteen rats intoxicated with fresh mixture of carbon tetrachloride (CCl_4) 25% and corn oil (1:3 of CCl_4 in corn oil) intraperitoneally at dose of 2.5ml/kg body weight twice weekly for one month.

Group 3: Considered as standard group

Fifteen rats were treated orally with silymarin (Hepaticum)® 100mg/kg body weight orally once daily for one month.

Group 4: Considered as Moringa oleifera test group

Fifteen rats were administered 1ml of hydroethanolic extract of *Moringa oleifera* seeds 250mg/kg b.wt. orally and carbon tetrachloride 2.5ml/kg bwt, intraperitoneally twice weekly for one month.[16]

Group 5: Considered as Moringa oleifera test group

Fifteen rats were administered 1ml of hydroethanolic extract of *Moringa oleifera* seeds orally 500mg/kg b.wt. and carbon tetrachloride 2.5ml/kg bwt, intraperitoneally twice weekly for one month.[17]

Group 6: Considered as Moringa oleifera control group

Fifteen rats were administered 1ml of hydroethanolic extract of *Moringa oleifera* seeds 250mg/kg bwt, orally for one month.

Group 7: Considered as Moringa oleifera control group

Fifteen rats were administered 1ml of hydroethanolic extract of *Moringa oleifera* seeds 500mg/kg bwt, orally for one month.

Blood sample

Blood sample and liver tissue were taken at day 1, 15 and 30 post-treatment in all groups. Two blood sample were taken from each rat in each group from retro-orbital sinus plexus of median canthus of the rat's eye using capillary tubes. The first blood sample was collected in test tube containing EDTA as anticoagulant for hematological studies.

The second blood sample was collected in test tube without anti coagulant and left in room temperature for an hour, then centrifuged at 3,000rpm for 15 minutes to collect serum for biochemical studies.

Preparation of liver homogenate immediately after blood sampling. Livers were collected for biochemical and histopathological examinations. Liver tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. Then it was homogenized by electric homogenizer. The homogenate was centrifuge at 3,000rpm for 5 minutes. Then the homogenate centrifuged again in cooling centrifuge for excluding debris from the homogenate.

Serum biochemical analysis

Aspartate, alanine aminotransferase, protein and albumin level were determined in serum spectro-photometrically by specific kits (Centronic Company®, Germany), while total bilirubin and triglyceride concentrations in serum were determined by using Dri Chem (model NX500i, Fuji film, Japan).

Determination of lipid peroxidation (malondialdehyde)

Lipid peroxidation in the liver was ascertained by the production of malondialdehyde (MDA). MDA, as a marker of lipid peroxidation, was measured colorimetrically in liver homogenate according to the method of Ohkawa et al, using commercially available kits (Boi diagnostic Company®).[18] Thiobarbituric acid reacts with MDA in acidic medium at 95°C for 30 min to form thiobarbituric acid reactive product, and the absorbance of the resultant pink product can be measured at 534nm.

Haematological study

Hemoglobin concentration, erythrocytic count, white blood cells and lymphocyte count were counted by using automatic blood cell counter (model HA-VET CLINDIAG).

Histopathology study

Autopsy samples were taken from the liver of rats in different groups and fixed in 10% formol saline for histopathological study.

Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS 16 Inc. Released, 2009). Compare between means were conducted by (general linear model repeated measure) followed by Tukey post hoc. (Probability values ($P \leq 0.05$) were considered significant.

RESULTS

Description of the extract

MOSE (30%) was a dark brown mass. The extraction process gave a yield of 12.5%.

Phytochemical analysis of Moringa oleifera seeds

The phytochemical evaluation of *Moringa oleifera* seeds showed that alkaloids, terpenoids and gums were present in high amount, glycosides, saponins, flavonoids, tannins, resin and proteins occurred in moderate concentration, while anthraquinones, fixed oil and fat appeared in low amount, whereas cardic glycosides and phlobatannins were absent in the seeds (Table 1).

Effects of MOSE on biochemical parameters

Effect of MOSE on serum AST and ALT level

CCl_4 induced a significant ($P<0.05$) increase (306.90, 94.60) in the level of serum AST, ALT respectively, compared to the control group (159.40, 50.40) on first day post administration. On day 1, 15 and 30, administration

of MO at both doses with CCl_4 and silymarin and CCl_4 for one month significantly reduced the levels of serum AST, ALT compared to CCl_4 group. On day 1, 15 and 30, 500 mg MO decreased the levels of serum AST, ALT toward normal values (Table 2).

Table 1: Results of phytochemical screening of seeds of *Moringa oleifera*.

	Phytochemical constituents	Test	Results
1	Alkaloids	Mayer's test	White precipitate
2	Alkaloids	Wagner's test	Brown precipitate
3	Glycosides and/or carbohydrates	Molisch's test	Bluish violet zone formation
4	Glycosides and/or carbohydrates	Benedict's test	Formation of green, yellow or brick red color precipitate
5	Cardiac glycosides	General lab test	Absence of brown ring of the interface
6	Anthraquinones	General lab test	Presence of red colour in the ammoniacal phase
7	Saponins	Froth test	Formation of froth and emulsion development
8	Flavonoids	General lab test	Yellow coloration
9	Tannins	Ferric chloride test	Brownish green colour
10	Phlobatannins	Hydrochloric acid test	Absence of deposition of a red precipitate
11	Gums/mucilages	Lab test	White or cloudy precipitate
12	Resins	Distalled water test	Formation of a precipitate
13	Terpenoids	General lab test	A reddish-brown colouration of the interface
14	Proteins	Biuret test	Appearance of a pink color in the ethanolic layer
15	Fixed oils and fats	Spot test	Appearance of oil stains

Table 2: Effects of MOSE (250 and 500mg/kg bwt, orally) and silymarin (100mg/kg bwt, orally) for one month on serum aspartate aminotransferase and alanine aminotransferase (U/L) in normal and intoxicated rats by CCl_4 (2.5ml/kg bwt, IP) (n=15).

