Original Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

A COMPARATIVE STUDY OF THE EFFECTS OF THREE *MORINGA* SPECIES ON OBESITY- INDUCED OXIDATIVE STRESS STATE IN LIVER TISSUE

ELHAM M.YOUSSEF ELABD[1*], ZAHRAN, H. A[2], ABOELFETOH M. ABDALLA[3]

[1]Biochemistry Department, National Research Center, Egypt.
[2]Fats and Oils Department, National Research`````` Center, Egypt.
[3]Horticulture Technology Department, National Research Center, Egypt.

ABSTRACT

Excessive consumption of a diet rich in fat leads to obesity and progressive forms of liver abnormalities due to oxidative stress. In the present study, antiobesity and antioxidant activity of oil extracted from seeds of three *Moringa* species, Moringa *peregrina*, *Moringa stenopetala, and Moringa oleifera* were evaluated and confirmed with histopathological examination of rat liver tissues. Results showed that administration of high fat diet induced liver damage, increased body weight, liver biomarkers, lipid peroxide levels while reduced antioxidant enzyme levels. Treatment with the three types of *Moringa* oil resulted in a significant (P<0.05) change in body weight, a significant (P<0.05) increase in antioxidant enzymes and a significant decrease in liver biomarker levels. However, histopathological examination confirmed that M. *stenopetala* significantly showed hepatocyte restoration. In conclusion, *Moringa peregrina*, *Moringa stenopetala, and Moringa oleifera* have antiobesity and antioxidant activity, however, *M.stenopetala* has served as the most effective type in reduction adiposity and hepatic steatosis.

KEYWORDS: **Moringa, oxidative stress, liver, anti-oxidant, obesity. high fat diet**

***Corresponding Author**

ELHAM M.YOUSSEF ELABD

Biochemistry Department, National Research Center, Egypt.

Received on : 31-01-2017
Revised and Accepted on : 20-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b572-584

INTRODUCTION

Obesity-induced oxidative stress has been related to serious metabolic dysfunctions including liver diseases. Available evidence suggests that excessive intake of either fat and or carbohydrate diet efficiently stimulates fat storage as triglycerides (TG) in hepatic tissue and other non-adipose tissues.[1-3] Such dysregulated fat accumulation promotes oxidative stress by excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[4,5]In an attempt to protect the living cell and to maintain cellular redox homeostasis, a highly effective antioxidant defense system in living organisms attenuates lipid peroxidation by scavenging free radicals . Antioxidant activity has been shown to be dependent upon two well-known essential forms of anti-oxidants, enzymatic and non-enzymatic .The major enzymatic antioxidant component includes superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx).[6-8] While the non-enzymatic antioxidant includes low-molecular-weight compounds, such as vitamins: C (ascorbic acid) and E (α-Tocopherol), carotenoids (β-carotene) and others, like flavonoids and phenolic compounds.[9,10] Recently, a great number of natural plants has shown to be considered as new approach to combat obesity and associated liver disease. The bioactive compounds that are responsible for relieving such stress are believed to be polyphenols and flavonoids compounds.[11,12] *Moringa* plant, a small genus comprising 13 species of trees and shrubs, has attracted growing interest recently in this field. Various parts of this plant such as leaves, roots, seed, bark, fruit, flowers have broad medicinal properties .However, results of previous laboratory animal study showed limited evidences about the anti-obesity and antioxidant potential of seed oil extracted from *moringa* species .Therefore, our study is to focus on the antioxidant activity of oil extracted from seeds of three *Moringa* species, *pere*grina, *stenopetala*, and *oleifera* and to compare their effects on weight gain ,lipid profile ,and liver oxidative stress induced by high fat diet (HFD).

MATERIAL AND METHODS

Experimental animals.

Thirty male Wistar rats were obtained from the modern veterinary house Cairo, Egypt. The animals were randomly divided into 5 groups of 6 animals each. They had free access to either a normal diet (fat content 3% of energy), or HFD (fat content 20% based on previous studies (Nascimento et al, 2008; Francia-Farje et al,2010).[13,14] It consists of beef tallow 15% and corn oil 5%).Three groups were administrated (0.5 ml/Kg) moringa oil orally for 8 weeks after HFD feeding .The other two groups were control (normal diet fed group) and group IV(only HFD fed group).

1- Group I: Rats received HFD then treated with *M.peregrina* oil.

2-Group II: Rats received HFD then treated with *M.stenopetala* oil.

3-Group III: Rats received HFD then treated with *M.oleifera* oil.

4-Group IV: Rats received HFD only.

5- Control group: Rats received normal diet.

Body weights of all groups were recorded at the beginning and at the end of the experiment. At the end of the treatment weeks, animals were fasted overnight, blood samples were collected in the morning from the retro-orbital for the biochemical parameters assays. All animals were then sacrificed by cervical dislocation. For histology, a small portion of liver tissue was preserved immediately in 10% buffered formalin fixative. The rest of the liver tissue was kept in -20 °C for hepatic antioxidants and malondialdehyde (MDA) level analysis. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of the Committee.

Moringa seed oil extraction.

Moringa seeds were provided from Horticulture Technology Department at the National Research Center, Egypt .The oil extraction from ground Moringa kernels was performed in fats and oils department ,National Research Center , (Giza) Egypt according to the Soxhlet extraction method with a modified preparatory step , preheating at 60^{o} C for 1 hour , to facilitate the extraction from seeds. Commercial hexane was used as extraction solvent at a ratio of 1:10 based on method described by Bhatnagar and Gopala Krishna[15], 2013.

Chemical quality properties and characterization of Moringa oil.

Acidity % was measured according to the method adapted from IUPAC (1987). [16] While

Peroxide value (PV) and iodine value were estimated by using Wijs procedures described in Association of Official Analytical Chemist AOAC (2000).17

Gas chromatography analysis of fatty acids.

Preparation of fatty acid methyl esters (FAME).

Fatty acids methyl esters were prepared according to the method recommended by Li and Watkin (1998).[18] The fatty acids methyl esters FAME was reconstituted in a volume of hexane appropriate for the conditions of GC analysis and FAME concentration. Sample vials with Teflon caps were transferred to GC for analysis.

