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THE AMELIORATIVE ROLE OF *MORINGA OLEIFERA* ON THE ABNORMALITIES INDUCED BY ASPARTAME IN RATS: II. LIVER AND KIDNEY STATUS IN RAT

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ABSTRACT

This study aimed to investigate the ameliorative effect of *Moringa oleifera* (MO) aqueous leaves extract against the damage induced by the sweetener aspartame (AM) on liver and kidney functions and oxidative stresses in adult male albino rats. AM and MO was administered (75 and 500 mg/ Kg body weight) as a daily oral dose, respectively. Rats were divided into five groups; namely, saline control (Sc), AM, Protection (Pr), Improvement (Im) and treatment (Tr). Results of the study suggested that MO treatments had a hepato renal protection as indicated by the enzymatic and non-enzymatic function tests and oxidant antioxidant status compared to AM administered group ($P<0.05$). Interestingly, the group of the Tr design exerted almost the most protection of liver and kidney compared to the other treated groups (Pr and Im). The aqueous leaves extract of MO administration indicated both preventative as well as curative hepato- and renal protective activities.

KEYWORDS: aspartame, *Moringa oleifera*, liver, kidney, oxidative stress.

INTRODUCTION

Sweeteners are paid special attention among food additives as their use enables a sharp reduction in sugar consumption and a significant decrease in caloric intake while maintaining the desirable palatability of foods and soft drinks.[1] Sweeteners are also of primary importance as part of nutritional guidance for diabetes, a disease with increasing incidence in developing as well as developed countries.[2]

Aspartame (L-aspartyl L-phenylalanine methylester) (AM) is a dipeptide artificial sweetener composed of the amino acids phenylalanine and aspartic acid plus a small quantity of methanol.[3] It is 200 times sweeter than sucrose. Since its approval, AM has been used in more than 6000 different type of products including soft drinks, dessert mixes, frozen desserts, yoghurt, chewable multi-vitamins, breakfast cereals, tabletop sweeteners and pharmaceuticals[4], and consumed by millions of people around the world.[5]

Since after ingestion AM, is very efficiently hydrolyzed one of the main concerns derives from the products of hydrolysis, comprising about phenylalanine (50%), aspartic acid (40%) and methanol (10%), and from the presence of degradation products such as 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DPK) and β-aspartame.[6]

The metabolism of xenobiotic to a large extent takes place in the liver. The byproducts of such metabolism sometimes are more toxic than the initial substance. This could lead to hepatic damage and the emergence of hepatic disorders.[7]The kidney is one of the organs responsible for the maintenance of constant extracellular environment through the excretion of such purine catabolite as urea, creatinine, blood urea nitrogen and uric acid as well as electrolyte balance.[8]The byproducts include oxygen containing molecules that damage vital cells components through oxidation of cellular molecules such as lipids, proteins and DNA. In order to prevent the potential effects of reactive oxygen species (ROS), organisms have evolved multiple systems of antioxidant defense including both enzymatic and non-enzymatic strategy, and are essential for the cellular metabolism and function.[9]

Recently, the use of herbal natural products has gained interest among the world population. Many of the herbs have been developed into herbal supplement. Among those herbs, is *Moringa oleifera Lam* (MO) belongs to the genius *Moringacea*.The plant is native to South Asia and with high potential medicinal value.[10] In addition, the MO leaf extract is relatively safe for consumption within 4000 mg/Kg, since within this dose administered to rats, there was no adverse effect.[11]

Therefore, the objective of this study is to evaluate the possible protection of aqueous extract of MO against hepato- and renal toxicities and the oxidant- antioxidant disturbance induced by AM.

MATERIALS AND METHODS

Chemicals

- AM was purchased from Ajinnomoto sweeteners Europe.
- Alanine aminotransferase (ALT), Asparate aminotransferase (AST) and alkaline phosphatase (ALP) assay enzymatic kits were purchased from Spinreact Co., Spain.
- All the other chemicals were of analytical grade obtained from Gamma Trade Co., Cairo, Egypt.