Groups	Time after the end of administration					
	Serum AST			Serum ALT		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
Control	159.40± 19.12^{b}	160.04± 3.18^{b}	160.28± 3.19^{bd}	50.40± 0.583^{bd}	50.50± 0.539^{bd}	50.58± 0.523^{b}
CCl_4 2.5ml/kg	306.90± 6.36$^{C\ acdefg}$	293.00± 6.63$^{C\ acdefg}$	240.30± 3.17$^{AB\ acdefg}$	94.60± 4.75$^{C\ acdefg}$	100.60± 1.94$^{C\ acdefg}$	74.20± 5.01$^{AB\ acdefg}$
100mg silymarin + CCl_4	171.32± 3.30^{b}	164.90± 1.35^{Cb}	152.80± 1.59^{Bbd}	59.75± 1.72^{b}	52.45± 0.590^{bd}	50.32± 0.086^{b}
250mg MO + CCl_4	180.38± 6.33 $^{C\ b}$	173.84± 1.74^{b}	169.80± 3.74 ^{abcefg}	66.47± 5.05$^{C\ abfg}$	65.13± 1.78$^{C\ abcefg}$	55.12± 0.362$^{AB\ b}$
500mg MO + CCl_4	170.74± 3.16^{b}	160.56± 3.18^{b}	158.60± 0.970^{Bbcd}	53.41± 2.05^{b}	45.26± 3.44^{bd}	44.01± 4.00^{b}
250mg MO	159.00± 2.45^{b}	160.08± 0.037^{b}	160.04± 0.722^{bd}	49.21± 0.825^{bd}	49.06± 0.979^{bd}	50.08± 0.680^{b}
500mg MO	158.36± 3.28^{b}	159.94± 0.194^{b}	160.26± 2.14 ^{bd}	48.50± 1.136^{bd}	48.78± 1.03^{bd}	49.16± 0.869^{b}

Values are mean±SE. Means within a column followed by different superscript letters (a (control), b (CCl_4), c (silymarin), d (250mg MO+ CCl_4), e (500mg MO+ CCl_4), f (250mg MO), g (500mg MO) were significantly different ($p \leq 0.05$), while values within a row followed by different superscript letters (A(D1), B (D15), C (D30)) were significantly different ($p \leq 0.05$)

Effect of MOSE on serum total protein and albumin

The concentrations of total protein and albumin were significantly ($P<0.05$) reduced in CCl_4 group compared to control group on day 1, 15 and 30. Treatment of (500mg MO and CCl_4) and (silymarin with CCl_4) for one month significantly increased level of total protein and albumin on first day post administration. The level of total protein was completely restored to normal in MO 500mg and CCl_4 group in all day post treatment. Administration of 500mg

MO alone for one month were significantly increased level of total protein and albumin compared to control group on first day post administration, also level of albumin increased significantly on day 15 and 30 (Table 3).

Table 3: Effects of MOSE (250 and 500mg/kg bwt, orally) and silymarin (100mg/kg bwt, orally) for one month on serum total protein and albumin (g/dl) in normal and intoxicated rats by CCl_4 (2.5ml/kg bwt, IP) (n=15).

Groups	Time after the end of administration					
	Total protein			Albumin		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
Control	6.40 ± 0.070	6.53± 0.052^{bcd}	6.43± 0.036^{bd}	3.57± 0.049^{bdfg}	3.55± $0.053^{C\ bdeg}$	3.43± $0.065^{B\ bdg}$
CCl_4 2.5ml/kg	4.37± $0.399^{acdefg\ BC}$	3.77± $0.230^{A\ acdefg}$	3.74± $0.204^{A\ acdefg}$	2.43± 0.052^{acdefg}	2.40± 0.036^{acdefg}	2.42± 0.036^{acdefg}
100mg silymarin + CCl_4	5.77± 0.045^{bfg}	5.86± 0.022^{abfg}	6.12± 0.070^{bfg}	3.19± 0.040^{bdfg}	3.56± 0.025^{bdeg}	3.55± 0.022^{bdg}
250mg MO + CCl_4	5.67± 0.125^{bfg}	5.75± 0.045^{abefg}	5.94± 0.023^{abefg}	2.99± $0.035^{C\ abcefg}$	3.07± $0.022^{C\ abcefg}$	3.19± $0.047^{ABabcefg}$
500mg MO + CCl_4	6.14± 0.165^{bg}	6.22± 0.035^{bdg}	6.39± 0.049^{bd}	3.38± 0.052^{bdfgB}	3.89± $0.032^{AC\ abcdeg}$	3.47± $0.019^{B\ bdg}$
250mg MO	6.67± 0.037^{bcd}	6.66± 0.051^{bcd}	6.58± 0.101^{bcd}	4.09± $0.038^{BC\ abcdeg}$	3.71± $0.044^{AC\ bdg}$	3.46± 0.040^{bdgAB}
500mg MO	7.53± $0.024^{BC\ abcde}$	6.85± $0.039^{A\ bcde}$	6.79± $0.072^{A\ bcd}$	4.46± $0.053^{BC\ abcdef}$	4.15± $0.044^{ACabcdef}$	3.76± $0.029^{AB\ abcdef}$