Separation and identification of fatty acid methyl esters by gas liquid chromatography.

The methyl ester of the fatty acids standard compounds were analyzed with a Perkin Elmer Auto System XL (GC) gas chromatography equipped with flame ionization detector (FID), Fused silica capillary column ZB-Wax (60 m x 0.32 mm id). The oven temperature was programmed in two stages as follows: First, the column temperature was increased at 40°C , held at 40°C for 5 min and then from 40 to 220°C at rate 3°C /min. Detector and injector temperature were generally 250, 230°C; respectively. The carrier gas (helium) flow rate was 1 ml/min.

Biochemical assays in serum and liver homogenate.

Hepatic marker enzymes (aspartate transaminase (AST), alanine transaminase (ALT)), serum total cholesterol (Tc), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc) triacylglycerol (TAG) and glucose were all assayed

using kits purchased from Biodiagnostic Co., Cairo, Egypt.Lipid peroxidation (MDA), antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were estimated using kits purchased from Biodiagnostic Co.,Cairo,Egypt.

STATISTICAL ANALYSIS

Data were analyzed by SPSS version 16, described as means ± standard error of mean for measurement, and compared by one-way ANOVA. When P<0.05, the difference was considered statistically significant.

RESULTS

Moringa seed oil (Extract) characterization

In vitro antioxidant analysis of the oil extracted from the three studied *Moringa* species has shown a considerable antioxidant activity with some variations among them. Both *M.stenopetala* and *M.peregrina* have more phenolic compounds and DPPH (diphenylpicrylhydrazyl) scavenging activity than *M. oleifera* ,while *M.Stenopetal* has more pamitic and lionleic acid percentages.(Table *1, Fig.1,2,3,4).*

Effect of studied Moringa seed oil extracts on liver enzymes, lipid profile, and weight change.

Tc ,LDLc, AST, ALT ,glucose, and body weights significantly (P<0.05) increased, while HDL significantly (P<0.05) decreased in rats fed on HFD (Group 4) compared with rats fed on normal diet (control group) . Post-administration of any of the *Moringa* seed oil extracts improved glucose, lipid profile and liver enzymes significantly (P < 0.05) compared with rat group fed on HFD (Group 4), (Table 2,Fig.5,6).

Effect of studied Moringa seed oil on hepatic antioxidants and MDA level.

A significant (P<0.05) increase in MDA and a significant (P<0.05) decrease in antioxidant enzymes levels in rats fed HFD (Group 4) compared with those fed on the normal diet.

After treatment with the three species of *Moringa* seed oil extract(Group 1,2,3), the MDA significantly (P<0.05) decreased and the antioxidant enzymes levels significantly (P<0.05) increased compared with rats fed on HFD .Two of the three types of *Moringa* seed extract oil , *M. peregrina* and *M. stenopetala* (Group 1 &2) were found to cause changes in levels of antioxidant enzymes and MDA towards the normal control levels as shown in Table 3,Fig.7.

Histopathological examination of liver tissue

The assessment of histological profiles of rat livers (Fig.8) showed that the control group has normal liver profiles with no histopathological changes (A). In group receiving HFD for 8 weeks there was a marked distortion of the liver cytoarchitecture with marked fatty change and steatosis (B). Post-treatment of HFD fed rats with different types of *Moringa* oil exhibited a remarkable improvement in their histological profiles especially those treated with *M. stenopetala*, which is considered to have the most effective action on restoring the liver tissue among the other studied seed oil extracts. Their examined sections revealed normal hepatic lobule with no histopathological changes (C).Whereas the examined sections of rats treated with *M. peregrina* revealed slight granular degeneration of hepatocytes and slight congestion of hepatic sinusoids (D). Moreover examined liver sections from rats treated with *M.oleifera* showed slight vacuolation of hepatocytes and dilatation of hepatic sinusoids (E).

Table1
Summary of means and SEMs. of Moringa seed oil (Extract) characterization.

Parameter	M. peregrina	M. stenopetala	M. oleifera
Total Phenol Content (mg/kg)	12.6±0.85	12.19 ± 0.56	10.7± 0.15
DPPH Scavenging Activity (mMolTrolox equivalent/kg)	172 ± 0.33	163.1 ± 0.14	101 ± 0.7
Acidity (%)	0.19 ± 0.02	0.25 ± 0.01	0.21 ± 0.03
Peroxide value (meq./kg)	2.1 ± 0.11	1.8 ± 0.31	2.3 ± 0.23
Iodine value (g/100g)	67.3 ± 0.37	65.6 ± 0.75	66.3 ± 0.8
Fatty acid composition (%):			
Palmitic acid	10.0	11.6	7.6
Linoleic acid	0.42	0.80	---
Oleic acid	78.33	72.34	74.62
Linolenic acid	2.75	6.14	6.06
Stearic acid	4.85	4.72	5.63
Arachedic acid	1.42	1.55	2.29
Behenic acid	2.15	2.87	3.84

Table 2
Summary of group means and S.E.Ms. of biochemical parameters in studied

Parameters	Group1	Group 2	Group 3	Group 4	Control
Tc (mg/dl)	159 ± 9.4[b]	146±10.76[b]	166± 12.09[b]	196 ± 11.5[a]	108± 7.69
TG (mg/dl)	144 ± 1.63[b]	125 ± 2.67[b]	130 ± 8.90[b]	177 ± 12.90[a]	89± 5.19
HDLc (mg/dl)	36 ± 4.72[b]	38 ± 2.32[b]	40 ± 1.21[b]	30 ± 1.87[a]	69 ± 1.47
LDLc (mg/dl)	90 ± 9.01[b]	67 ± 5.43b	92 ± 16.77[b]	179 ± 12.54[a]	117± 6.31
Glucose (mg/dl)	111 ± 14.57[b]	115 ± 6.02[b]	108 ± 4.23[b]	166 ± 20.25[a]	71± 2.16
AST (U/L)	72.83 ± 8.66[b]	58 ± 4.45[b]	73 ± 11.44[b]	175 ± 18.05[a]	19± 1.72
ALT (U/L)	71 ± 17.01[b]	51 ± 7.33[b]	88± 3.21[b]	201 ± 16.17[a]	19± 2.16
Initial weight (g)	106 ± 8.16[b]	100 ± 5.65[b]	110.83 ± 7.4[b]	111 ± 8.16[a]	118± 6.22
Final weight (g)	216 ± 12.11[b]	220±11.40[b]	231± 9.31[b]	256 ± 14.02[a]	210± 18.97

Data are presented as the mean ± S.E.M; a P < 0.05 compared with control, b P <0.05 compared with Group 4, n=6.

