

THE HEPATOPROTECTIVE IMPACT OF MORINGA OLEIFERA LEAVES EXTRACT AGAINST SODIUM VALPROATE- INDUCED LIVER TOXICITY IN ADULT RATS

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ABSTRACT

Objective: Valproic acid and its salts (sodium valproate, VPA) are mood stabilizing and anticonvulsant agents. VPA use has been complicated by a high incidence of hepatic injury. Moringa oleifera (MO) leaves extract exhibits the greatest antioxidant activity. Mepacure (MEPA) is used as reference drug. The aim of present study is to investigate the possible effect of Moringa oleifera leaves extract against the toxicity of VPA in albino rats. **Design:** A total number of 120 rats were equally divided into 5 groups, MO and/ or MEPA were administrated for 3 periods (2, 4, 6) weeks in sodium valproate treated rats. Liver enzymes activities of (ALT, AST and γ -GT), total protein as

well as (TNF- α), oxidative stress parameters; (GSH, GSSG, NO and MDA) contents were determined. In addition, determination of gene expression (Bax and B-cell lymphoma 2 (Bcl2)) levels in liver tissue. **Results:** sodium valproate revealed an increase in the liver enzymes activities with decrease in total protein content. Also, an elevation in TNF- α level, increase in liver MDA, GSSG and NO contents and decrease in GSH content, increasing gene expression of (Bax) and decreasing gene expression of (Bcl2) in liver tissue. Moringa attenuated the serum activities of liver markers enzymes, restored the normal redox status and inhibited the inflammatory factor. Also, ameliorated apoptotic signals. Histopathological examination confirmed the ameliorative effect for the Moringa leaves extract against VPA induced liver injury. **Conclusion:** These data suggest that moringa leaves extract is a

promising pharmacological agent for preventing the potential hepatotoxicity of sodium valproate.

KEYWORDS: Sodium valproate, *Moringa oleifera*, Hepatotoxicity, Gene Expression, Oxidative stress.

INTRODUCTION

Liver is involved in the detoxification of drugs; where some of them may cause hepatotoxicity.^[1] The liver is the primary organ for drug metabolism and elimination for many antiepileptic drugs (AEDs). Hepatotoxicity induced by antiepileptic drug can lead to an acute liver failure which could imperatively require liver transplantation.^[2] Valporic acid is a medicine widely prescribed as an anticonvulsant and mood-stabilizing agent in the treatment of epilepsy, mania and bipolar disorders as well as the management of migraine headaches.^[3] Sodium valproate (Depakine, Valproic acid, VPA) therapy is responsible for a number of fatal cases of hepatic failure.^[4] Flavonoids are a prevalent group of naturally occurring antioxidants and anti-inflammatory agents in plants. They abundantly exist in vegetables, fruits, nuts, seeds, leaves, flowers and barks of plants.^[5] Antioxidants play an important role in reducing oxidative damage to tissues, and therefore they are used in treating and preventing many diseases.^[6] *Moringa oleifera* (MO) is widely used in traditional medicine, where its leaves and immature seed pods are used as food products in human nutrition. Leaves extract exhibit the greatest antioxidant activity, which protects the liver from progression of fibrogenesis in intoxicated subjects.^[7] A wide variety of polyphenols and phenolic acids as well as flavonoids, glucosinolates, and possibly alkaloids is believed to be responsible for the observed effects.^[8] *Moringa* leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids and fatty acids.^[9] Moreover,^[8] reported that aqueous, hydroalcohol, or alcohol extracts of MO leaves have a wide range of extra-biological activities including antioxidant through which protect different tissues (liver, kidneys, heart, testes, and lungs), analgesic, antiulcer, antihypertensive, antidiabetic and anti-dyslipidemic, radioprotective, and as immune-modulator. In addition, Mepacure is a nutraceutical product that protects the liver cells and used in the treatment of chronic hepatitis, cirrhosis and fatty degeneration.^[10] So, the present study aimed to investigate the hepatoprotective effect of *Moringa oleifera* leaves extract against sodium valproate-induced liver toxicity in adult rats.

MATERIAL AND METHODS

Animals

Adult male Sprague Dawley rats weighting 140-170 g were used. The animals were brought from laboratory animal breeding of national organization of drug control and research (NODCAR), Giza, Egypt. Animals were kept in plastic cages at room temperature (22-25°C) and humidity (55%) under a 12 hrs dark-light cycle. All animals were fed standard basal diet and accommodated with laboratory conditions for two weeks before starting the experiment. The rats were allowed free access to food and drinking water *ad libitum*. All animals were handled and treated according to the guidelines for animal experiments which were approved by Ethical Committee of Medical Research of the National Research Centre, Cairo, Egypt.