Values are mean±SE. Means within a column followed by different superscript letters (a (control), b (CCl4), c (silymarin), d (250mg MO+ CCl4), e (500mg MO+ CCl4), f (250mg MO), g (500mg MO) were significantly different ($p \leq 0.05$), while values within a row followed by different superscript letters (A(D1), B (D15), C (D30)) were significantly different ($p \leq 0.05$)

Table 4: Effects of MOSE (250 and 500mg/kg bwt, orally) and silymarin (100mg/kg bwt, orally) for one month on serum total bilirubin (mg/dl) and triglyceride (mg/dl) in normal and intoxicated rats by CCl_4 (2.5ml/kg bwt, IP) (n=15).

Groups	Time after the end of administration					
	Total bilirubin			Triglyceride		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
Control	0.540± 0.024^{bde}	0.580± 0.020^{bde}	0.560± 0.024^{b}	30.20± 0.374^{b}	30.80± 0.374^{bd}	31.00± 0.316^{b}
CCl_4 2.5ml/kg	1.64± $0.024^{acdefg\ BC}$	1.46± $0.024^{acdefg\ AC}$	1.22± $0.037^{AB\ acdefg}$	106.80± $7.31^{C\ acdefg}$	104.00± $2.45^{C\ acdefg}$	93.40± $2.62^{acdefg\ AB}$
100mg Silymarin + CCl_4	0.480± 0.020^{Cbde}	0.540± 0.024^{bd}	0.580± $0.020^{A\ b}$	32.80± 0.200^{b}	30.80± 0.200^{bd}	30.60± 0.245^{b}
250mg Mo + CCl_4	0.720± 0.020^{abcfg}	0.680± 0.020^{bceg}	0.640± 0.024^{b}	42.00± $4.90^{C\ b}$	37.60± $2.18^{C\ abcefg}$	34.00± $0.447^{AB\ bfg}$
500mg Mo + CCl_4	0.640± $0.024^{abcefg\ BC}$	0.480± 0.020^{Abd}	0.540± $0.024^{A\ b}$	31.20± 0.374^{b}	30.60± 0.245^{bd}	30.40± 0.245^{b}
250mg Mo	0.530± 0.006^{bde}	0.580± 0.020^{b}	0.540± 0.024^{b}	29.40± 0.245^{b}	30.20± 0.538^{bd}	29.60± 0.245^{bd}
500mg Mo	0.508± 0.012^{bde}	0.548± 0.022^{bd}	0.560± 0.024^{b}	28.40± 0.245^{b}	30.60± 0.245^{bd}	30.20± 0.200^{bd}

Values are mean±SE. Means within a column followed by different superscript letters (a (control), b (CCl_4), c (silymarin), d (250mg MO+ CCl_4), e (500mg MO+ CCl_4), f (250mg MO), g (500mg MO) were significantly different ($p \leq 0.05$), while values within a row followed by different superscript letters (A(D1), B (D15), C (D30)) were significantly different ($p \leq 0.05$).

Effect of MOSE on serum total bilirubin and triglyceride

On first day post administration, the concentrations of total bilirubin in serum were increased in CCl_4 group (1.64), Mo

250mg and CCl_4 group (0.72) and Mo 500mg and CCl_4 group (0.64) and these increases were significantly ($P<0.05$) compared to control group (0.52), while concentrations of total bilirubin in Mo at both doses with CCl_4 group decreased toward normal level on day 30 (Table 4). The concentrations of triglyceride in serum were significantly ($P<0.05$) increased in CCl_4 group compared to control group on first day post administration. In groups (standard by silymarin 100mg/kg and CCl_4, MO 250 and 500mg/kg and CCl_4, triglyceride concentrations were significantly decreased compared to intoxicated group by CCl_4. The same results were recorded on day 15 and 30 post administration (Table 4).

Table 5: Effects of MOSE (250 and 500mg/kg bwt, orally) and silymarin (100mg/kg bwt, orally) for one month on serum malondialdehyde level (nmol/ml) in normal and intoxicated rats by CCl_4 (2.5ml/kg bwt, IP) (n=15).

Animal's groups	Time after the end of administration					
	Serum MDA			Hepatic MDA		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
Control	11.94± 0.842^{b}	11.88± 0.860^{b}	11.94± 0.820^{b}	54.60± 0.327^{bd}	54.74± 0.329^{bd}	55.08± 0.107^{bd}
CCl_4 2.5ml/kg	18.74± 0.719$^{C\ acdefg}$	18.26± 0.322$^{C\ acdefg}$	15.46± 0.360$^{AB\ acdefg}$	93.82± 0.765^{acdefg}	95.42± 1.03^{acdefg}	92.06± 0.665^{acdefg}
100 mg silymarin + CCl_4	12.14± 0.528^{b}	11.82± 0.282^{b}	11.93± 0.100^{b}	58.76± 0.362^{bdfg}	56.32± 0.306^{bd}	55.74± 0.273^{bd}
250mg MO + CCl_4	13.11± 0.664^{b}	12.53± 0.431^{b}	12.36± 0.075^{b}	70.36± 0.756^{abcefg}	72.82± 1.87^{abcefg}	69.86± 0.944^{abcefg}
500mg MO + CCl_4	12.09± 0.405^{b}	11.77± 0.461^{b}	11.88± 0.307^{b}	58.60± 2.55^{bdfg}	56.30± 0.397^{bd}	55.64± 0.087^{bd}
250mg MO	11.26± 0.698^{b}	11.79± 0.739^{b}	11.86± 0.761^{b}	53.34± 0.609^{bcde}	54.20± 0.326^{bd}	55.20± 0.317^{bd}
500mg MO	10.84± 0.330^{b}	11.99± 0.296^{b}	11.92± 0.289^{b}	50.98± 0.438$^{C\ bcde}$	50.36± 3.73$^{C\ bd}$	54.98± 0.220^{ABbd}