Table 3
Summary of group means and S.E.Ms. of MDA and antioxidant enzymes in studied groups.

Parameters	Group1	Group 2	Group 3	Group 4	Control
Lipid peroxide (MDA)(nmol\g)	59.80 ± 9.83[b]	45.88 ± 4.52[b]	69.15 ± 11. 5[b]	81.05 ± 6.78[a]	40.33 ± 5.78
Glutathione Peroxidase (U\g)	24.17 ± 6. 9[b]	37.82 ± 5.9[b]	21.07 ± 3.37[b]	12.01 ± 1.8[a]	40.12 ± 2.13
Catalase (U\g)	5.05± 0.4[b]	6.66 ± 0.2[b]	3.8± 0.6	3.57 ± 0.27[a]	6.97 ± 0.72
Superoxide dismutase(U\g)	11.33 ± 1.42[b]	12.69 ± 2.1[b]	8.29 ± 1.2[b]	5.28 ± 0.42[a]	15.27 ± 2.9

Data are presented as the mean ± S.E.M; a P < 0.05 compared with control, b P <0.05 compared with Group 4, n=6.

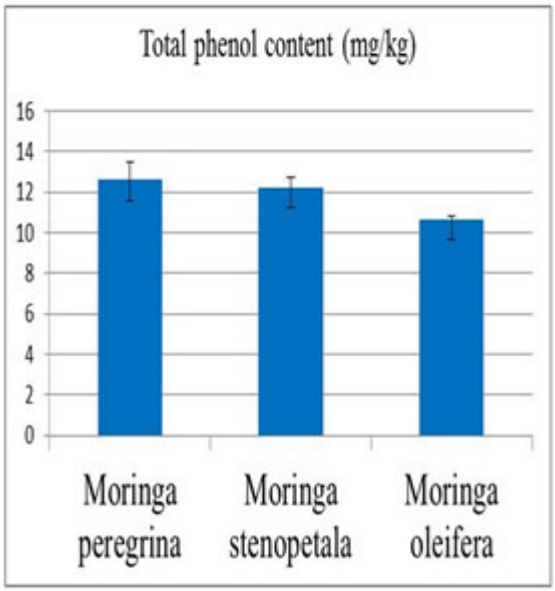
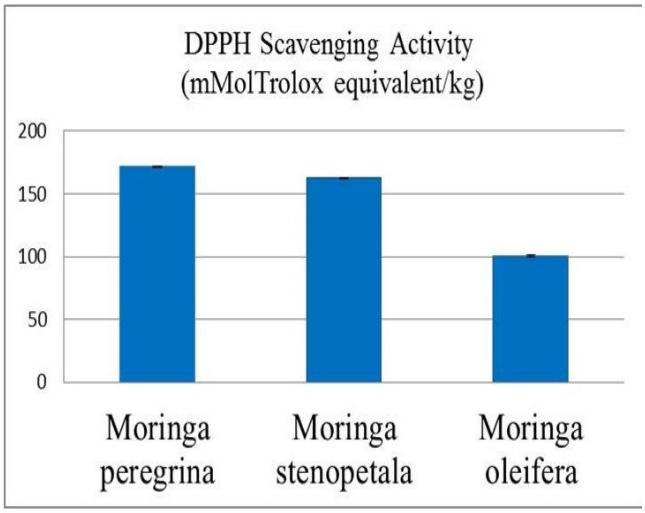
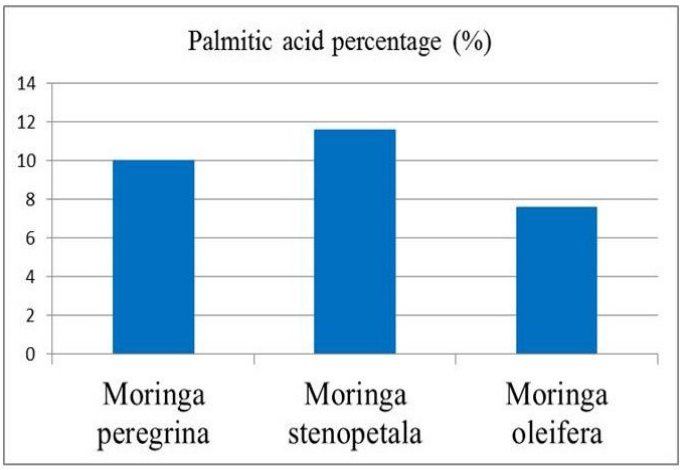


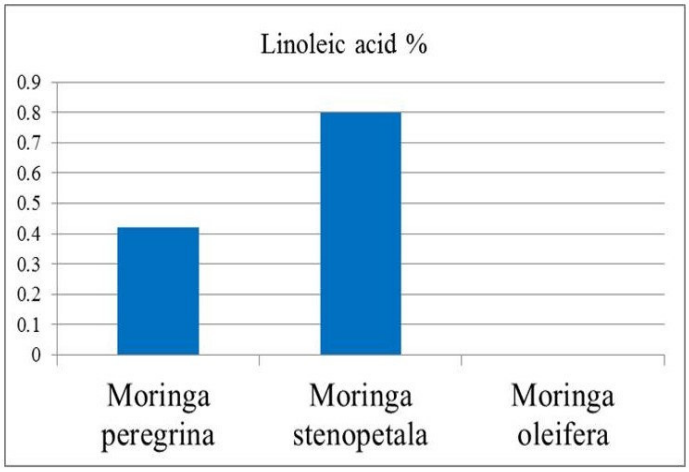
Figure 1
Total phenol content(mg/Kg) in Moringa peregrina, Moringa stenopetala,and Moringa oleifera seeds oil