Drugs and Dosages

A- Sodium valproate

Sodium Valproate (VPA) Sanofi-France was purchased from Global Napi Pharmaceuticals, Egypt, and was dissolved in distilled water (500 mg/kg b.wt. /day).^[11]

B- Mepacure capsules

Mepacure[®] capsules: each capsule contains 30 mg Dimethyl Dicarboxylate Biphenyl (DDB) and 50 mg silymarin. Drug was purchased from the local market as a capsule form manufactured by Arab Company for Pharmaceutical and Medicinal plants (MEPACO), Egypt, and administered orally in a dose (13.5 mg/kg b.wt. silymarin and 8.1 mg/kg b.wt. DDB/ day).^[12]

C- Moringa oleifera leaves

Moringa oleifera leaves extract was purchased from National Research Center (NRC, Giza, Egypt), and administered orally in a dose (500 mg/kg b.wt. /day).^[7]

Preparation of ethanolic moringa leaves extract

Ethanolic extract of Moringa oleifera leaves was prepared as follows: The leaves were washed thoroughly with distilled water and dried under room temperature at (29 -35°C) for three weeks, after which the leaves were pulverized into coarse form with high speed milling machine. The coarse form (1000 g) was then macerated in absolute ethanol and left to stand for 48h. Then, the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature was between 40 and 45°C to avoid denaturation

of the active ingredients. The concentrated extract was diluted to 1000 ml using a polysaccharide as a carrier and stored in the refrigerator.^[13]

Experimental Design

The animals were divided into five groups, each with 24 animals, 8 rats per cage as in the following design

Group 1:(Control) group received 1ml/100 g b.wt of vehicle (2% tween 80 in water) daily.

Group 2 (VPA): Sodium valproate- treated group in which the rats were treated orally with sodium valproate (500 mg/kg b.wt / day) for 6 weeks for induction of chronic hepatotoxicity.

Group 3 (MO+VPA): Rats received orally Moringa leaves extract (500 mg/kg b.wt. /day) along with sodium valproate (500 mg/kg b.wt. / day) for 6 weeks.

Group 4 (MEPA+ VPA): Rats received orally Mepacure (13.5 mg/kg b.wt silymarin and 8.1 mg/kg b.wt DDB/ day) along with sodium valproate (500 mg/kg b.wt. / day) for 6 weeks.

Group 5 (MO+MEPA+VPA): Rats received orally Moringa leaves extract (500 mg/kg b.wt./ day) along with sodium valproate (500 mg/kg b.wt./ day) and Mepacure (13.5 mg/kg b.wt silymarin and 8.1 mg/kg b.wt DDB/ day) for 6 weeks.

In all treatments described in this study, drugs were prepared in water containing 2% tween 80 and the appropriate dose of each drug was administered orally by gastric intubations to each rat daily for six weeks.

Sample collection and biochemical analysis

Patches of 8 rats from each group were decapitated at the end of the 2nd, 4th and 6th week of the study. Blood samples were collected and incubated at 37°C for 10 min. Serum was separated using cooling centrifugation at 5000 rpm for 10 min and stored at -20°C for measuring the biochemical parameters. Total protein.^[14] activities of alanine transaminase (ALT), aspartate transaminase (AST)^[15] using commercial kits purchased from Chema Diagnostica (ITALY), Gamma-Glutamyltransferase (γ -GT)^[16] using reagent kits purchased from Spectrum Diagnostics Company (EGYPT), Tumor necrosisfactor alpha (TNF- α) were detected by using ELISA procedure.^[17] Liver samples were quickly removed from each animal and washed in an ice-cold isotonic saline. One part was removed and immediately immersed in 10% buffered formalin for histopathological examinations. The second part was

homogenized in ice-cold Tris-HCl buffer (150 mM KCl, 50 mM Tris, pH 7.4), at homogenate concentration of 10% (w/v), centrifuged at 1000 rpm to remove nuclei and cell debris. The supernatant was used to determine malondialdehyde (MDA) by HPLC.^[18] The third portion was homogenized in 5% sulphosalicylic acid to make 10% homogenate and immediately used for the determination of reduced glutathione (GSH) and oxidized glutathione (GSSG) content of liver tissue by HPLC.^[19] The fourth portion was homogenized in 70% methanol and centrifuged 1000 rpm for the HPLC determination of nitric oxide (NO).^[20] The remaining part of livers was immediately snap frozen in liquid nitrogen and then stored at -80°C for the evaluation of gene expression of Bax and Bcl2, total RNA was isolated using Qiagen tissue extraction kit (Qiagen, USA) according to instructions of manufacture.

Statistical analysis

Data were presented as means \pm S.E. using SPSS ANOVA test version 11.5, $P \leq 0.05$ was considered significant difference.