Plant material and extraction: The MO dried leaves were obtained from the local market. They were crushed into a course powder using a laboratory electric blender. The powder was soaked in distilled water at room temperature for 24 h. It was then filtered using sieve cloth, then filter paper and the filtrate was used for the oral daily administration of rats.[12]

Experimental animals and design: Adult male albino rats "Sprague-Dawely strain" weighing 140-180 g were obtained from Research of Bilharzias Institute. Academic of Scientific Research and Technology, Cairo, Egypt. Rats were acclimatized to laboratory conditions for 3 days, maintained at constant 24^{o} C with 12 h light-dark cycle and fed with balanced rodent pellet diet and water *ad libitum*. After acclimatization, the rats were randomized into five experimental groups (n = 12); namely, saline control (Sc), AM, protection (Pr), improvement (Im) and treatment (Tr) groups.

AM mixed in sterile saline was orally daily administered (75 mg/ kg body weight) [13] for 2 weeks.

The MO aqueous extract was orally daily administered (500 mg/ kg body weight)[12] for 2 weeks.

Sc group: Rats were administered orally daily saline for 2 weeks.
AM group: Rats were administered orally daily AM dose for 2 weeks.
Pr group: Rats were administered orally daily MO dose for 2 weeks, and then AM orally daily dose for another 2 consecutive weeks.
Im group: Rats were administered orally daily AM dose for 2 weeks, then MO orally daily dose for another 2 consecutive weeks.
Tr group: Rats were administered orally daily MO and AM doses for 2 weeks.

Sample collection: At the end of the experimental duration, rats were killed for blood, liver and kidney samples collection. Blood was centrifuged (10 min 3000 rpm at 4^{o}C) for serum samples. Livers were immediately removed, rinsed with ice cold saline, and blotted dry, then accurately weighed.

Biochemical determinations: Liver function tests were determined in serum samples by the following biochemical parameters: alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[14], alkaline phosphatase (ALP) was estimated according to the method provided by Kind and King[15], lactate dehydrogenase (LDH) was estimated according to Rec[16] also the concentration of each of the total and direct bilirubin were estimated according to the method of Monnet.[17]

Kidney function tests were also determined by estimating the serum concentration of urea and creatinine according to the methods of Fawcett and Scott[18] and Newman and Price.[19]
Preparation of liver and kidney tissues for analysis: Accurately weighed pieces of liver and kidney tissues were perfused with phosphate buffered saline solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells, then homogenized in 5-10 ml cold buffer (i.e., 100 mM potassium phosphate, pH 7.0 containing 2mM EDTA) per gram tissue. Centrifuged at 4000 rpm at 4ºC for 15 min, the resultant supernatants were used for the assay of malondialdehyde (MDA) [20], total antioxidant capacity (TAC) [21]levels and catalase (CAT) activity.[22]

Statistical analysis

The data were statistically analyzed by SPSS version 9.0 statistical packages. Data were presented as a mean ± S.D.; statistical differences between groups were performed using Student's t-test. Differences considered significant when $P < 0.05$.[23]

RESULTS and DISCUSSION

Liver function test

AM is one of the widely consumed artificial sweetener and health conscious societies are increasingly concerned about its safety. The effect of AM on human is probably dependent on its metabolite components.[7] Upon ingestion, AM is immediately absorbed from the intestinal lumen and metabolized. Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and latter to formate.[24]

The results of table (1) showed that, AM administration to albino rats increased ALT, AST, ALP and LDH activities and total and direct bilirubin levels, indicating severe hepatotoxicity. Administration of MO either pre-, post or parallel with respect to AM, reduced the elevated parameters and the great reduction was recorded for the Tr group.