Values are mean±SE. Means within a column followed by different superscript letters (a (control), b (CCl_4), c (silymarin), d (250mg MO+ CCl_4), e (500mg MO+ CCl_4), f (250mg MO), g (500mg MO) were significantly different ($p \leq 0.05$), while values within a row followed by different superscript letters (A(D1), B (D15), C (D30)) were significantly different ($p \leq 0.05$).

Table 6: Effects of MOSE (250 and 500mg/kg bwt, orally) and silymarin (100mg/kg bwt, orally) for one month on hemoglobin concentrations (g/dl) and red blood cells count (106/ µl) in normal and intoxicated rats by CCl4 (2.5ml/kg bwt, IP) (n=15).

Animal's groups	Time after the end of administration					
	Hb			RBCs		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
Control	14.26 ± 0.068^{bg}	14.36 ± 0.068^{b}	14.12 ± 0.037^{b}	6.34± 0.025^{bg}	6.31± 0.067^{b}	6.29± 0.050^{b}
CCl_4 2.5ml/kg	10.56 ± 0.256^{acdefg}	10.26 ± ^{acdefg}0.117	10.26 ± 0.068^{acdefg}	5.35± 0.124^{acdefg}	5.23± 0.085^{acdefg}	5.50± 0.086^{acdefg}
100mg silymarin + CCl_4	13.60 ± 0.918^{bg}	13.66 ± 0.204^{bg}	13.98 ± 0.332^{b}	6.08± 0.118^{bg}	6.11± 0.095^{bg}	6.13± 0.061^{b}
250mg MO + CCl_4	13.32 ± 0.761^{bg}	13.38± 0.593^{bg}	14.04 ± 0.286^{b}	6.07± 0.083^{bg}	6.11± 0.091^{bg}	6.07± 0.089^{b}
500mg MO + CCl_4	14.00 ± 0.446^{bg}	13.78 ± 0.369^{b}	14.18 ± 0.116^{b}	6.23± 0.073^{bg}	6.14± 0.053^{bg}	6.29± 0.095^{b}
250mg MO	14.62 ± 0.058^{b}	14.52 ± 0.080^{b}	14.44 ± 0.040^{b}	6.43± 0.025^{bg}	6.37± 0.017^{b}	6.37± 0.049^{b}
500mg MO	15.56 ± 0.081^{abcde}	15.04 ± 0.147^{bcd}	14.68 ± 0.156^{b}	7.05 ± 0.139$^{BC\ abcdef}$	6.59± 0.089^{Abcde}	6.41± 0.061^{Ab}

Values are mean±SE. Means within a column followed by different superscript letters (a (control), b (CCl_4), c (silymarin), d (250mg MO+ CCl_4), e (500mg MO+ CCl_4), f (250mg MO), g (500mg MO) were significantly different ($p \leq 0.05$), while values within a row followed by different superscript letters (A(D1), B (D15), C (D30)) were significantly different ($p \leq 0.05$)

Table 7: Effects of MOSE (250 and 500mg/kg bwt, orally) and silymarin (100mg/kg bwt, orally) for one month on white blood cells count (103/mm3) and lymphocyte count (k/ul) in normal and intoxicated rats by CCl4 (2.5ml/kg bwt, IP) (n=15).

Animal's groups	Time after the end of administration					
	WBCs			Lymphocyte		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
Control	14.48± 0.458^{bcd}	14.88± 0.623^{d}	14.76± 0.455	12.05± 0.520^{bd}	12.39± 0.486	12.42± 0.458
CCl_4 2.5ml/kg	24.74± 0.719$^{BC\ acefg}$	17.24± 0.502^{AC}	12.48± 1.23$^{AB\ g}$	17.91± 1.29$^{BC\ aefg}$	14.06± 0.472^{AC}	10.50± 0.908^{AB}
100 mg silymarin + CCl_4	20.06± 0.662$^{BC\ abef}$	17.06± 0.919^{A}	15.50± 0.638^{A}	15.92± 0.783^{C}	14.00± 0.503	12.57± 0.500^{A}
250mg MO + CCl_4	21.28± 1.24$^{C\ aefg}$	18.96± 0.549$^{C\ aef}$	15.92± 0.497^{AB}	16.77± 1.315$^{C\ af}$	15.13± 0.817^{e}	13.14± 0.545^{A}
500mg MO + CCl_4	16.04± 0.844^{bcd}	14.14± 0.831^{d}	14.48± 0.968	12.78± 0.475^{b}	10.90± 1.29^{d}	12.12± 0.750
250mg MO	15.40± 0.822^{bcd}	14.38± 0.927^{d}	14.86± 0.671	12.70± 0.724^{bd}	12.32± 0.833	12.92± 0.524
500mg MO	16.84± 0.359^{bcd}	16.04± 0.520	14.94± 0.639^{b}	13.83± 0.401^{b}	13.53± 0.465	12.08± 0.656