RESULTS

Liver Functions

The oral administration of VPA during the time course of the experimental periods (2, 4 and 6 weeks) induced a significant increase in ALT, AST and GGT activity, with a noticeable decrease in total protein content compared with their respective control group. Meanwhile, the administration of MO displayed a significant ($p > 0.05$) reduction in the enzyme activity of ALT, AST and GGT when compared with VPA treated group, as well as the MEPA treatment to VPA induced a gradual decrease in ALT, AST and GGT. While, the administration of MO extract or MEPA caused a significant increase in total protein levels compared to their respective control groups. The depicted data also revealed that, the co-administration of MO and MEPA restored the enzymatic activity and increased the level of total protein (Table 1).

Tumor necrosis factor (TNF- α) in serum

data revealed that oral administration of sodium valproate induced a significant ($p > 0.05$) elevation in (TNF- α) level in a time dependent manner as compared to control group amounted to (389%, 439% and 457%) for 1st, 2nd and 3rd intervals, respectively. Meanwhile, the continuous administration of MO for the same periods showed a significant reduction in TNF- α level as compared with VPA treated group. (Figure 1).

Oxidative stress parameters

In addition, sodium valproate increased levels of hepatic MDA, NO and GSSG and decreased levels of GSH in comparison to control group. The administration of moringa and/or mepacure displayed a significant ($p > 0.05$) reduction in GSSG, MDA and NO level and increase in GSH when statistically compared with VPA treated group throughout the experiment. The depicted data also revealed that, the co-administration of MO plus MEPA antagonized the oxidizing effect of VAP (Table 2).

Gene expression

Furthermore, VPA provoked apoptotic response through increasing gene expression of pro-apoptotic Bax and decreasing gene expression of anti-apoptotic Bcl2 in liver tissue. Meanwhile, MO administration suppressed the elevation in the gene expression of Bax. Likewise, MEPA treated group exerted a significant reduction in the gene expression of Bax but MO group was more potent than MEPA group in this effect. The data also revealed that administration of MO with MEPA recorded a significant ($p > 0.05$) reduction in the gene expression of Bax and significant increase in the anti-apoptotic gene bcl2 and proved the protection role of MO extract against the injurious effect of sodium valproate (Figure 2, 3).

Histopathological results

The biochemical results were supported by histopathological results showing strict changes in liver sections of VPA group in a time dependent manner. These include marked congestion in portal vessels and central vein associated with inflammation in lymphocytes and plasma cells, also focal hepatocyte degenerative changes. Meanwhile, administration of MO and/or MEPA showing gradual enhancement in the histopathological alternations resulted from VPA administration through (1st, 2nd and 3rd) intervals. These enhancements include nil inflammation response and congestion indicating reduction in hepatic damage (Figure 4).

Table 1: Effect of *Moringa oleifera* (MO, 500mg/kg,p.o) and/or Mepacure (MEPA, 21.6mg/kg, p.o) treatments on serum liver functions in rats treated with Sodium Valproate.

Parameters	Intervals	Control	VPA	MO+VPA	MEPA +VPA	MO +MEPA +VPA
ALT U/L	2weeks	33.71 ±0.77 ^{bcde}	50.85 ±1.10 ^{acde}	39.41 ±1.39 ^{ab}	41.36 ±1.26 ^{ab}	38.99 ±1.82 ^{ab}
	4weeks	33.83 ±0.54 ^{bd}	63.17 ±2.46 ^{acd}	36.35 ±0.31 ^b	37.18 ±0.52 ^{ab}	35.95 ±0.44 ^b
	6weeks	33.64 ±0.55 ^b	65.80 ±1.74 ^{acde}	34.47 ±0.61 ^b	35.13 ±0.30 ^b	34.98 ±1.11 ^b
AST U/L	2weeks	31.54 ±1.18 ^{bcde}	49.63 ±2.09 ^{acde}	38.85 ±0.55 ^{ab}	40.05 ±1.13 ^{ab}	39.14 ±1.44 ^{ab}
	4weeks	31.49 ±0.75 ^{bcde}	64.78 ±1.10 ^{acde}	35.11 ±1.15 ^{ab}	37.25 ±1.04 ^{ab}	36.53 ±0.46 ^{ab}
	6weeks	31.17 ±1.11 ^b	68.85 ±0.37 ^{acde}	33.45 ±0.51 ^b	35.33 ±0.41 ^b	32.47 ±0.87 ^b
GGT U/L	2weeks	31.94 ±0.92 ^{bcde}	59.63 ±1.65 ^{acde}	48.49 ±0.96 ^{ab}	49.44 ±1.63 ^{ab}	43.83 ±1.62 ^{ab}
	4weeks	31.97 ±3.69 ^{bcde}	116.34 ±1.01 ^{acde}	40.03 ±1.55 ^{ab}	39.37 ±1.17 ^{ab}	37.90 ±1.13 ^{ab}
	6weeks	32.16 ±3.63 ^b	122.47 ±2.41 ^{acde}	33.02 ±0.79 ^b	34.49 ±0.76 ^b	33.43 ±1.21 ^b
TP g/dl	2weeks	6.44 ±0.17 ^{bcde}	5.00 ±0.10 ^{acde}	5.53 ±0.09 ^{ab}	5.66 ±0.07 ^{ab}	5.86 ±0.16 ^{ab}
	4weeks	6.49 ±0.12 ^b	4.29 ±0.09 ^{acde}	6.21 ±0.22 ^b	6.36 ±0.21 ^b	6.31 ±0.26 ^b
	6weeks	6.53 ±0.16 ^b	4.25 ±0.07 ^{acde}	6.36 ±0.24 ^b	6.38 ±0.12 ^b	6.55 ±0.10 ^b