Table (1): Values of liver function tests for adult male albino rats administered different experimental doses of AM and /or MO

	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Sc	52.13 ±2.58	31.83 ±2.04	249.65±1.04	747.72 ±12.05	0.387±0.06	0.145±0.02
AM	85.03 ±2.84	68.04 ±3.81	567.55±4.22	1786.08±66.12	0.713±0.08	0.165±0.01
Pr	65.79 ±2.71	50.71 ±2.92	308.42±4.18	1099.63±26.33	0.610±0.06	0.142±0.01
Im	67.83 ±1.34	53.98 ±1.81	458.50±5.29	1034.11±50.71	0.560±0.03	0.145±0.01
Tr	58.33 ±2.07	45.33 ±2.08	439.03±5.54	994.29 ±10.74	0.457±0.03	0.145±0.01

Values are the mean ± S.D., n = 12.

It is clear that, AM consumption caused liver injury which was marked by the increase in ALT, AST, ALP and LDH activities in serum. It is certainly possible that the enhanced activities of these enzymes observed in this study were due to methanol, the byproduct of AM metabolism, which was previously reported to produce altered oxidant/antioxidant balance and surface change density which cause leakage of ALT and AST.[25] ALP is a membrane associated enzyme and an increased activity of ALP is an indication of liver damage. The consequent increase in level of LDH activity confirmed that damage has been inflicted on the plasma membrane which might have resulted in the compromise of its integrity.[26] Disruption of the ordered lipid-bilayer of the membrane structure probably due to the presence of ROS produced due to oxidative stress leading to escape of detectable quantity of these enzymes out of the cell into the extracellular fluid. The ROS might have oxidized the polyunsaturated fatty acids which make up the lipid bilayer resulting in its disruption. Increased bilirubin production may be as a result of decreased uptake, conjugation or increased bilirubin production related to increased free radical production by methanol metabolism of AM.[8] Furthermore, Shaheen and Afifi[27] illustrated alterations in liver functions of wistar rats administered 500 mg AM/Kg body weight/day expressed as ALT, AST and gamma glutamyltransferase.

The reversal of elevated serum intracellular enzyme activities and total and direct bilirubin concentrations by MO extracts administered by either of the three routes suggested that the plant extract has role in preserving structural integrity of hepatocellular membrane, thus

prevented enzyme leakage into circulation as earlier postulated by Saalu et al.[28] These results suggested that MO extract reduced the toxicity due to elimination of the toxic products of AM in rats.

Previous study had reported that MO leaves extract has significantly restored the elevation of ALT and AST as well as ALP levels that was induced by isoniazide to the normal level.[29] Meanwhile, another study had demonstrated that MO leaves extract had prevented the release of these enzymes from hepatocytes into the blood stream when induced with high level of ethanol administration in rats.[30] Moreover, MO preserved the structural integrity of hepatocytes when challenge with acetaminophen and subsequently preventing enzyme leakage into plasma.[31]

Kidney function test: Urea is the major excretory product of protein metabolism. Urea is carried by plasma to the kidney, where it is filtered from the plasma by the glomerulus. Creatinine is a nitrogenous product which is produced from the metabolism of creatinine in the skeleton muscles. Creatinine concentration not only assesses impairment of kidney function but also serve as a clinical chemistry endpoint to detect treatment related to toxic effects of extracts/compounds on the kidney in experimental models.[32]

In the present investigation, there was an elevation in serum urea and creatinine concentrations as a result of AM administration. Whereas, the MO administration either pre-, post or parallel to AM showed a statistically significant reduction ($P<0.05$) compared to AM and control groups. Moreover, there was no significant difference among the three treated groups.

Table (2): Values of kidney function tests for adult male albino rats administered different experimental doses of AM and /or MO

	Urea (mg/dl)	Creatinine (mg/dl)
Sc	27.79 ± 1.15	0.50 ± 0.07
AM	28.08 ± 0.82	1.20 ± 0.07
Pr	25.88 ± 0.64	0.798 ± 0.07
Im	24.19 ± 0.72	0.807 ± 0.06
Tr	25.96 ± 0.71	0.792 ± 0.06

Values are the mean ± S.D., n = 12.