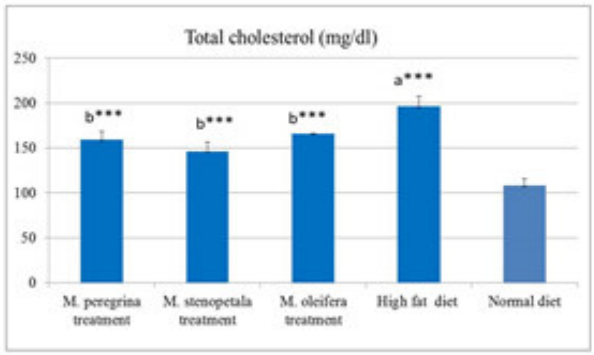
Firgure 2
DPPH scavenging activity (mMol Trolox eauivalent/kg) in *Morings peregrina* ,*Moringa stenopetala*,and *Moringa oleifera* seeds oil

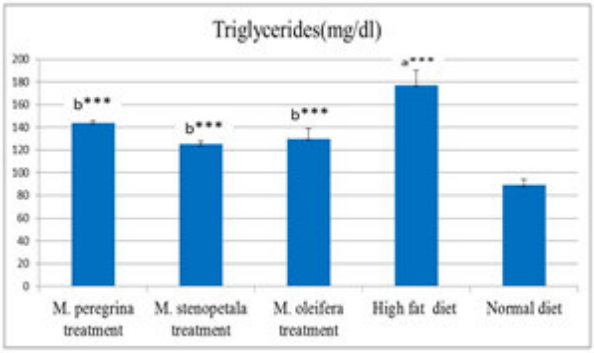


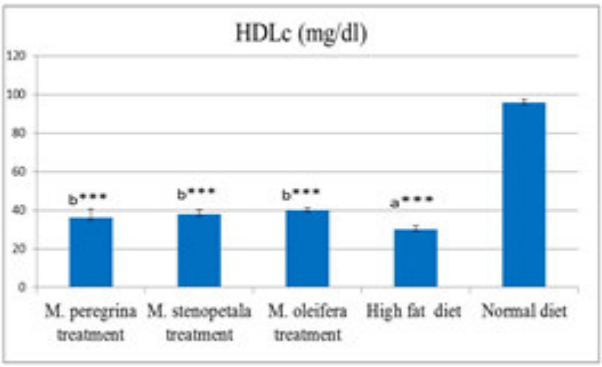
Firgure 3
Palmitic acid (%) *Moringa peregrina*,*Moringa stenopetala*,and *Moringa oleifera* seeds oil

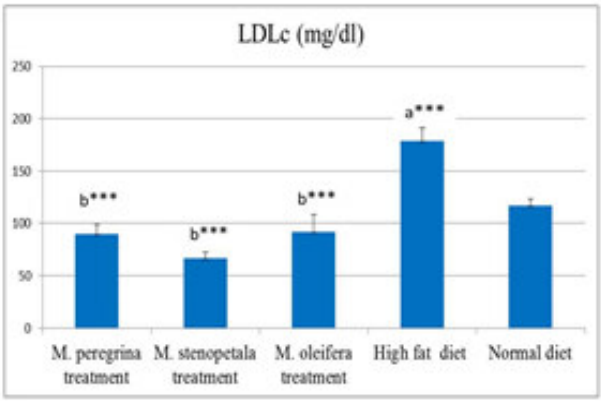


Firgure 4
Linoleic acid (%) *Moringa peregrina,Moringa stenopetala*,and *Moringa oleifera* seeds oil

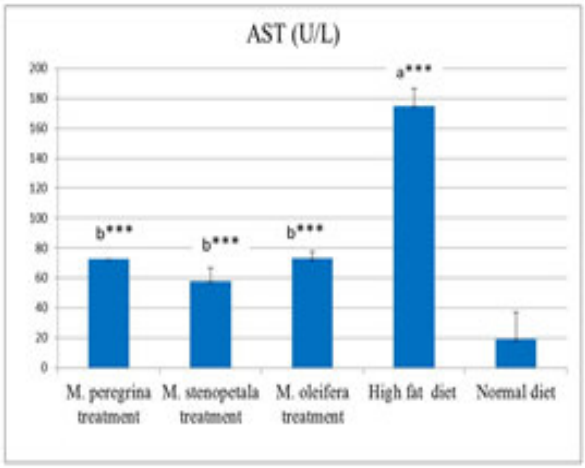


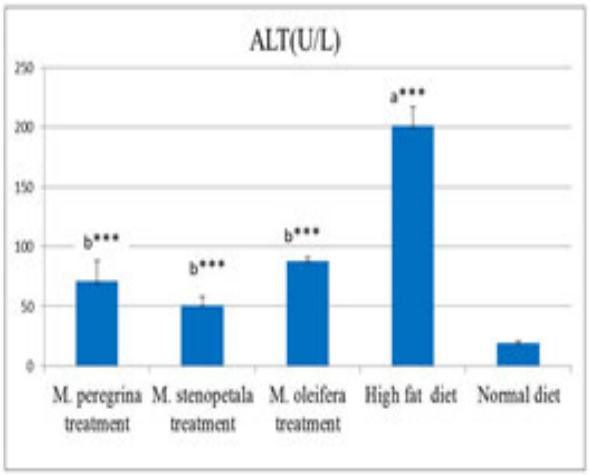






Firgure 5
Effect of *Moringa peregrina* ,*Moringa stenopetala*,and *Moringa oleifera* on Total cholesterol,Triglycerides,HDLc,and LDLc