Data were expressed as mean±S.E, differences were considered significant at P<0.05

a: significant difference from control in the same column

b: significant difference from VPA in the same column.

c: significant difference from MO in the same column.

d: significant difference from MEPA in the same column.

e: significant difference from MO+VPA+ MEPA in the same column.

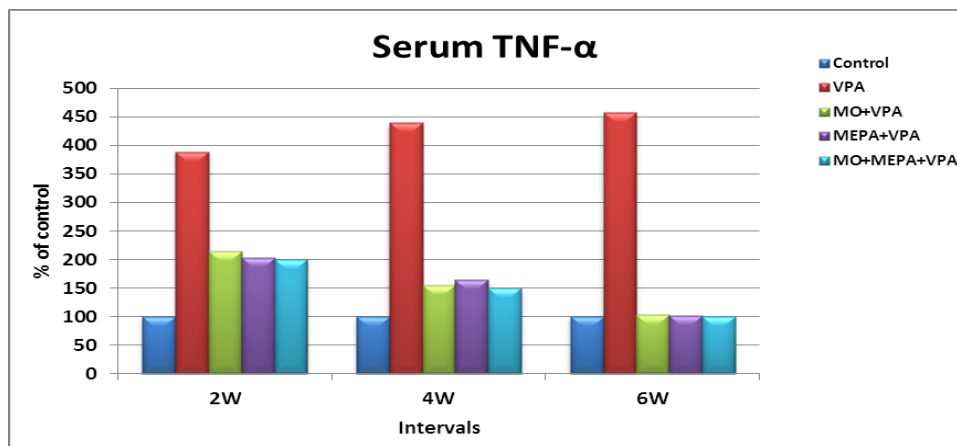


Fig. 1: Effect of *Moringa oleifera* (MO, 500mg/kg,p.o) and /or Mepacure (MEPA, 21.6mg/ kg, p.o) on serum Tumor Necrosis Factor level in rats treated with Sodium Valproate.

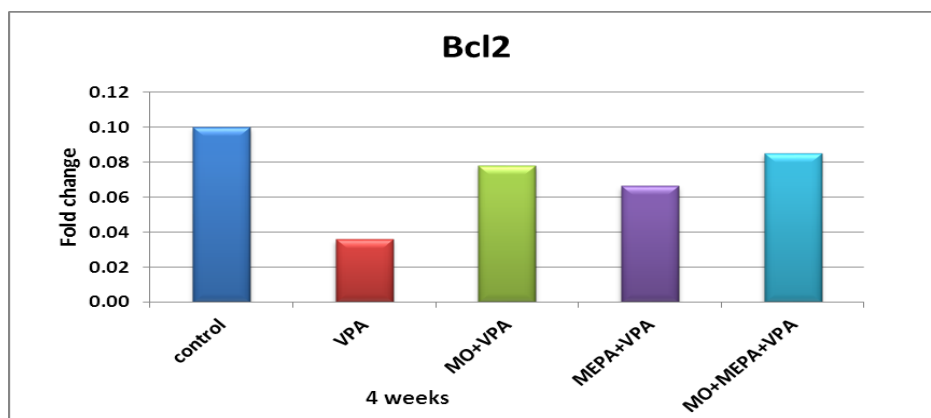


Fig. 2: Effect of *Moringa oleifera* (MO, 500mg/kg,p.o) and /or Mepacure (MEPA, 21.6mg/ kg, p.o) on Gene Expression (Bcl2) in rats treated with Sodium Valproate.