An increase in blood methanol during AM metabolism is associated with a marked increase in the free radical generation. The imbalance antioxidant system may damage the kidney functions and probably contribute to the increased serum urea and creatinine

concentrations.[33] This explanation is emphasized by Ashok et al [28], who reported a histopathological changes of renal cortex in rats administered 75 mg/Kg AM. There was a marked glomerular damage including the loss of normal architecture and reduction in their normal sizes. The epithelium of the proximal and distal convoluted tubes peeled off brush borders from the surface. Moreover, since 1970s, the experimental studies on animal reported an excess risk of bladder cancer in rodents treated with extremely high doses of saccharin.[34] In addition, there is a relationship between saccharin and bladder cancer risks in human.[35]

The normalization of urea and creatinine levels in rats treated with MO extracts indicates inhibition of kidney cell injury. This indicates that the aqueous MO extract has potent anti-nephropathy properties against renal damage induced by AM administration. As it was indicated by Alaezi et al.[11] that the MO leaf extract could be given to patients who have undergo kidney transplant as a remedy, since it is capable of regeneration renal cells.

Hepatic and renal oxidative stresses

It could be noticed from table (3) that, rats administered AM had significantly ($P<0.05$) increased hepatic MDA level by 2 folds compared to control. However there was a significantly decreased ($P<0.05$) CAT activity and TAC level compared to control group. Rats administered the aqueous MO leaves extract had a significantly improved hepatic MDA, CAT and TAC levels compared to AM group. Whereas, there was no significant difference between the hepatic oxidative stress levels of Pr and Tr groups.

Administration of AM to albino rats increased the renal MDA level significantly ($P< 0.05$) by about 3 folds compared to the control group as shown in table (3). However, there was a noticeable significant reduction ($P< 0.05$) in the renal CAT activity and TAC level compared to control group. The renal oxidative stress was improved by the administration of MO extract with no significant difference between the renal CAT and TAC levels recorded for Pr and Im groups. On the other hand, there was no significant difference between the values of renal MDA of groups Im and Tr.

Table (3): The Hepatic and renal oxidative stress markers of adult male albino rats administered different experimental doses of AM and /or MO

	Hepatic			Renal		
	MDA (nmol / g)	**TAC (mM / g)**	**CAT (U /g)**	**MDA (nmol / g)**	**TAC (mM / g)**	**CAT (U /g)**
Sc	21.86 ± 1.90	4.19 ± 0.39	15.69 ± 0.578	13.58 ± 0.68	2.70 ± 0.186	8.14 ± 0.661
AM	45.80 ± 1.74	1.15 ± 0.078	8.73 ± 0.666	37.30 ± 1.11	0.173 ± 0.01	2.70 ± 0.363
Pr	29.25 ± 2.48	1.58 ± 0.041	12.79 ± 0.371	23.40 ± 1.43	1.42 ± 0.034	3.95 ± 0.559
Im	32.60 ± 2.01	1.82 ± 0.058	11.23 ± 1.01	27.24 ± 0.654	1.28 ± 0.351	4.33 ± 0.137
Tr	27.18 ± 1.18	1.43 ± 0.076	13.85 ± 0.501	25.47 ± 0.883	1.19 ± 0.014	6.17 ± 1.15

Values are the mean ± S.D., n = 12.