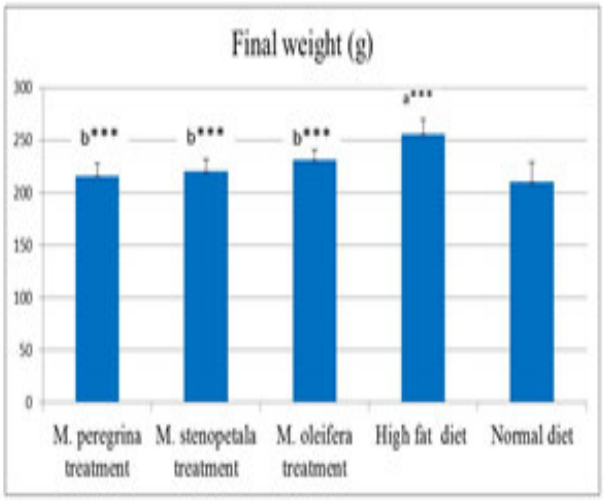
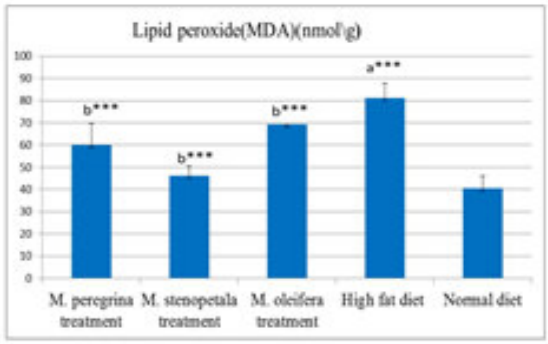
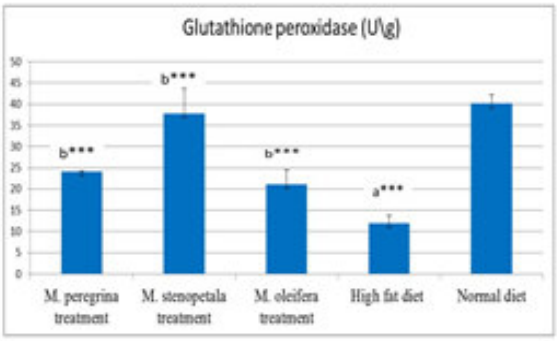
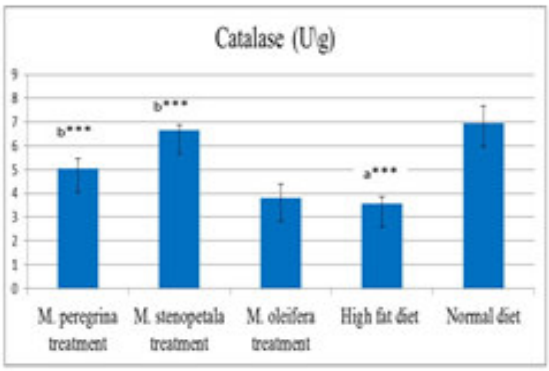


Figure 6
Effect of *Moringa peregrina* ,*Moringa stenopetala*,and *Moringa oleifera* on AST,ALT,and final weight.







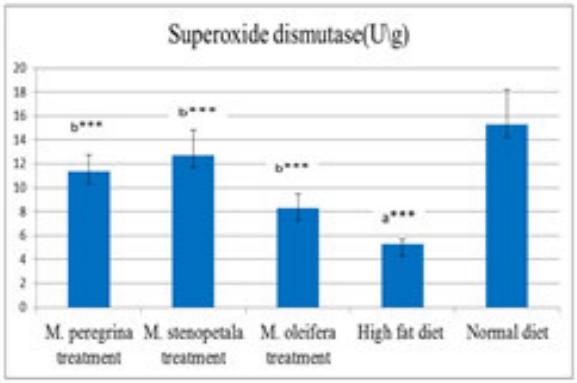


Figure 7
Effect of Moringa peregrina ,Moringa stenopetala,and Moringa oleifera on lipid peroxide,glutathione peroxidase,cstalase,and superoxide dismutase

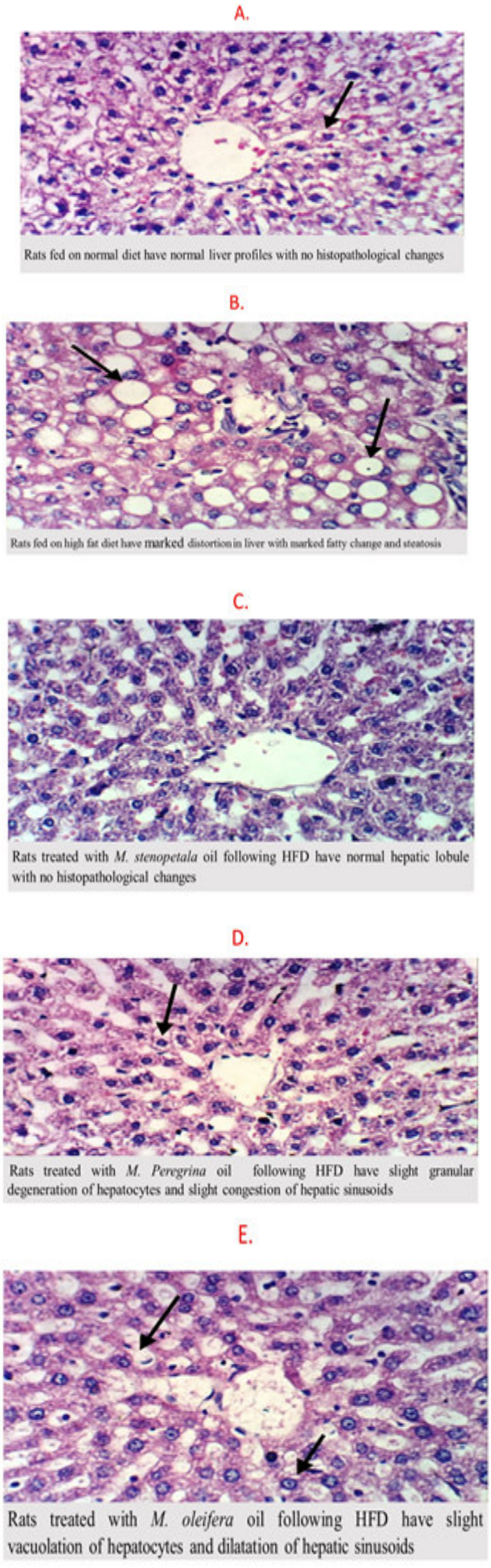


Figure 8
Changes in obese rat livers following treatment with Moringa stenopetala, Moringa peregrina and Moringa oleifera seeds oil