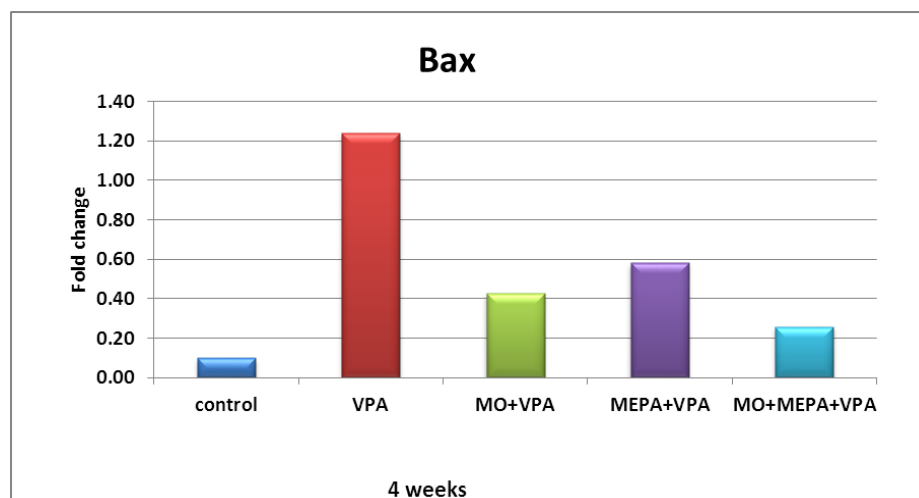


Fig. 3: Effect of *Moringa oleifera* (MO, 500mg/kg,p.o) and /or Mepacure (MEPA, 21.6mg/ kg, p.o) on Gene Expression (Bax) in rats treated with Sodium Valproate.

β -actin

Bcl2



Bax



Control	VPA	MO+VPA	MEPA+VPA	MO+MEPA+VPA
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Table 2: Effect of Moring a oleifera (MO, 500mg/kg,p.o) and /or Mepacure (MEPA, 21.6mg/ kg, p.o) treatments on Oxidative Stress Parameters in Sodium valproate-treated Rats.

Parameters	Intervals	Control	VPA	MO +VPA	MEPA +VPA	MO +MEPA +VPA
GSH $\mu\text{mol/g}$ tissue	2weeks	4.32 $\pm 0.03^{bcde}$	3.23 $\pm 0.11^{acde}$	3.61 $\pm 0.04^{abe}$	3.54 $\pm 0.02^{abe}$	3.75 $\pm 0.02^{abcd}$
	4weeks	4.28 $\pm 0.03^{bcde}$	2.76 $\pm 0.02^{acde}$	4.04 $\pm 0.01^{abd}$	3.90 $\pm 0.02^{abc}$	4.01 $\pm 0.02^{ab}$
	6weeks	4.25 $\pm 0.01^b$	2.56 $\pm 0.03^{acde}$	4.14 $\pm 0.04^b$	4.17 $\pm 0.06^b$	4.25 $\pm 0.03^b$
GSSG $\mu\text{mol/g}$ tissue	2weeks	1.00 $\pm 0.01^{bcde}$	1.53 $\pm 0.02^{acde}$	1.35 $\pm 0.01^{abd}$	1.28 $\pm 0.02^{abe}$	1.32 $\pm 0.01^{abcd}$
	4weeks	0.99 $\pm 0.01^{bcde}$	1.61 $\pm 0.01^{acde}$	1.25 $\pm 0.03^{abde}$	1.21 $\pm 0.01^{abcd}$	1.22 $\pm 0.02^{abc}$
	6weeks	1.01 $\pm 0.01^{bcde}$	1.65 $\pm 0.03^{acde}$	1.16 $\pm 0.02^{abe}$	1.10 $\pm 0.02^{abcd}$	1.09 $\pm 0.02^{abc}$
MDA $\mu\text{mol/g}$ tissue	2weeks	115.89 $\pm 0.99^{bcde}$	200.93 $\pm 1.01^{acde}$	140.04 $\pm 0.31^{abde}$	135.76 $\pm 1.03^{abc}$	137.33 $\pm 0.41^{abce}$
	4weeks	113.80 $\pm 1.22^{bcde}$	234.41 $\pm 0.83^{acde}$	127.64 $\pm 0.67^{ab}$	125.74 $\pm 0.59^{ab}$	126.59 $\pm 0.25^{ab}$
	6weeks	114.02 $\pm 0.76^{bc}$	241.19 $\pm 0.32^{acde}$	117.05 $\pm 0.25^{ab}$	116.00 $\pm 0.40^b$	115.87 $\pm 0.49^b$
NO $\mu\text{mol/g}$ tissue	2weeks	19.25 $\pm 0.04^{bcde}$	23.02 $\pm 0.03^{acde}$	21.67 $\pm 0.21^{abde}$	20.81 $\pm 0.22^{abc}$	21.02 $\pm 0.18^{abc}$
	4weeks	19.27 $\pm 0.06^{bcde}$	26.03 $\pm 0.14^{acde}$	20.75 $\pm 0.27^{abde}$	20.03 $\pm 0.02^{abc}$	19.87 $\pm 0.17^{abc}$
	6weeks	19.22 $\pm 0.03^{bcde}$	30.18 $\pm 0.20^{acde}$	19.89 $\pm 0.14^{abe}$	19.79 $\pm 0.20^{abc}$	18.57 $\pm 0.15^{abcd}$

Data were expressed as mean \pm S.E, differences were considered significant at $P < 0.05$

a significant difference from control in the same column

b significant difference from VPA in the same column.

c significant difference from MO in the same column.

d significant difference from MEPA in the same column.

e significant difference from MO+VPA+ MEPA in the same column.