Reactive oxygen species (ROS), namely superoxide and hydroxyl free radicals, together with hydrogen peroxide, are believed be directly toxic, ROS can initiate free radical-mediated chain reaction. ROS damage the building structures of the cell membrane, and genetic material by causing scission, carbonylation, fragmentation, cross-linking and oxidation. These structural changes lead to the decrease or loss of protein biological functionand altering physicochemical properties and potentially losing of enzymatic activities.[36] The increase level of lipid peroxidation is taken as direct evidence for oxidative stress.[37] Further high concentration of lipid peroxides has been reported to lead to cytotoxicity by damaging the polyunsaturated fatty acids in the cellular membrane tending to reduce membrane fluidity, which is essential for proper functioning of the cell. A marked increase in lipid peroxides (LPO) substantiates the generation of free radicals following AM consumption.[38] Hence the elevated level of LPO in liver and kidney after AM administration in this study could be due to the methanol released during AM metabolism and the formaldehyde formed during methanol metabolism. This is well supported by the report of Zararsiz et al.[39] who recorded a significant increase in LPO in the kidney of rats after treatment with formaldehyde. Similarly Ashok et al.[24] reported that there was a marked increase in LPO of the liver and kidney of rats administered AM, indicating changes in antioxidant status of these organs. Moreover, Choudhary and Devi [8] concluded that the oral administration of 40 mg/Kg AM to rats increased serum LPO. In addition, Shaheen and Afifi[27] concluded that there was an elevation in MDA incorporated by a decrease in the enzymatic antioxidants of wistar rats administered AM, indicating liver toxicity by AM.

The reduction in both of the hepatic and renal TAC content and CAT activity after AM administration is related to the increased level of the LPO expressed as MDA. Hence these enzymatic and non-enzymatic antioxidants react directly with the free radicals generated

from methanol and formaldehyde resulted from AM metabolism. This is in accordance with Gulecet al. [40] who indicated that formaldehyde exposure led to a decrease in CAT activity in the liver tissue compared to the control. Moreover, Mourad[38] demonstrated that CAT activity significantly decreased in the liver tissue after 2 and 4 weeks of AM administration. However, Abhilash et al. [7] recorded no significant change in the hepatic CAT activity of rats administered either 500 or 1000 mg/Kg body weight AM for 180 days.

In AM treated rats by any of the three routes of MO administration (Protection, Improvement or Treatment) exerted a beneficial effect on modulating the increased level of MDA and the decreased levels of the TAC and CAT in the liver and kidney tissues. These findings are related to the active antioxidant components in the aqueous leaves extract of MO. Several studies reported hepatoprotective effects of some of these antioxidants such as quercetin, rutin and ascorbic acid.[41-43] However, another mechanism may be the induction of hepatic enzymes and/or proteins involved in the protection of oxidative stress.[44]

MO leaves are able to protect the liver against different hepatotoxic drugs and toxins such as acetaminophen[31,32],antitubercular drugs[29],carbon tetrachloride[45],paracetamol[46], gentamicin[47] and alcohol.[28] Moreover extracts of MO leaves, seeds and roots are effective against cyclophosphamide induced toxicity [48], ulceration [49], fluoride toxicity [50] and in the improvement of hepato-renal function [51] MO leaf extract also ameliorates ionizing radiation induced lipid peroxidation in mice liver.[52] Also the leaves have been reported to have antihypercholesterolemic action [53] and those with other risk factors, such as hypertension [54] or diabetes mellitus [55] Reports have also described the plant to be highly potent anti-inflammatory agent [56], antitumor activity[57] and to be hepatoprotective against some antitubercular drugs.[29] These results provide an explanation for the finding in this study that MO administration improved the hepato-renal functions and oxidative stress in tissues induced by AM.

CONCLUSION

This study demonstrated that AM consumption affect the liver and kidney due to its metabolite methanol that act as a stressor to alter the antioxidant status and cause hepato-renal toxicity. Moreover, the findings suggested that the administration of the aqueous leaves extract of MO either pre-, post or parallel to AM possesses a potent protective and curative efficacy against liver and kidney injury in rats.