DISCUSSION

Adequate evidence has demonstrated that obesity induces steatosis and increases oxidative stress. This condition is characterized by reduction in antioxidant enzyme activities and increment in MDA .[19-23] A number of studies have examined drugs for the treatment of hepatic tissue injury.[24,25], while others have been directed towards new treatment strategies by using herbal medicine[26]. Moringa oleifera is among plants which has wide pharmaceutical actions. However limited studies reported about *Moringa* oleifera and other *Moringa* species as a potential therapeutic agent for the treatment of obesity and associated hepatic injury . Therefore, further research in this area and a closer look at the antioxidant activities of its species is still needed. The long period treatment with HFD promotes excess adipose tissue ,oxidative stress and other metabolic changes.[27-32] The present study thus proved that body weights, lipid profile, and MDA (the oxidative stress marker) were found significantly ($P<0.05$) increased in rats fed on HFD compared with control thus verifying the obesity status and oxidative stress. Vincent et al. 2001 and Olusi SO 2002, explained that the excess of lipid peroxidation in obesity is due to ROS generated from the progressive and cumulative cell damage.[33,34] Additionally, high triglyceridemia may contribute to the alteration in the oxidant- antioxidant balance, leading to an increment of the bioavailability of the free fatty acids and consequently the oxidative deterioration of lipids.[35] Further increase in superoxides, H_2O_2, hydroxyl radicals and lipid peroxidation consequently cause decrease in the effectiveness of antioxidant defenses, like SOD, CAT and GPx.[35,36] Consistent with this view, our data provide further evidence about the disturbance of the antioxidant enzyme activities SOD, CAT, and GPx levels of rats fed on HFD as compared with rats of the control group. High levels of circulating liver enzymes, AST and ALT in HFD group rats together with the assessment of the histological profiles of their liver tissue indicated a severe damage to its structural integrity, this might be due to altered permeability of membranes of the hepatocyte. This is agreed with previous studies which suggested potential mechanisms for the liver oxidative stress damage ; ROS and lipid peroxidation products deteriorated the respiratory chain in hepatocytes through oxidative damage to the mitochondrial genome.[37,38] These features, in turn, lead to the generation of more ROS together with lipid peroxidation products and activate stellate cells resulting in fibrosis.[39,40] Treating rats fed on HFD with the three types of *Moringa s*eed oil extracts caused significantly ($P<0.05$) increase in antioxidant enzymes , and significantly ($P<0.05$) decrease in body weights, Tc, TG, LDLc, liver biomarkers, and MDA levels (the marker of hepatic oxidative injury) which is confirmed by histopathological evidence .These results are in agreement with previous studies done by Denen Atsukwei et al in 2014 and Mustafa A et al in 2016 who concluded that *Moringa oleifera* plant acts as an exogenous antioxidant source, that can complement the antioxidant defense system in restoring the hepatic cell integrity.[41,42] The significant reduction in body weights following the treatment with the oil extracts is in agreement with Alberts 1980, Altschu 1964, and Patil et al. 2010 who explained that it may be referred to inhibition of cholesterol deposition in body tissues or inhibition of hydroxylmethylglutaryl-CoA reductase activity which is the key regulatory enzyme in cholesterol biosynthetic pathway.[43-44] According to Mathur et al[45]; the hypolipidaemic effect of different medicinal plants has been related to bioactive components like alkaloids, tannins, and cardiac glycosides. Temitope M and his colleagues in 2014 explained that tochopherols and other antioxidants in *Moringa oleifera* seed oil has a significant protective activity against oxidative damage and play a central role along with fatty acids in decreasing the MDA levels.[46] This is suggested to occur by suppression of reactive oxygen species formation, the inhibition of enzymes or chelating of elements involved in the free radical production, scavenge reactive species, and upregulate antioxidant defenses. We observed a significant difference of anti-oxidative activity percentages and variations in fatty acid levels among the three studied oil extracts. As can be seen in both *M.peregrina* and *stenopetala*, phenolic compounds and DPPH scavenging activity are higher than those in *M.oleifra*. This may explain the GPT significant decreasing in rats treated with them after receiving HFD for 8 weeks and approaching normal levels more than those treated with *M oleifera* seed extracted oil .This might have more effective action in modulating the structural integrity of the hepatic cell and its membrane damage caused by the HFD than *M.oleifera* might have. There was also a significant ($P<0.05$) increase in antioxidant enzymes and a significant ($P<0.05$) decrease in MDA levels in rat groups treated with the same two oil extracts than those treated with *M.oleifera* seed extract oil.The obtained biochemical analysis revealed that both *M peregrina,* and *M. stenopetala* significantly modulated the oxidative stress caused by feeding on HFD However, the histopathological studies presented that *M.stenopetala* have the most restoring effect on liver among the studied oil extracts as sections of this group revealed normal hepatic lobule with no histopathological changes. It can be suggested that the high percentages of palmitic and linoleic acids might contribute in decreasing hepatocyte oxidative damage as shown in previous work done.[47-49]

CONCLUSION

Data obtained from this study strongly confirm the antiobesity antioxidant activity of oil extracted from three *Moringa* species. However, in comparison to liver injury and pathological changes, *M. stenopetala* seems to have served as the most effective type in promoting and restoring liver functional integrity. Therefore, its potential is therefore believed to be largely attributable in reducing hepatic steatosis.

ACKNOWLEDGEMENTS

The study was financially supported by a grant from Science and Technological Development Fund in Egypt (STDF). The authors would like to thank Prof. Dr. Ferial A. Zaher at fats and oils department who provided