Values are mean±SE. Means within a column followed by different superscript letters (a (control), b (CCl_4), c (silymarin), d (250mg MO+ CCl_4), e (500mg MO+ CCl_4), f (250mg MO), g (500mg MO) were significantly different ($p \leq 0.05$), while values within a row followed by different superscript letters (A(D1), B (D15), C (D30)) were significantly different ($p \leq 0.05$)

Effect of MOSE on lipid peroxidation (serum MDA and hepatic MDA)

On first day post administration, CCl_4 significantly ($P<0.05$) elevated (18.74, 93.82) lipid peroxidation measured as MDA formation (serum and hepatic MDA) respectively, compared to the control group (11.94, 54.60). In treated group with MO at both doses significantly reduced ($P<0.05$) the levels of serum and hepatic MDA and respectively, compared to the CCl_4 group. Similar results were found with silymarin. On day 30, CCl_4 was decreased and this decrease was significant compared to day 1 and 15 post administration (Table 5).

Effects of MOSE on hematological parameters

The effect of the extract on hematological parameters of intoxicated rats by CCl_4 was clearly observed. The results showed that Hb concentrations were decreased significantly ($p<0.05$) in the CCl_4 group compared to control group on all day post administration (Table 6).

Similarly, RBCs count were decreased, and these decreases were significantly to control group (Table 6). Treatment with MOSE with CCl_4 at both doses for one month significantly raised ($p<0.05$) Hb and RBCs nearly to normal level compared to CCl_4 group. Administration of 500mg MOSE alone for one month were significantly increased Hb and RBCs compared to control group on first day post administration, while 250mg MO alone remained insignificant to control group.

On first day post administration, WBCs and lymphocyte count in CCl_4 and 250mg MOSE with CCl_4 groups significantly elevated ($p<0.05$) compared to control group. After day 15, WBCs and lymphocyte count decreased and return toward normal by day 30 in the above group. Treatment with 500mg MO and CCl_4 significantly decreased WBCs and lymphocyte count compared to CCl_4 group on day 1 (Table 7).

Effect of MOSE on histopathological profile

Histopathological examination revealed parallel findings with biochemical alteration in the liver (Figure 1 to 6).

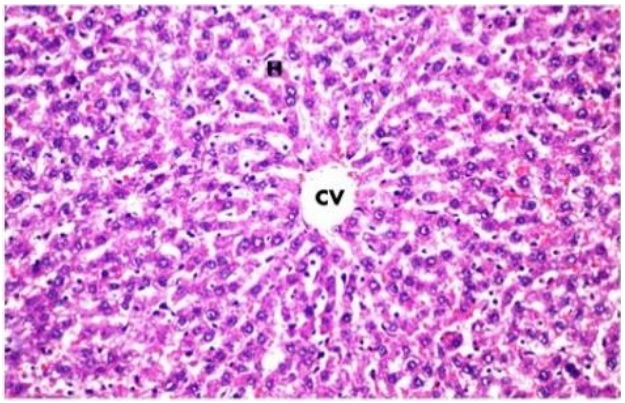

Figure 1: Liver section in normal group: Normal histological structure of the central vein (CV) and surrounding hepatocytes (H) in the parenchyma (H&E, × 40).

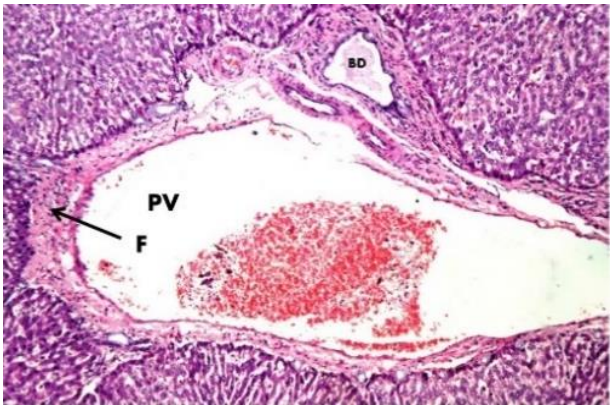

Figure 2: Liver section in CCL_4 group: Fibrosis (F) surrounding the portal vein on day 1 post administration (H&E× 16).

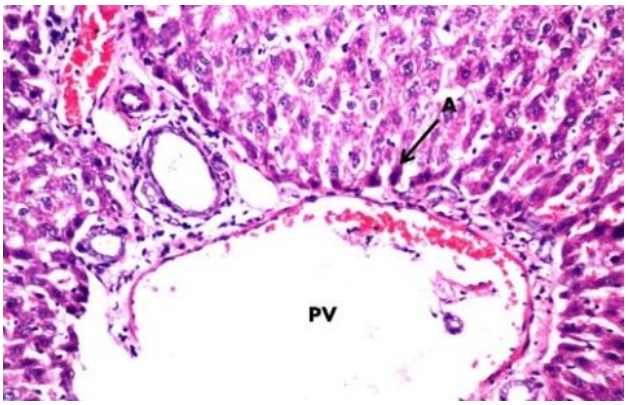

Figure 3: Liver section in silymarin group + CCl_4 group: Apoptosis (A) in some individual hepatocyte on day1 post administration (H&E×40).