REFERENCES

1. Vences-Mejia A, Labra-Ruiz N, Hernandez-Martinez N, Dorado-Gonzalez V, Gomez-Garduno J, Perez-Lopez I, Nosti-Palacios R, Carranza RC, Espinosa-Aguirre JJ.The effect of aspartame on rat brain xenobiotics-metabolizing enzymes. Human Exp.Toxicol, 2006; 25: 453-459.
2. Gougeon R, Spidel M, Lee K, Field CJ. Canadian diabetes association national nutrition committee technical review: non-nutritive intense sweeteners in diabetes management. Can. J. Diabetes, 2004; 128: 385-399.
3. Butchko HH, Stargel WW, Comer CP, Mayhew DA, Benninger C, Blackburn GL, deSonneville LM, Geha RS, Hertelendy Z, Koestner A, Leon AS, Liepa GU, Mc Martin KE, Mendenhall CL, Munro IC, Novotny EJ, Renwick AG, Sehiffman SS, Schomer DL, Shaywitz BA, Spiers PA, Tephly TR, Thomas JA, Trefz FK. Aspartame: review of safety. Regul.Toxicol.Pharmacol., 2002; 35: S1-S93.
4. Rencüzogullari E., Tuylu B.A., Topaktas M., Ila H.B., Kayraldiz A. and Arslan M. Genotoxicity of aspartame, Drug Chem. Toxicol., 2004; 27(3): 257-268.
5. Butchko HH, Stargel WW. Aspartame: scientific evaluation in the postmarketing period. Rgul.Toxicol.Pharmacol., 2001; 34: 221- 233.
6. Marinovich M, Galli CL, Bosetti C, Gallus S, La Vecchia C. Aspartame, low-calorie sweeteners and diseases: Regulatory safety and epidemiological issues. Food and Chemical Toxicol., 2013; 60: 109-115.
7. Abhilash M, Paul MVS, Varghese MV, Nair RH. Effect of long term intake of aspartame on antioxidant defense status in liver . Food Chem. Toxicol, 2011; 49: 1203-1207.
8. Choudhary AK, Devi RS. Serum biochemical responses under oxidative stress and aspartame in wistar albino rats. Asian Pac. J. Trop. Dis., 2014; 4(Suppl.1): S403- S410.
9. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology.Toxicol., 2000; 153: 83-104.
10. Ezejindu DN, Udemezue OO, Akingboye AJ. Hepatoprotective effects of *Moringa oleifera leaf* extract on the kidneys of adult wistar rats, AJER, 2014; 3(2): 157-161.
11. Alaezi CM, Okorochi EC, Nnodim JK. Histological and biochemical effects of *Moringa oleifera* leaf extract on the liver of wistar rats, WJPLS, 2015; 1(2): 129-148.
12. Aja PM, Nwafor EJ, Ibiam AU, Orji OU, Ezeani N, Nwali BU. Evaluation of anti-diabetic and liver enzymes activity of aqueous extracts of *Moringa oleifera* and *Brideliaferruginea* leaves inalloxan induced diabetic rats. Int. J. Biochem. Res. Rev, 2013; 3(3): 248-258.

13. Ashok I, Sheeladevi R,Wanhhar D. Long term effect of aspartame (Artificial sweetener) on membrane homeostatic imbalance and histology in the rat brain. Free Rad. and Antiox., 2013; 3: 542-549.
14. Reitman A, Frankel S. Colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. Am. J. of Clin.Pathol, 1957; 28: 56–63.
15. Kind N, King A. Elimination of plasma phosphate by determination of hydrolysed phenol with aminoantipyrine. J. Clin. Pathol., 1954; 7 : 322–326.
16. Rec G. (DGKC) Recommendations of the German Society of clinical chemistry: Standarization of methods for the determination of enzyme activities in biological fluids. J. Clin. Chem. Biochem., 1970; 8: 658-660.
17. Monnet L. Determination of bilirubin. Annal. Biol. Clin., 1963; 21: 717.
18. Fawcett JK, Scott JE. Determination of urea.J.Clin.Pathol., 1960; 13: 156-159.
19. Newman DJ, Price CP. Renal function and Nitrogen Metabolites.Tietz Textbook of Clinical Chemistry 1999, 3rd.ed., Pheladelephia: W.B. Saunders CompanyP.1204.
20. Draper H, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 1990; 186: 421-431.
21. Koracevic D , Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 2001; 54:356-361
22. Aebi H. Catalase in vitro. Method Enzymol, 1984; 105: 121–126.
23. Levesque R. SPSS programming and data management: A guide for SPSS and SAS users. 2007, 4th ed. SPSS Inc, Chicago, IL.
24. Ashok I, Wankhar D, Sheeladevi R, Wankhar W. Long-term effect of aspartame on the liver antioxidant status and histopathology in wistar albino rats. Biomed. Preventative Nutr., 2014; 4(2): 229-305.
25. Parthasaraathy NJ, Srikumar R, Manikandan S, Devi RS. Methanol-induced oxidative stress in rat lymphoid organs. J. Occup. Health, 2006; 48: 20-27.
26. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ, 2005; 172(3): 367-379.
27. Sheheen NEM, Afifi MSH.The protective role evaluation of N-acetyl-cysteine and folic acid against aspartame-induced hepatotoxicity in albino rats. World J. Pharm.Sci., 2014; 2(12): 1614-1619.