expertise that strongly assisted the research. They would also like to thank the research members in horticulture technology department for providing them with the *Moringa* seeds.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Gaggini M, Morelli M, Buzzigoli E, DeFronzo R.A., Bugianesi E, Gastaldelli A . Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients 2013; 5:1544–1560.
2. Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014; 2: 901–910.
3. Gaggini M, Saponaro C ,Gastaldelli A. Not all fats are created equal: Adipose vs. Ectopic fat, implication in cardiometabolic diseases. Horm. Mol. Biol. Clin. Investig 2015; 22: 7–18.
4. Milagro FI, Campión J, Martínez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. Obesity (Silver Spring) 2006; 14: 1118-23.
5. Savini I, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity associated oxidative stress: Strategies finalized to improve redox state. Int J Mol Sci 2013; 14:10497-538.
6. Halliwell B, Gutteridge JM. Lipid peroxidation: a radical chain reaction. Free radicals in biology and medicine. 1989;2:188-218.Halliwell, B. and Gutteridge, J.M.C. The chemistry of oxygen radicals and other derived species. In: Halliwell, B. and Gutteridge, J.M.C. (eds). Free Radicals in Biology and Medicine. 2nd edn. Oxford .Clarendon Press; 1989. p. 22-85 .
7. Frie B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci 1988; 37:569–71.
8. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annals of botany. 2003 Jan 2;91(2):179-94 E, Fagerstedt KV. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress a Review. Ann Bot 2002; 91:179–179.
9. Sies H. (ed.) Antioxidants in Disease, Mechanisms and Therapy, Academic Press, New York, 1996, pp. 1-691.
10. Kagan VE, Kisin ER, Kawai K, et al. Towards mechanism based antioxidant interventions. Ann N Y Acad Sci 2002; 959:188-98.
11. Augustyniak A, Waszkiewicz E, Skrzydlewska E. Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. Nutrition. 2005 Sep 30;21(9):925-32.Augustyniak A., Waszkiewicz E., Skrzydlewska E. Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. Nutrition 2005; 21: 925–932.
12. Singh, R.S.G.; Negi, P.S.; Radha, C. Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of Moringa oleifera seed flour. J. Funct. Foods 2013; 5: 1883–1891.
13. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Luvizotto RA, Nogueira CR, Cicogna AC. A hypercaloric pellet diet cycle induces obesity and co-morbidities in Wistar rats. Arq Bras Endocrinol Metabol. 2008;52(6):968–974
14. Francia-Farje LA, Silva DS, Volpato GT, Fernandes GS, Carnietto N, Cicogna AC, Kempinas Wde G. Sibutramine effects on the reproductive performance of pregnant overweight and nonoverweight rats. J Toxicol Environ Health A. 2010; 73 (13–14):985– 990.
15. Bhatnagar A.S. and A.G. Gopala Krishna. Natural antioxidants of the Jaffna variety of *Moringa Oleifera* seed oil of Indian origin as compared to other vegetable oils. Grasas y aceites 2013; 64:537-545.
16. Paguot C. Standard methods for the analysis of oils, fats and derivatives/International Union of Pure and Applied Chemistry; preparedfor publication by C. Paguot. International Union of Pure and Applied Chemistry. Commission on Oils, Fats and Derivatives .IUPAC, (1987). In International Union of Pure and Applied Chemistry, standard methods for the analysis of oils, fats and derivatives (7th Ed.). Oxford, UK: Blackwell Scientific Publications.
17. Trucksess M, Weaver C, Oles C, D'Ovidio K, Rader J. Determination of aflatoxins and ochratoxin A in ginseng and other botanical roots by immunoaffinity column cleanup and liquid chromatography with fluorescence detection. Journal of AOAC International. 2006 May 1;89(3):624-30. AOAC, (2005). Official Methods of Analysis of the Association of Official Analytical Chemists, 18th Ed., AOAC International, Gaithersburg, MD, USA.
18. Li Y, Watkins BA. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 1998; 33: 417-425.
19. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. Science 1993; 259 (5091):87-91.
20. Milagro FI, Campión J, Martínez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. Obesity (Silver Spring) 2006; 14 : 1118-23.
21. Jaskiewicz K, Rzepko R, Sledzinski Z. Fibrogenesis in fatty liver associated with obesity and diabetes mellitus type 2. Dig Dis Sci 2008; 5 : 785-8.