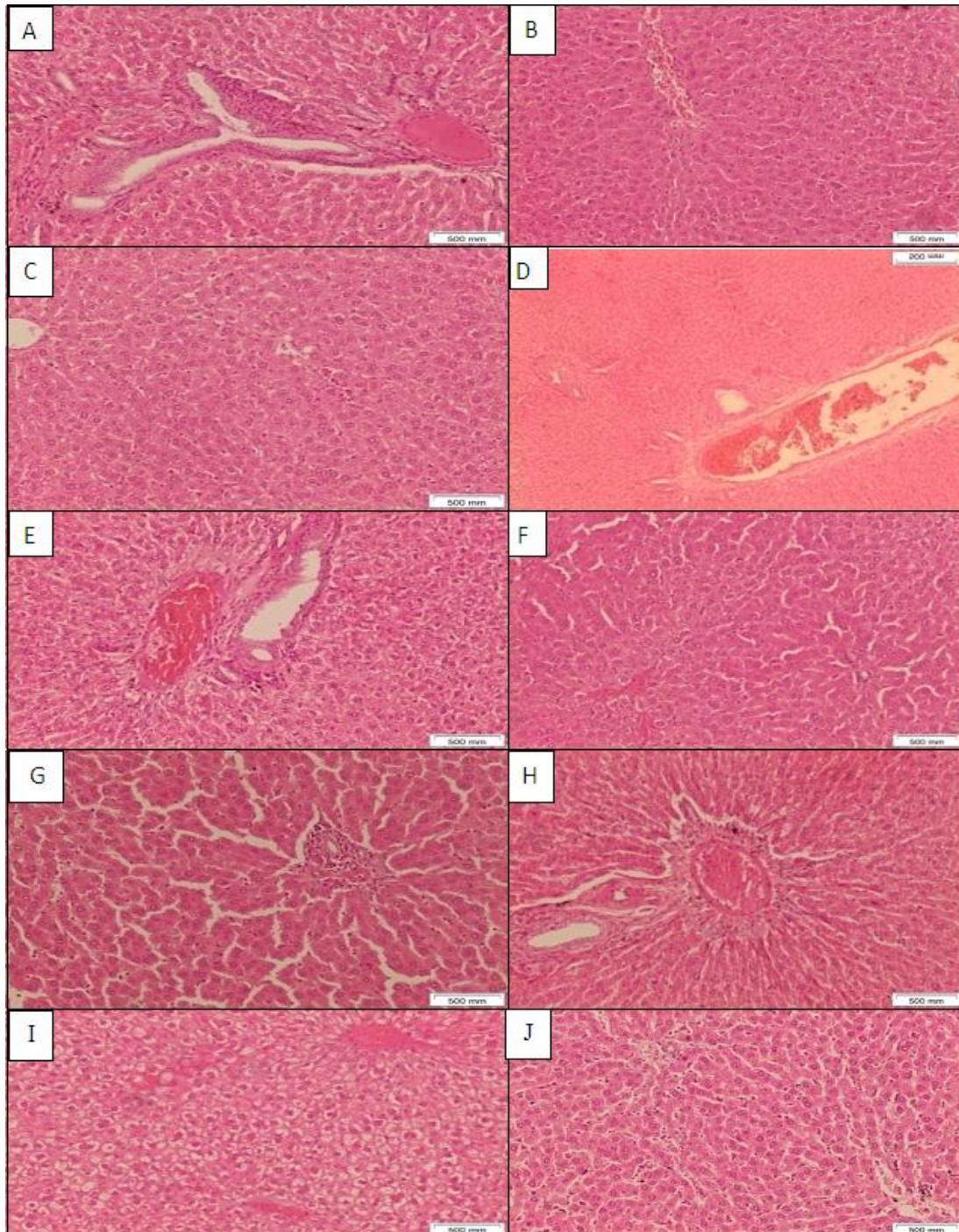


Fig. (4): Liver sections of male rats showing histopathological changes (A): Photomicrograph of liver tissue of treated rat with VPA for 2 weeks showing congested portal vessels and central vein associated with minimal inflammatory cells lymphocytes