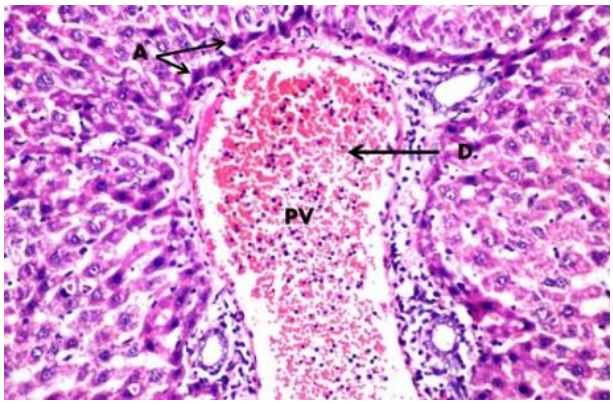

Figure 4: Liver section in treated 250mg MO+CCl_4 group: Sever dilatation and congestion (D) in the portal vein associated with few inflammatory cells infiltration in the portal area while the adjacent hepatocytes had apoptosis (A) on day 1 post administration (H&E×40).

In the CCl_4 group, severe histopathological changes such as fibrosis and fatty degeneration were showed on day 1 (Figure 2) 15 and30 post administration. In standard group with silymarin, dilatation in the portal vein and apoptosis in some individual hepatocyte was observed on day 1 (Figure 3), while the liver histology of rats showed no histopathological alteration on day 15 and 30. In treated group with 250 mg MO and CCL_4, sever dilatation and congestion in the portal vein and inflammatory cells infiltration were examined on day 1 (Figure 4),while focal necrosis detected on day 15 and 30 post administration. Liver of rats after treatment with 500mg MO and CCl_4 exhibited mild congestion in the portal vein and sinusoids (Figure 5), whereas liver of rats returned to normal on day 15 and 30. However, MO a lone did not induce any morphological changes (Figure 6).

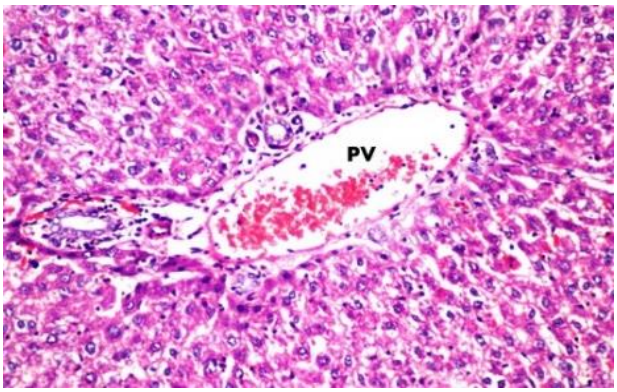

Figure 5: Liver section in treated 250mg MO+CCl_4 group: Mild congestion in the portal vein (PV) and sinusoids on day 1 post administration (H&E×40).

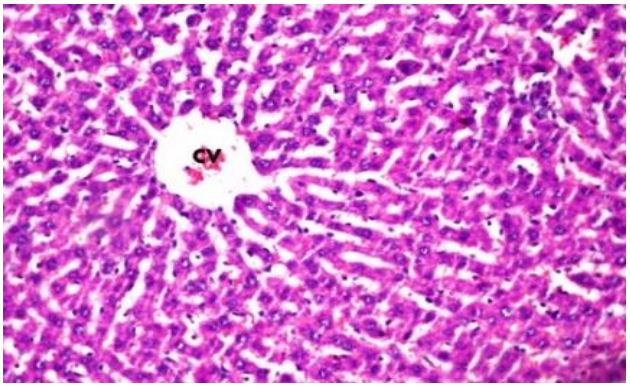

Figure 6: Liver section in control 250mg MO: Normal histological structure on day1 post administration (H&E×40). Similar result was found with 500mg MO.

DISCUSSION

The result of phytochemical screening of MOSE revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, resin, proteins, terpenoids, gums, anthraquinones, fixed oil and fat. The results obtained were consistent with previous studies on seeds of MO.[19] However, Emmanuel et al, reported absence of tannins, phenols, carbohydrates and resins in aqueous, methanol and ethyle acetate seed extract. In this investigation, cardic

glycosides was absent in MOSE.[20] This was in contrast with the finding of Auwal et al.[19]

Ncube et al, reported that the variations in the existence of the phytochemicals may be due to the choice of solvent used in extraction.[21] During extraction, solvents may have diffused into the plant material and solubilised compounds with similar polarity.

Administration of 2.5ml/kg CCl_4 induced hepatocellular damage as (fatty degeneration and fibrosis) causing leakage of AST and ALT into the circulation that is normally cytoplasmic in location. ALT was found in the hepatic parenchymal cells of the liver in large amount and regarded as more liver specific to test hepatocellular damage, while AST was found in mitochondria particularly in the centrilobular region of the liver.[22] Thus, AST and ALT are considered the better markers for detecting liver damage. Markers of hepatocellular damage (AST and ALT) increased significantly in the group intoxicated with CCl_4 alone. Whereas, the extract markedly reduced the activities of these liver function enzymes, of which administration of 500mg/kg MO appeared to have the best result when compared with silymarin.

Data showed that CCl_4 treatment significantly decreased serum total protein and albumin level compared to control group. Significant decrease in serum albumin had been associated with active cirrhosis and biliary liver damages leading to reduction in the number of hepatocytes which in turn, may result in decreased hepatic ability to synthesize protein.[23] The protective effect of MO was evident when the enhancement of total protein and albumin level was observed in rats given MO in combination with CCl_4.