28. Saalu LC, Ogunlade B, Ajayi GO, Oyewopo AO, Akunna GG, Ogunmodede OS. The hepato-protective potentials of *Moringa oleifera* leaf extract on alcohol-induced hepato-toxicity in wistar rats. Am. J. Biotechnol. Mol. Sci., 2012; 2(1): 6-14.
29. Pari L, Kumar NA. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. J. Med. Food, 2002; 5(3): 171-177.
30. Nadro MS, Arungbemi RM, Dahiru D. Evaluation of *Moringa oleifera* leaf extract on alcohol-induced hepatotoxicity. Trop. J. Pharmaceut. Res., 2005; 5(1): 539-544.
31. Fakurazi S, Hairuszah I, Nanthini U. *Moringa oleifera* Lam prevents acetaminophen induced liver injury through restoration of glutathione level. Food Chemical Toxicol., 2008; 46: 2611-2615.
32. Sharifudin SA, Fakurazi S, Hidayat MT, Hairuszah I, Moklas MAM, Arulselvan P. Therapeutic potential of *Moringa oleifera* extracts against acetaminophen-induced hepatotoxicity in rats. Pharmaceut.Biology, 2013; 51(3): 279-288.
33. Parthasarathy NJ, Srikumar R, Devi RS. Effect of methanol intoxication on rat neutrophil functions. J. Immunotoxicol., 2005; 2:115-121.
34. Wihrauch MR, Diehl V. Artificial sweeteners- do they bear a carcinogenic risk? Ann. Oncol., 2004; 15: 1460-1465.
35. Andreatta MM, Muňoz SE, Lanieri MJ, Eynard AR, Navarro A. Artificial sweeteners consumption and urinary tract tumors in Cordoba. Argentina Prev. Med., 2008; 47: 136-139.
36. Cakatay U, Kayali R, Uzun H. Relation of plasma protein oxidation parameters paraxonase activity in the ageing population. Clin. Exp. Med., 2008; 8: 51-57.
37. Skrzydlewska E, Elas M, Farbiszewski R, Roszkowska A. Effect of methanol intoxication on free radical induced protein oxidation. J. Appl. Toxicol., 2000; 20(3): 239-243.
38. Mourad IM. Effect of aspartame on some oxidative stress parameters in liver and kidney of rats. Afr. J. Pharma. Pharmacol., 2011; 5(6): 678- 682.
39. Zararsiz I, Sarsilmaz M, Tas U. Protective effect of melatonin against formaldehyde-induced kidney damage in rats. Toxicol. Indian Health, 2007; 23(10): 573-579.
40. Gulec M, Gurel A, Armutcu F. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. Mol. Cell Biochem., 2006; 290(1-2): 61-67.
41. Lanteri R, Acquaviva R, Di Giacomo C, Sorrenti V, Li Destri G, Santangelo M, Vanella L, Di Cataldo A. Rutin in rat liver ischemia reperfusion injury: effect on DDAH/NOS pathway. Microsurgery, 2007; 27: 245.