and plasma cells. Hx&E X100.(B):control liver tissue shows the normal structure with preserved lobular architecture and normal hepatic plates. Hx&E X100. (C):MO for 2weeks showing minimal inflammatory cells and vascular congestion no hepatocyte damage noted. Hx&E X100.(D):MEPA for 2weeks showing normal liver architecture very nil inflammation and much less vascular congestion. Hx&E X100.(E): MO+MEPA for 2weeks showed a picture of almost normal liver devoid of inflammatory infiltrate or congestion. Hx&E X100.(F):VPA for 4 weeks showing marked congestion in portal vessels and central vein associated with mild inflammatory response and focal hepatocyte degenerative changes. Hx&E X40. (G):MO+MEPA for 4 weeks showed no congestion nil inflammatory cells. Hx&E X100.(H):MO for 6 weeks showing nil inflammation and congestion. Hx&E X100.(I):VPA for 6 weeks showing marked congestion in portal vessels and central vein associated with inflammatory and focal hepatocyte degenerative changes. Hx&E X100 (J):MO+ MEPA for 6 weeks showing nil inflammation and congestion almost like normal Hx &E X100.

DISCUSSION

Valproic acid is broadly used as a major drug in the treatment of epilepsy, one of the most important adverse effects of VPA is mild to severe hepatotoxicity.^[21] In this study hepatotoxicity induced by sodium valproate treatment has been clearly observed and documented particularly with chronicity of the treatment characterized by elevation of ALT, AST, GGT and reduction in total protein in a time dependent manner.

The elevation of liver functions in rats treated with sodium valproate in the present study was suggested by^[22] who suggested that the elevated levels of AST and ALT may be due to the direct damage in hepatocytes or due to the oxidative stress leading to apoptosis of hepatocytes. Also, VPA use has been complicated by a high incidence of hepatic injury which was found to be associated with oxidative damage.^[23]

In agreement with the results represented in this study,^[24,25] reported that the treatment with VPA alone caused a significant increase in serum ALT and AST activities and a significant decrease in serum albumin, and total protein levels. Also, Saleh et al^[2] reported that serum total protein, albumin and globulin were decreased after chronic administration of VPA. Okdah and Ibrahim^[26] have reported that AST and bilirubin increased in serum of animal given VPA. These results may due to its oxidative stress which has been recognized to be involved in etiology of several liver diseases.^[26]

Administration of Sodium valproate time dependently induced significant increase in oxidative stress parameters MDA, NO and GSSG and depletion in GSH content in liver tissue. These results were in accordance with Tong *et al.*^[27] who said that valporic acid drug induce hepatotoxicity by a multiple step mechanism, it may hypothesize to involve the generation of toxic metabolites and/or reactive oxygen species. The reactions of toxic metabolites with glutathione in mitochondria produce a localized depletion of glutathione that would result oxidation stress. Oxidative stress precedes the onset of steatosis and necrosis in liver.^[27] Also, this fact was in good keeping with Szalowska *et al.*^[28] who indicated that the reactive VPA metabolites (i.e., 4-ene-VPA and its subsequent metabolite, 2,4-diene-VPA) may mediate the hepatotoxicity by inhibiting mitochondrial β -oxidation of fatty acids.

The other possible explanation for elevation liver functions in rats treated with sodium valproate in the present study might be due to the fact that VPA has two hepatotoxic components; 1- Hypoglycin which leads to Jamaican vomiting sickness and micro-vesicular liver steatosis; 2- Pantoic acid which inhibits beta oxidation and causes micro-vesicular liver steatosis.^[21] Mitochondria are prominent in hepatocytes and cell becomes granular and eosinophilic.^[29] The harmful effect of VPA treatment was reflected by the increase in TNF- α level, which is a pro-inflammatory cytokine. The increase in TNF- α secretion triggers a series of intracellular events that result in activation of apoptosis and accelerating hepatic cell death during liver injury.^[30] VPA treatment induced significant elevation in the level of pro-apoptotic gene Bax and reduction in the level of anti-apoptotic gene Bcl2. Changes in the expression of Bcl-2 family proteins are usually thought to lead to apoptosis through the intrinsic route. The present results are in agreement with Chateauvieux *et al.*^[31] who reported that VPA induced apoptotic via, extrinsic and intrinsic apoptotic pathway and has a central role in Bcl2 inhibition.