Higher serum total bilirubin level was detected in groups that received CCl_4 alone compared to control group. Rise in the level of total bilirubin than normal must have been due to liver damage and fibrosis formed in the portal area which in turn, impaired bilirubin excretion.[23] Following administration of MO extract with CCl_4 for one month, there was a significant reduction in total bilirubin level toward normal value compared to CCl_4 group. Elevated level of serum triglycerides in CCl_4 group were restored to normal level by the administration of the MO extract.

In this study, CCl_4 induced massive damage to liver tissue in the form of extreme fibrosis in portal area, congestion and dilatation in central vein and fatty change in hepatocytes. Similarly, CCl_4 has also been reported to produce excessive fibrosis, cellular inflitaration and vaculor degeneration of hepatocytes.[24] The mechanism of CCl_4 injury involves oxidative damage by generation of reactive oxygen species (ROS) from biotransfornation of CCl_4 to CCl3. The high level of ROS is known to cause destruction of antioxidant enzyme activities and considerably leads to oxidative stress. Oxidative stress in turn induces oxidative degeneration of membranes of hepatic cell to cause lipid peroxidation of the lipid membranes and leakage of biomarkers like malondialdehyde.[25]

The study results are consistent with a previous study in which the level of serum and hepatic MDA greatly increased in CCl_4 group compared to control group. Treament of MO at both doses significantly reduced elevated level of MDA. Administration of MO showed protection against lipid peroxidation and suggested the capacity of MO to rapidly detoxify reactive toxic metabolites of CCl_4. These results are in accordance with Uma et al.[26]

The benefits of MO extract are confirmed by histopathalogical observations. Our findings revealed that treatment with MO at dose of 500mg and CCl_4 appeared to have a remarkable effect than 250mg MO, as evidenced by return the levels of AST, ALT, total protein, albumin, triglyceride and MDA toward normal level. However, 500mg MO and CCl_4 results of biochemical parameters nearly similar to silymarin and may be better in histopathological examination in which the ability of 500mg MO to reverse the apoptotic hepatocellular injury induced by the CCl_4 to a large extent compared to silymarin.

In the present investigation, the amelioration role of MOSE against CCl_4 intoxication may be attributed to potential involvement of constituents such as flavonoids, tannin, alkaloids and saponin (evident by preliminary phytochemical screening of MO seeds). These constituents are reported to have antioxidant, scavenging properties and inhibition cytochrome p-450 aromatase.[27-31] Antioxdiants provide protection or remediation by scavenging reactive oxidative species (ROS) that damage DNA and initate diseases.[31] Thus, the ability of MO itself to act as a free radical scavenger by trapping reactive oxygen species and hindering interaction with polyunsaturated fatty acids could also clarify lipid peroxidation inhibition and therefore could reduce the risk of cancer and degenerative diseases.[32]

The present results showed that CCl_4 administration significantly decreased the RBC count and Hb level however, the level of WBC and lymphocyte count significantly increased ($P < 0.05$) as compared to control. The results observed agree with that reported by Eshak et al, and Elshater et al, who found that the administration of CCl_4 to rats led to significant decrease of RBC counts and Hb level and significant increase of WBC counts in respect to control.[24,34] The depression in Hb content and RBCs count might be attributed to the toxicity of CCl_4. This toxicity lead to decrease in the Hb concentration and RBCs counts. Similarly, Elshater et al, revealed that the depression in RBCs count and Hb level due to CCl_4 treatment could be attributed to disturbed hematopoiesis, excessive destruction of erythrocytes, reduction in the rate of their formation.[33] On the other hand, the WBCs count significantly elevated in CCl_4 group compared to control group on day 1 post administration. This may be attributed

to the defensive mechanism of immune system, so the ability of free radical to increase WBCs count indicates that these radicals to an extent affected the defense mechanism of treated rats.[34]

The treatment with MOSE showed a remarkable enhance of the hematological parameters especially in high dose of (500mg/kg). This could be due to the phytoconstituents in the extract. These constituents as flavonoids are known to have antioxidant properties and vasculo-protector against CCl_4 by reducing the accumulation of toxic CCl_4 derived metabolites.[35] Also, these constituents are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow.

CONCLUSION

In conclusion, the present study proved that MOSE especially at dose of 500mg can prevent hepatotoxicity and hemotoxicity induced by CCl_4 in rats. The presence of bioactive compounds in *Moringa oleifera* seeds is suggestive of its protective mechanism in preventing CCl4 induced liver injuries. This phytocompounds have antioxidant and free radical scavenging activity.