42. Kanter M. Proective effect of quercetin on liver damage induced by biliary obstruction in rats. J. Mol. Histol., 2010; 41: 395.
43. Ahu T, Yun CH, Oh DB. Tissue-specific effect of ascorbic acid supplementation on the expression of cytochrome P450 2E1 and oxidative stress in streptozotocin-induced diabetic rats.Toxicol.Lett., 2006; 166: 27.
44. Das N, Sikder K, Ghosh S, Fromenty B, Dey S. *Moringa oleifera*Lam leaf extract prevents early liver injury and restores antioxidant status in mice fed with high-fat diet. Indian J. Exp. Biol., 2012; 50: 404-412.
45. Nanjappaiah HM, Hugar S. Prophylactic and curative effects of *Moringa oleifera* Lam pods in CCl_4 damaged rat liver. Indian J. Nat. Prod.Resour., 2012; 3(4): 541-546.
46. Buraimoh AA, Bako IG, Ibrahim FB. Hepatoprotective effect of ethanolic leave extract of *Moringa oleifera* on the histology of paracetamol induced liver damage in wistar rats. Int. J. Anim. Veter. Adv., 2011; 3(1): 10-13.
47. Ouédraogo M, Lamien-Sanou A, Ramdé N, Ouédraogo AS, Zongo SP, Goumbri O, Duez P, Guissou PI. Protective effect of *Moringa pleifera*leaves against gentamicin-induced hepatotoxicity in rabbits. Exp. Toxicol. Pathol., 2013; 65(3): 335-339.
48. Gupta A, Gautam MK, Singh RK, Kumar MV, Rao CV, Goel RK, Anupurba S. Immunomodulatory effect of *Moringa oleifera* Lam extract on cyclophosphamide induced toxicity in mice. Indian J. Exp. Biol., 2010; 48: 1157.
49. DebnathS, Guha D. Role of *Moringa oleifera* on enterochroaffin cell count and serotonin content of experimental ulcer model. Indian J. Exp. Biol., 2007; 45: 726.
50. Ranjan R, Swarup D, Patra RC, Chandra V. Tamarindusindica L. and *Moringa oleifera* M. extract administration ameliorates fluoride toxicity in rabbits. Indian J. Exp. Biol., 2009; 47: 900.
51. Mazumder UK, Gupta M, Chakrabarti S, Pal D. Evaluation of hematological and hepatorenal functions of methanolic extract of *Moringa oleifera* Lam root treated mice. Indian J. Exp. Biol., 1999; 37: 412.
52. Sinha M, Das DK, Datta S, Ghosh S, DeyS. Amelioration of ionizing radiation induced lipid peroxidation in mouse liver by *Moringa oleifera* leaf extract. Indian J. Exp. Biol., 2012; 50: 209.
53. Ghasi S, Nurobodo E, Ofili JO. Hypocholestrolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high fat diet fed wistar rats. J. Ethnopharmacol., 2000; 69: 21-25.

54. Faizi S, Siddiqui BS, Saleem R, Aftab K, Shaheen F, Gilani AH. Hypotensive constituents from the pods of *Moringa oleifera*. Planta Med., 1998; 64(3): 225-228.
55. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats. J. Ethnopharmacol. 2003; 84(1): 105-108.
56. Ezeamuzle IC, Ambederomo AW, Shode FO, Ekwebelem SC. Antiinflammatory effects of *Moringa oleifera* root extract. Int. J. Pharmacogen, 1996; 34: 207-212.
57. Murakami A, Kitazona Y, Jiwajinda S, Koshimizu K, Ohigashi H. Niaziminin, a thiocarbamate from the leaves of *Moringa oleifera*, holds a strict structural requirement for inhibition of tumor promoter-induced Epstein-Barr virus activation. Planta Med. 1998; 64: 319-323.