On the other hand, Moringa leaves ethanolic extract treatment showed potent effect against liver damage in rats induced by sodium valproate. MO treatment attenuated the serum activities of liver markers enzymes AST, ALT and GGT, increased total protein level, restored the normal redox status and inhibited the inflammatory factor (TNF- α). Also, significantly ameliorated apoptotic gene expression Bax and Bcl2. This hepatoprotective effect is might interpreted due to its high antioxidant activity. The major bioactive compounds of phenolics were found to be flavonoid groups such as quercetin and kaempferol. Previous studies have confirmed that flavonoids exert their anti-inflammatory

effects by modulating the inflammatory cells, inhibiting the T lymphocyte proliferation, inhibiting pro-inflammatory cytokines (TNF- α and IL-1), or controlling enzymes derived from the arachidonic acid pathway.^[32] Also, the drumstick leaves are found to be a potential source of natural antioxidants.^[33]

The present findings on Moringa effect are in agreement with Sreelatha and Padma^[34] they reported that aqueous extract of Moringa oleifera exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and inhibition of lipid peroxidation. In addition, the free radical scavenging effect of Moringa oleifera leaf extract was comparable with that of the reference antioxidants. These results in agreement with Ezejindu *et al.*^[35] who said that the effect of moringa on AST, ALT, alkaline phosphatase and bilirubin levels in serum and lipid peroxidation levels in liver mediates its hepatoprotective activity. In this line, M. oleifera leaves aqueous extract was observed to have a therapeutic action against radiation hazards through enhancing of liver enzyme activities (AST, ALT and ALK), decreasing the malondialdehyde (MDA), and reduction of genetic alterations (micronuclei and DNA damage) in irradiated rats by gamma irradiation.^[36] Sreelatha *et al.*^[37] stated that moringa leaves extract showed a dose-dependent inhibition of cell proliferation associated with induction of apoptosis as well as morphological changes and DNA fragmentation. The present data recorded significant decrease in the hepatic level of the gene expression of Bax and significant increase in hepatic Bcl2 level in the group of MO with MEPA. The induction of direct liver cellular damage, ROS and oxidative stress are known as apoptosis triggers and modulators.^[38] ROS-induced apoptosis requires the participation of other cell death signaling pathways, including c-Jun N-terminal kinase (JNK) which regulate the expression of various apoptosis proteins implicated in hepatotoxicity.^[38] A previous study indicated that quercetin was able to attenuate the toxicant-induced apoptosis by the inhibition of JNK activation, indicating that quercetin can prevent apoptosis by altering the expression of Bax, Bcl-2 and caspase-3.^[39]

In this line, quercetin in Moringa flowers provides significant protection against liver damage.^[40] Immunohistochemical studies revealed that liver fibrosis was retracted by moringa plant.^[41] So, the present study suggested that Moringa oleifera significantly attenuated the up-regulation of Bax and markedly prevented the down-regulation of hepatic Bcl-2 expression in sodium valproate-treated rats. In addition, administration of mepacure drug (Silymarin + DDB) with VPA significantly alleviated the liver biomarker enzymes

activities, which were reflected by a significant reduction in serum levels of AST, ALT and GGT, as well as significant elevation of serum levels of total protein. It also suppressed the oxidative stress and reduced the cytokine (TNF- α). Also, ameliorated apoptotic gene expression Bax and Bcl2. These findings are in concordance with that reported by Wassfy et al^[42] who mentioned that Silymarin and Dimethyl Dicarboxylate Biphenyl (DDB) were significantly treated the liver and kidneys after CCL4 induced toxic hepatitis in rats, and improved liver functions as regards to AST, ALT, ALP, serum bilirubin and GGT in patients suffering from HCV infection. The same was held true with the findings of Mishra et al^[43] who indicated that silymarin is beneficial in reducing the damage of hepatocytes and regenerating the normal function of the liver after being exposed to CCl4 hepatotoxication. Mepacure was found to significantly ameliorate the rimactazid drug-induced alterations in the levels of total protein, albumin, bilirubin, glucose, triglycerides, total cholesterol and the activities of AST and ALP.^[12] Mepacure may be protects the liver cells by acting as a cell membrane stabilizer by blocking the entrance of harmful toxins and helping to remove these toxins from the liver cells.^[10] Moreover, effects of DDB may protect hepatocytes by stimulating the hepatic mitochondrial reduced glutathione (GSH) antioxidant system via activation of GSH related enzyme. The histopathological observation include marked congestion in portal vessels and central vein associated with inflammation in lymphocytes and plasma cells, also focal hepatocyte degenerative changes.^[26] Meanwhile, administration of MO and/or MEPA showing gradual enhancement in the histopathological alternations include nil inflammation response and congestion indicating reduction in hepatic damage resulted from VPA administration through different intervals. These findings are in agreement with Buraimoh et al^[44] which reported hepatoprotective effect of ethanolic extract of *Moringa oleifera* against paracetamol induced liver damage in rats. Sadek et al^[45] reported that histopathological appearances were extraordinarily enhanced in livers of rats that were treated with *Moringa oleifera* leaves ethanolic extract against diethyl nitrosamine induced hepatocellular carcinoma.

CONCLUSION

Results of the present study suggested that moringa leaves extract protects rat liver from sodium valproate- induced oxidative stress, probably via its antioxidant activity, anti-inflammatory and antiapoptotic effects. So, moringa leaves extract is a promising pharmacological agent for preventing the potential hepatotoxicity of sodium valproate.

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