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REFERENCES

1. Fakurazi S, Sharifudin SA, Arulselvan P. Moringa oleifera hydroethanolic extracts effecively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules. 2012;17:8334-50.
2. Sabir SM, Rocha IB. Water-extractable phytochemicals from Phyllanthus niruri exhibit distinct in vitro antioxidant and in vivo hepatoprotective activity against paracetamol-induced liver damage in mice. Food Chemistry. 2008;III:845-51.
3. Mehta J, Shukla A, Bukhariya V, Charde R. The magic remedy of Moringa oleifera: an overview. International Journal of Biomedical and Advanced Research. 2011;2(6):215-27.
4. Siddhuraju P, Becker K. Antioxidantproperties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. J Agr Food Chemis. 2003;51:2144-55.
5. Ogbunugafor HA, Eneh AN, Ozumba MN, Igwo-Ezikpe, J, Okpuzor IO, Igwilo SO, et al. Physico-chemical and antioxidant properties of Moringa oleifera seed Oil. Pak J Nutrit. 2011;10(5):409-14.
6. Caceres A, Saravia A, Rizzo S, Zabala L, De-Leon E, Nave F. Pharmacological properties of Moringa oleifera screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol. 1992;36(3):233-7.
7. Fahey J. Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Trees Life J. 2005;1(5).
8. Dahot MU. Vitamin contents of flowers and seeds of Moringa oleifera. Pak J Biochemi. 1988;21:1-24.
9. Harborne JB. Phytochemical methods, a guide to modern techniques of plant analysis. London: Chapman and Hall, Ltd.; 1973:49-188.
10. Balbaa SI. Chemistry of crude drugs. laboratory manual. Faculty of Pharmacy, Cairo University, Al-Shaab Printing House. 1986;195.
11. Evans WC, Evans D. Trease and Evans Pharmacognosy, 15th Ed, W.B. Saunders, Michigan; 2002:585.
12. Sofowara AE. Medicinal plants and traditional medicine in Africa. 2nd ed. Ibadan, Nigeria: Spectrum books Ltd.; 1993:289.
13. Whistler RL, BeMiller JN. Industrial gums: Polysaccharides and their derivatives. 3rd Ed, Academic Press, UK; 1993:642.
14. Gahan PB. Plant histochemistry and cytochemistry. Academic Press, Florida; 1984.
15. Kokate CK. Practical pharmacognosy, 4th Ed, Vallabh Prakashan, New Delhi, India; 2008:107-111.
16. Chinedu AA, Olanrewaju S, Alani SO, Olaide AO. Effect of the ethanolic leaf extract of Moringa oleifera on insulin resistance in streptozotocin induced diabetic rats. J Pla Scienc. 2014;2(6-1):5-12.
17. Jangir RN, Jain GC. Antidiabetic and antioxidant potential of hydroalcoholic extract of Moringa oleifera leaves in streptozotocin-induced diabetic rats. Eur J Pharma Med Res. 2016;3:438-50.
18. Ohkawa H, Ohishi N, Yagi K. Assay for liquid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979;95:351-8.
19. Auwal MS, Tijjani AN, Sadiq MA, Saka S, Mairiga IA, Shuaibu A, et al. Antibacterial and haematological activity of Moringa oleifera aqueous seed extract in wistar albino rats. Sok J Veteri Scienc. 2013;11(1):28-37.
20. Emmanuel S, Olajide O, Abubakar S, Idowu I, Orishadipe A, Thomas S. Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of Moringa oleifera seeds. Ame J Ethnomed. 2014;1(5):346-54.
21. Ncube N, Afolayan A, Okoh A. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol. 2008;7(12):1797-806.
22. Amacher DE. Serum transaminase elevations as indicators of hepatic injury following the

administration of drugs. Reg Toxicol Pharmacol. 1998;27:119-30.
23. Shukla A, Bhatia SJ. Outcome of patients with primary hepatic venous obstruction treated with anticoagulants alone. Ind J Gastroenterol. 2010;29:8-11.
24. Eshak MG, Hassanane MM, Farag IM, Shaffie NM, Abdalla AM. Evaluation of protective and therapeutic role of Moringa oleifera leaf extract on CCl_4-induced genotoxicity, hemotoxicity and hepatotoxicity in rats. Int J Pharm Tech Research. 2015;7(2):392-415.
25. Shim JY, Kim MH, Kim HD, Ahn JY, Yun YS, Song JY. Protective action of the immunomodulator ginsan against carbon tetrachloride-induced liver injury via control of oxidative stress and the inflammatory response. Toxicol Applied Pharmacol. 2010;242(3):318-25.
26. Uma N, Fakurazi S, Hairuszah I. Moringa oleifera enhances liver antioxidant status via elevation of antioxidant enzymes activity and counteracts paracetamol-induced hepatotoxicity. Mal J Nutriti. 2010;16(2):293-307.
27. Korkina LG, Afanas'ev IB. Antioxidant and chelating properties of flavonoids. Adv in Pharmaco. 1997;38:151-63.
28. Shekhar TC, Anju G. Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. leaves. Ame J Ethnomedic. 2014;1(4):244-9.
29. George F, Zohar K, Harinder PS, Makkar KB. The biological action of saponins in animal systems: a review. Bri J Nutrit. 2002;88:587-605.
30. Rausch W, Liu S, Gille G, Radad K. Neuroprotective effects of ginsenosides. Acta Neuro Experimen J. 2006;66:369-75.
31. Sathya TN, Aadarsh P, Deepa V, Balakrishna MP. Moringa oliefera Lam, leaves prevent cyclophosphamide-induced, micronucleus and DNA damage in mice. Int J Phytomed. 2010;2:147-54.
32. Prasanna V, Sreelatha S. Synergistic effect of Moringa oleifera attenuates oxidative stress induced apoptosis in Saccharomyces cerevisiae cells: evidence for anticancer potential, Int J Phar and Bio Scienc. 2014;5(2):167-77.
33. Elshater AA, Salman MMA, Moahmed S. The hepato-ameliorating effect of Solanum nigrum against CCL_4 induced liver toxicity in albino rats. Egy Acad J Bio Scienc. 2013;5(10):59-66.
34. Patrick-lwuanyanw KC, Wegwu MO, Ayalogu EO. Prevention of CC14 induced liver damage by ginger, garlic and vitamin E. Pak J Bio Scienc. 2007;10(4):617-21.
35. Mada SB, Inuwa HM, Abarshi MM, Mohammed HA, Aliya A. Hepatoprotective effect of Momordica charantia extract against CCL_4 induced liver damage in rats. Bri J Pharma Resear. 2014;4(3):368-80.

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