22. Baker RG, Hayden MS, Ghosh S. NF-kB, inflammation, and metabolic disease. Cell Metab 2011; 13: 11-22.
23. Kesh SB, Sikder K, Manna K, Das DK, Khan A, Das N, et al. Promising role of ferulic acid, atorvastatin and their combination in ameliorating high fat diet-induced stress in mice. Life Sci 2013; 92: 938-49.
24. Keith G Tolman and Anthony S Dalpiaz. Treatment of non-alcoholic fatty liver disease. Ther Clin Risk Manag. 2007; 3(6): 1153–1163.
25. De Oliveira CP, Stefano JT, De Siqueira ER, Silva LS, de Campos Mazo DF, Lima VM, Furuya CK, Mello ES, Souza FG, Rabello F, Santos TE. Combination of N-acetylcysteine and metformin improves histological steatosis and fibrosis in patients with non-alcoholic steatohepatitis. Hepatology Research. 2008 Feb 1;38(2):159-65.
26. Tao W, Deqin Z, Yuhong L, Hong L, Zhanbiao L, Chunfeng Z, Limin H & Xiumei G. Regulation effects on abnormal glucose and lipid metabolism of TZQ-F, a new kind of Traditional Chinese Medicine, J Ethnopharmacol 2010; 128(3):575-82.
27. Zou Y, Li J, Lu C, Wang J, Ge J, Huang Y, et al. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. Life Sci 2006; 79:1100-7.
28. Begriche K, Knockaert L, Massart J, Robin M A & Fromenty B. Mitochondrial dysfunction in nonalcoholic steatohepatitis (NASH): are there drugs able to improve it?, Drug Discov Today Dis Mech 2009; 6:e11-e23.
29. Chidambaram J & Carani-Venkatraman A. Cissus quadrangularis stem alleviates insulin resistance, oxidative injury and fatty liver disease in rats fed high fat plus fructose diet. Food Chem Toxicol 2010; (8-9): 2021-2029.
30. Tao W, Deqin Z, Yuhong L, Hong L, Zhanbiao L, Chunfeng Z, Limin H & Xiumei G. Regulation effects on abnormal glucose and lipid metabolism of TZQ-F, a new kind of Traditional Chinese Medicine, J Ethnopharmacol 2010; 128(3):575-82.
31. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J, Bollheimer LC. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. Journal of molecular endocrinology. 2006 Jun 1;36(3):485-501.Buettner, R., K. G. Parhofer, M. Woenckhaus, C. E. Wrede, L. A. Kunz-Schughart, J. Scholmerich, et al. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types 2006 ; 36(3):485-501.
32. Leopoldo AS, Sugizaki MM, Lima-Leopoldo AP, do Nascimento AF, Luvizotto RD, de Campos DH, Okoshi K, Dal Pai-Silva M, Padovani CR, Cicogna AC. Cardiac remodeling in a rat model of diet-induced obesity. Canadian Journal of Cardiology. 2010 Oct 1;26(8):423-9. Leopoldo, A. S., M. M. Sugizaki, A. P. Lima-Leopoldo, A. F. do Nascimento, A. M. Luvizotto Rde, De Campos D. H. S., et al. Cardiac remodeling in a rat model of diet induced obesity. Can. J. Cardiol 2010; 26 : 423–429.
33. Vincent HK, Powers SK, Dirks AJ, Scarpace P. Mechanism for obesity-induced increase in myocardial lipid per-oxidation. Int J Obes 2001; 25 :378–378.
34. Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotecticve enzymes in humans. Int J Obes. 2002; 26:1159–1159.
35. Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M . Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women? ARYA Atherosclerosis Journal 2007; 2 : 189–192.
36. Hafize U, Dildar K, Remisa G, Kagan Z, Mustafa. Plasma Protein Carbonyl and Thiol Stress Before and After Laparoscopic Gastric Banding in Morbidly Obese Patients Obesity Surgery 2007; 17 :1367–1373.
37. Keaney JF, Larson MG, Vasan RS, Wilson PWF, Lipinska I, Corey D, et al: Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham study. Arterioscler Thromb Vasc Biol 2003; 23:434-434.
38. Sonta T, Inoguchi T, Tsubouchi H, Sekiguchi N, Kobayashi K, Matsumoto S, Utsumi H, Nawata H: Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. Free Radic Biol Med 2004; 37:115-115.
39. Begriche K, Knockaert L, Massart J, Robin M A & Fromenty B. Mitochondrial dysfunction in nonalcoholic steatohepatitis (NASH): are there drugs able to improve it?, Drug Discov Today Dis Mech 2009; 6:e11-e23.
40. Alberts AW, Chen J, Curon G, Hunt V, Huff J, Hoffman C, et al . A highly potent competitive inhibitor of hydroxyl methyl glutaryl coenzyme A reductase and cholesterol-lowering agent. Proc Natl Acad Sci 1980 ; 77:3957.
41. Denen A, Ejike DE, Moses *DA, Seriki SA, Chiamaka NU. Hypolipidaemic effect of ethanol leaf extract of Moringa oleifera Lam. in experimentally* induced hypercholesterolemic wistar rats. Int. J. Nutr. Food Sci. 2014;3:355-60.Denen Atsukwei, Ejike Daniel Eze, Moses Dele Adams, Seriki Samuel Adinoyi, Chiamaka Nnenna Ukpabi. Hypolipidaemic effect of ethanol leaf extract of *Moringa oleifera* Lam. in experimentally induced hypercholesterolemic wistar rats. International Journal of Nutrition and Food Sciences 2014; 3(4): 355-360.
42. Mustafa A. Hammam, Gaber A. Kalil , Salah M. El-Sayed, Ibrahim A. Ahmed. Effects of *Moringa oleifera* Lam (Moringaceae) Seeds in Rats Fed with High Fat Diet. J Pharm Chem Biol Sci, 2016 May 4(1):76-87 .Journal of Pharmaceutical, Chemical and Biological Sciences 2016; 4(1):76-87.
43. Patil RH, Prakash K, and Maheshwari VL. Hypolipidemic. effect of *Celastrus paniculatus* in experimentally induced hypercholesterolemic Wistar rats. Indian J Clin Biochem 2010; 25:405-410.
44. Mathur NT, Varma M, and Dixit VP. Hypolipidemic and antiatherosclerotic effect of *Celastrus*

paniculatus seed extract (50% E-OH) in cholesterol fed rabbits. Indian Drugs 1993 ;30: 76

45. Olatosin TM, Akinduko DS, Uche CZ. Antioxidant capacity of Moringa oleifera seed oil against CCl4-induced hepatocellular lipid peroxidation in wistar albino rats. J Exp Biol. 2014;4:514-8. .Tayal V, Kalra BS, Agarwal S, Khurana N, Gupta U. Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits. Indian J Exp Biol 2007; 45 :1031–6.
46. Gaullier JM, Halse J, Høye K, Kristiansen K, Fagertun H, Vik H, Gudmundsen O. Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. The American journal of clinical nutrition. 2004 Jun 1;79(6):1118-25. Jean-Michel Gaullier, Johan Halse, Kjetil Høye, Knut Kristiansen, Hans Fagertun, Hogne Vik et al. . Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. Am J Clin Nutr 2004 ;79 :1118–25.
47. Müller C1, Gardemann A, Keilhoff G, Peter D, Wiswedel I, Kropf S, Schild L. Palmitate protects hepatocytes from oxidative stress and triacylglyceride accumulation by stimulation of nitric oxide synthesis in the presence of high glucose and insulin concentration .Free Radic Res 2010; 44 :1425-34.
48. Ebrahimi-Mameghani M, Jamali H, Mahdavi R, Kakaei F, Abedi R, Kabir-Mamdooh B. Conjugated linoleic acid improves glycemic response, lipid profile, and oxidative stress in obese patients with non-alcoholic fatty liver disease: a randomized controlled clinical trial. Croatian medical journal. 2016 Aug 15;57(4):331-42.Mehrangiz Ebrahimi-Mameghani, Haleh Jamali, Reza Mahdavi, Farzad Kakaei, Rana Abedi, and Bita Kabir-Mamdooh. Conjugated linoleic acid improves glycemic response, lipid profile, and oxidative stress in obese patients with non-alcoholic fatty liver disease: a randomized controlled clinical trial. Croat Med J 2016; 57: 331–341.

Reviewers of this article

Prof Monira A.Abd Elkader

Biochemistry Department,
Genetic Engineering and Biotechnology Division
National Research Centre,Dokki,Giza,Egypt.

Asst.Prof.Dr. Sujata Bhattacharya

Assistant Professor, School of Biological and Environmental Sciences, Shoolini University, Solan (HP)-173212, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript