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HEPATOPROTECTIVE EFFECTS OF *MORINGA OLEIFERA* EXTRACTS ON ACETAMINOPHEN-INDUCED OXIDATIVE DAMAGE IN RATS

[117]

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ABSTRACT

This study aimed to evaluate the in-vitro antioxidant activities of *Moringa Oleifera* Lam (MO) extracts, and their protective effects in acetaminophen (APAP)- induced liver injury in rats caused by oxidative damage. The antioxidants activities of ethanolic, aqueous and hexane extracts of different MO edible parts were investigated by DPPH radical scavenging capacity and malondialdehyde (MDA) assays. The ethanolic extracts of different parts of MO were found to have higher antioxidant capacity compared to the aqueous and hexane extracts. The flowers ethanolic extract has the highest total antioxidants capacity among the other different parts of MO follo wed by leaves, pods, roots and finally seeds. HPLC-MS scanning of ethanolic leave extracts showed the presence of flavonoid derivates Apigenin, quercetin and kaempferol in addition to chlorogenic acid. In the hepatoprotective study, either leaves or pods extracts (300mg/Kg bw or 600mg/Kg bw stomach tube orally) were administrated to rats one hour prior to administration of a single dose of APAP (4g/Kg bw by stomach tube orally). The hepatoprotective activity of MO leaves and pods extracts were followed for 21 days by observed in the levels of liver markers such as alanine aminotransferase (ALT) and the levels of oxidative damage markers including superoxide dismutase (SOD) and malondialdehyde (MDA) and catalase (CAT), wich analysed and compared between groups pretreated with MO extracts +APAP to those treated with APAP alone. The outcome of this parameters indicate reduction in the severity of liver damage in group treated with MO extracts + APAP and compared to those treated with APAP alone . Also, histopathological examination of liver tissues of rats treated with MO extracts showed an improvement at the end of experiment. The results of this study indicate the hepatoprotective properties of MO leaves and pods ethanolic extracts against liver injury and thereby signify its traditional use.

INTRODUCTION

Liver diseases are a major global concern, and this type of disease / disorder still has extremely poor prognosis and high mortality because of the lack of effective preventive /treatment options. The population of Egypt has a heavy burden of liver disease, mostly due to chronic infection with hepatitis C virus (HCV**) (Christina, et al 2000).**

Oxidative stress due to high levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is a common mechanism contributing to initiate initiation and progress of hepatic injury in variety of liver disorders. Various indogenous factors (such as alcohols, drugs, environmental toxins ,virus and UV light) may cause oxidative stress in liver **(Sha Li, et al 2015)**. Moreover systemic oxidative stress arising during liver disease can also cause damage to extra-hepatic organs, such as brain impairment and kidney failure (**Palma, et al 2014**). Application of natural antioxidants signifies a rational curative strategy to prevent and cure liver diseases involving oxidative stress. Several studies showed that hepatoprotective effects of medicinal plants associated with natural antioxi-

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dants (**Sharida Fakuraz, et al 2012).** *Moringa Oleifera* Lam (MO) is a highly valued plant, having remarkable range of Pharmacological properties in addition to significant nutrition value. The various parts of MO plant such as roots, leaves, flowers, gum bark, fruit, seeds and seed oil have been used for various aliments in the indigenous medicine of south Asia, especially in India, including the treatment of inflammation and infectious diseases along with cardiovascular and hepatorenal disorder **(Anwar, et al 2007)** The methanolic extract of MO edible parts showed strong *in vitro* antioxidant properties, which is due to the presence of various bioactive compounds such as chlorogenic acid, rutin, quercetin glucoside **(Atawodi et al 2010)** Moreover, the extract of Moringa oleifera leaves and other parts of plant have been shown to have potent antioxidant action *in vivo* **(Ashok and Pari, 2003; Streelatha and Padma, 2011; Sharida, et al 2012).** Acetaminophen (paraacetamol) is most widely used in the world as an analgesic and antipyretic drug for humans, that is save at therapeutic dosages. However, it's also known to cause hepatic necrosis and renal failure in humans **(Jaeschke and Bajt, 2006)** and animals **(Sharida Fakuraz, et al 2008)**. Oxidative stress is reported to play a role in the pathogenesis in acetaminophen – induced liver and renal damages. Therefore, models of acetaminophen – induced in liver damage in mice / rate were used to study antioxidant and hepatoprotective effects of natural antioxidant and medicinal plants.

The objective of this study were to (1) evaluate and compare in vitro total antioxidant capacity of different parts (leaves, Flowers, roots, pods and seeds) of *Moringa oleifera* Lam (MO) extracts, (2) evaluate the adverse effects induced by oral of acetaminophen (APAP) in an overdose on hepatotoxicity of experimental rats, (3) evaluating the anti-hepatotoxic potential of ethanolic extracts from MO leaves and pods on APAP-induced liver toxicity in experimental rats.

MATERIALS AND METHODS

Plant collection

Moringa Oelifera (MO) plant were collected from farm of the Egyptian scientific society farm of *Moringa* at National Research Center; Giza, Egypt., August 2016. The collected parts (leaves, seeds, roots and pods) were washed thoroughly with distilled water and air- dried.

Preparation of MO extracts

Different parts of MO (leaves, flowers, seeds, roots and pods) were stepwise extracted using hexane (60-80 %), ethanol (95-96%) and distilled water. One Kg of each dried powder part and 103.45 g of fresh flowers were susceptible for extraction by soaking overnight at room temperature three times. Extracts were then evaporated to dryness at 40-45ºC at reduced pressure by using rotary evaporator. Extracts of hexane, ethanol and distilled water were taken for analysis of antioxidant activities.

Determination of (DPPH) radical scavenging activity

DPPH radical scavenging activity was measured by determins the decrease in absorbance of the ethanolic DPPH solution at 517 nm in the presence of MO extracts as described by **Brand-Wiliams, et al (1995)**. In brief 0.5 ml extract sample (12 mg/ml) was added to 0.3ml ethanolic solution of DPPH (0.05mM) and the total volume made up to 3ml with ethanol. The reaction mixture were vortexed and allowed to stand for 100min at room temperature in the dark before the absorbance was measured using solvent ethanol as blank. The percentage of free radical scavenging activity was calculated from the following equation:-

$$\textit{\% inhibition} = \frac{A\ cont - A\ sample}{A\ Standard} \times 100$$

Wheres: A_{cont} = absorbance of standared DPPH solution used in experiment with all reagent except sample.

Determination of lipid peroxidation (LPO) measurement of MDA- TBARS.

Malondialdehyde (MDA) was measured by thiobarbituric acid reactive substances (TBARS). TBARS of Oil-MO extracts mixtures was determined according to **Tamura and Shibamoto (1991).** The reaction mixture consists of 0.5 ml of oil-extract mixture (0.1mg of sunflower oil- 12 mg MO extract), 1ml thiobarbituric acid (TBA) 0.3% and 1ml trichloroacetic acid (TCA) 10%. mixture was heated for 30 min at 95°C in water bath. After cooling, 1ml chloroform was added, and the reaction mixtures were centrifuged at 1000×g to give clear supernatants. Absorbance of the supernatants was measured at 532 nm. The extinction coefficient of TBA-malondialdehyde product of

1.56×105M-1cm-1 was used to convert absorbance values into concentrations of mmol MDA/g oil.

Identification of compounds in MO extracts by HPLC-MS

Qualitative analyses was carried out by high performance liquid chromatography using an Agilent LC-MS 1200. Phenolics and flavonoids were characterized in ethanolic extracts of leaves of MO by chromatographic comparisons with standards (retention time, UV-Vis spectral features) and MS scanning according to the procedure described by **(Chandra, et al 2001)** with some modifications. Samples were analysed using a Zorbax SB- C18 (5µm, 4.6 mm x 250 mm) column at 30°C. The following solvents in water with a flow rate of 1 ml min-1were used: A (0.5% aqueous phosphoric acid (v/v)) and B (water/acetonitrile/glacial acetic acid/phosphoric acid, 50:48.5:1.0: 0.5 (v/v/v/v)). The solvent gradient was % B, initial, 20%; 26 min. 60%; 30 min. 20%; 35 min. 20%.

Hepatoprotective effects of MO extracts

Experimental animals

Male albino rats of weighing 130-150 g were obtained from the animal house of National Organization for Drug Control and Research(NOD CAR). Animals were housed in an ambient temperature of 25°C + 3.2°C on light/dark cycle of 12/12 hours. All rats were kept in clean polypropylene cages and administered food and water.

Induction of hepatic stress by acetaminophen treatment

The acetaminophen (APAP) were dissolved in dimethyl sulfoxide (DSMO) at a concentration 4 g APAP/kg bw. 11 groups with 6 rats each received 1 mL oral administration of single dose of (APAP) dissolved in DMSO one day after treatment with MO extracts. Four groups received ethanolic extract of MO at 300 mg or 600 mg pods or leaves / 1 ml DMSO starting one day and continuous for 21 days before treating with APAP.

Blood samples from the retro-orbital were taken weekly through fine capillary glass tubes according to **Schermer's (1967).**

Liver functions tests

The following parameters were measured in serum, alanine aminotransferase (ALT), aspartate aminotransferase (AST),according to **Reitman and Frankel (1957)**, ɣ-glutamyl transferase (GGT) according to **Shaw, M. et al (1983)** , alkaline phosphatase (ALP) according to **Belfield and Goldberg (1971)** and total bilirubin according to **Walter and Gerade (1970)**.

Determination of oxidative-stress markers

The biological system contains some enzymes such as catalase (CAT) and superoxide dismutase (SOD) which contribute in the defense process against reactive species. The level of these enzymes change under oxidative stress. Catalase activity in serum was determined according to the method described by **Sinha A.K.(1972).** The activity of SOD enzyme in serum was determined according to the procedure of **Kiran, K.K. et al (2017)**

Determination of malondialdehyde (MDA).

MDA in serum samples was determined according to the procedure of **Kiran, K.K. et al (2017)**.

Determination of urea

Urea in serum was determined according to the procedure of **Fawcett and Soctt, et al (1960).**

Determination of creatinine

creatinine in serum was determined according to the procedure of **Bartles, et al (1972).**

Histopathological examination.

AT the end experiment (after 21 days), the rats were killed, and the liver was removed and stored in 10% formalin for histological evaluation.

Statistical analysis

The recorded data were treated statistically using the one way analysis of variance (ANOVA).The means were compared by Duncan's Multiple Range Test at $p<0.05$. Statistical analysis were performed using (**SAS, 2006. Data Analysis of Various, Cary, NC).**

RESULTS AND DISCUSSIONS

1-HPLC-MS analysis

The ethanolic extract of MO was analyzed by HPLC-MS. five main fragment ion peaks (354,431, 463, 505 and 447) were observed which represent chlorogenic acid, Apigenin-8-C—glucoside, Quercetin-3-O-β-D-glucoside,Quercetin-3-O-acetyl glucoside and kaempferol-3-O-glucoside respectively. The antioxidant activity of MO ethanolic extract is due to the presence various bioactive compounds such as Quercetin glucoside, kaempferol glucoside, chlorogenic acid and rutin **(Atawodi, et al 2010)**.

2- DPPH scavenging activity of the different extracts of MO

Table (1) illustrates the DPPH free radical scavenging activity of the ethanolic , aqueous and hexane extracts of different parts (leaves, Flowers, roots, pods and seeds) of the *Moringa oleifera* lam (MO) extracts at constant concentration of (12 mg/ml extract). The highest antioxidant activity in term of % inhibition was detected in ethanolic extracts followed by aqueous and finally hexane extract. Among the ethanolic extracts, the flowers have higher scavenging activity (94.910%) than standard substance vitamin C (94.000%) followed by leaves (93.100%) and pods (90.786%). Generally, it could be concluded that (1) ethanolic extract possessed significant highest antioxidant activity comparing to aqueous and hexane extracts. (2) the flowers, leaves and pods extract has also significant highest antioxidant activities compared to other parts of the plant. It has been reported that the chemical constituents are varying among the different parts of the same plant (**Abdul K. et al 2009)**, and using different solvents due to variation of the solubilities of different bioactive compound **(Koruthu, et al 2011).**

3- Effect of MO leaves and pods ethanolic extracts in the liver function in APAP induced toxicity

Liver cells are highly susceptible to oxidative stress because it is a major organ attacked by reactive oxygen species (ROS). Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The metachondrion , microsomes and peroxisomes in parenchymal cells can produce ROS. Moreover, Kupffer cells, hepatic stellate cells and endothelial cells are potentially more exposed or sensitive to oxidative stress- related molecules **(Sanchez-Valle, et al 2012).** A disease process-which usually including oxidative stress- may cause changes in cell membrane permeability or increase cell death, resulting in release of intracellular enzymes. Increasing degree of tissue damage, increased the amount of released cellular components. Analyzing blood for cellular components represent a convenient way for clinicians to assess damage occurring elsewhere in the body.

3.1. Effects of (MO) leaves and pods ethanolic extracts on liver function enzymes in serum, ALT, AST, GGT and ALP.

The leaves and pods ethanolic extracts were evaluated for their potential antioxidant activity to study their hepatoprotective effect on APAP-induced hepatoxicity in rats. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma (Ɣ) glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein and total bilirubin in the serum are used as a biochemical markers for evaluation of hepatic injury. The liver enzymes ALT, AST, GGT, ALP are concerned with intra cellular metabolism; they are released from the liver when cells become necrotic as in viral or toxic hepatic or cirrhosis, secreted into the serum at elevated levels in the presence of liver cell damage **(Price and Steven, 2000).** The results in **Table (2)** showed that, ALT, AST, GGT and ALP activity were increased significantly after oral administration with (APAP 4g/kg b.w) as compared with normal control rats after three weeks. All liver function markers enzymes activity were decreased in hepatotoxic induced rats treated with *MO* leaves or pods extracts at levels of 300 and 600mg/kg. b.w. It concluded that *Moringa oleifera* lam extract has concentration dependent reducing effect on serum ALT, AST, GGT and ALP enzymes. Obtained results are in agreement with those of **(Govindarajan Karthivashan, et al 2016); (Nevine, et al 2015) and (Mariam, et al 2014-2015).**

Table 1. The% of DPPH radical scavenging inhibition activity of extracts of MO (at constant conc. of 12 mg extract/ ml)

The percentage of DPPH scavenging activity (% inhibition) Mean values ± S.E.				
	Ethanolic extracts	**Aqueous extracts**	**Hexane extracts**	**Vit C**
Flowers	94.916±2.031^{a}	72.666±1.763^{d}	17.333±0.881^{h}	
Leaves	93.101±0.935^{ab}	75.500±0.500^{d}	43.000±2.309^{f}	
Pods	90.786±1.015^{abc}	86.333±1.201^{bc}	40.333±1.452^{f}	**94.000±1.171^{a}**
Roots	84.803±2.952^{c}	58.333±1.201^{e}	25.333±2.603^{g}	
Seeds	62.150±5.500^{e}	63.333±2.603^{e}	12.666±1.452^{h}	

Means with the same letters are not significant at 5%.

Table 2. Effect of ethanolic extract of MO leaves and pods on serum (ALT), (AST), GGT and ALP activity

Treatment	**ALT U/ml**		**AST U/ml**		**GGT U/ml**		**ALP IU/L**	
	Zero time	**Week 3**	**Zero time**	**Week 3**	**Zero time**	**Week 3**	**Zero time**	**Week 3**
Normal Control	51.434±	40.269±	54.767±	45.508±	8.299±	8.874±	173.182±	161.165±
% Change	0.957^{a}	0.662^{a}	1.258^{a}	0.993^{b}	1.477^{a}	0.433^{b}	5.943^{c}	8.201^{b}
	125.4%	40.3%	119.5%	40.7%	195. 5%	19.2%	86.2%	31.3%
Negative Control	49.390±	38.931±	53.917±	44.017±	6.369±	7.141±	165.528±	138.228±
(DMSO)	1.048^{a}	1.045^{b}	0.404^{a}	0.790^{b}	1.223^{abc}	0.676^{b}	3.897^{c}	3.701^{b}
% Change	120.4%	38.9%	117.6%	39.4%	150%	15.4%	82.4%	26.8%
Positive Control	41.021±	100.027±	45.845±	111.833±	4.246±	46.304±	200.870±	514.915±
(APAP)4g/kg	0.520^{c}	1.625^{a}	0.399^{b}	1.548^{a}	0.553^{c}	2.856^{a}	0.300^{b}	63.592^{a}
b.w	100%	100%	100%	100%	100%	100%	100%	100%
% Change								
MOLE 300mg/kg	43.658±	35.603±	47.717±	40.878±	6.948±	3.667±	173.975±	122.322±
b.w	0.203^{b}	0.770^{c}	0.719^{b}	0.252^{c}	0.598^{ab}	0.22^{c}	8.005^{c}	7.423^{bc}
% Change	106.4%	35.6%	104.1%	36.6%	163.6%	7.9%	86.6%	23.85%
MOLE 600mg/kg	50.050±	33.880±	54.175±	40.300±	5.211±	3.088±	218.578±	119.445±
b.w	0.611^{a}	0.743^{cd}	0.441^{a}	0.714^{c}	0.704^{bc}	0.548^{c}	6.575^{b}	6.858^{bc}
% Change	122.0%	33.9%	118.2%	36.0%	122.7%	6.7%	108.8%	23.2%
MOPE	51.187±	32.165±	55.797±	36.838±	6.755±	3.005±	261.900±	112.952±
300mg/kg b.w	0.776^{a}	1.089^{d}	0.668^{a}	0.946^{d}	0.612^{abc}	0.275^{c}	19.124^{a}	4.499^{bc}
% Change	124.8%	32.2%	121.7%	32.9%	159.1%	6.5%	130.4%	21.9%
MOPE	45.247±	28.837±	54.458±	34.673±	7.861±	2.509±	200.605±	64.342±
600mg/kg b.w	1.335^{b}	0.118^{e}	1.590^{a}	0.755^{d}	0.516^{a}	0.435^{c}	0.579^{b}	4.103^{c}
% Change	110.3%	28.8%	118.8%	31.0%	185.1%	5.4%	99.9%	12.5%

a, b, c, d , e Values of 6 rats means ± S.E., Means with the same letter are not significant different, % change from positive control (100% toxicity), at P<0.05

3.2. Effects of (MO) leaves and pods ethanolic extracts on serum total bilirubin (T.BIL) and total protein

The results in **Table (3)** showed that, (T.BIL) was increased to (6.001mg/dl) after oral administered with (APAP 4g/kg b.w) as compared with normal control rats (3.738mg/dl) after 3 weeks, and in hepatotoxic induced rats the T. Protein was significantly decreased compared with either negative or normal controls. T.BIL was noticed significant decrease after 3 weeks in groups that treated with MO leaves and pods extracts at doses of 300 and 600mg/kg.b.w. On the other hand, T. Protein in serum was increased in hepatotoxic induced rats treated with MO extract leaves and pods at the two doses used in experiment. The obtained results rerefers to the concentration dependent effect of extracts used either from leaves or pods. The ethanolic extract of pods was showed no significant difference compared with ethanolic extract of leaves on serum T.BIL and total protein. Obtained results agreed with **Marzan Sarkar, et al (2017) & Nevine, et al (2015).**

3.3. Effect of Moringa Oleifera lam(MO) leaves and pods ethanolic extracts on oxidative markers in serum , malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT).

Malondialdehyde (MDA) the toxic end product of lipid peroxidation in serum (**K Gayathri., et al 2011)** was studied in table (4). MDA was significantly elevated after treatment with (APAP 4g/kg b.w) by (0.341 nmol/ml) as compared with normal control rats (0.058 nmol/ml) after 3 weeks. Treatment with *Moringa Oleifera lam* extracts used in this experiment at the two doses 300 and 600mg/kg.b.w resulted in significant decrease in MDA in hepatotoxic induced rats. SOD is the first enzyme involved in the antioxidant defense by lowering the steady state $O_2^{\cdot-}$, it̓ s a member of a mutually supportive team of defense against reactive oxygen species (ROS) **(Nevin and Vijayammal, 2005)**. Data presented in **Table (4)** showed that, (SOD) was decreased significantly after treatment with (APAP 4g/kg b. w) compared with normal control rats after 3 weeks, SOD was recovered in hepatotoxic induced rats treated with *Moringa Oleifera* lam extracts and the elevation in SOD activity was corelated with either leaves or pods extract level. Similarly, *Moringa Oleifera* lam extracts of leaves and pods at levels of 300 and 600mg/kg.b.w. significantly decreased the catalase activity in serum by (20.552, 24.356, and 67.807, 71.426 mmol/min/μL) respectively. Our findings are in agreement with those of **(Sharida Fakurazi., et al 2012) and (Mariam., et al 2014-2015).**

4. Histopathological result of the pathological analysis of the biological experiment

Figures 1, 2, 3 and 4 showed results of the histopathological analysis of the liver for rats treated with 300 or 600 mg/kg b.w. of ethanolic extract of either leaves or pods of *Moringa Oleifera* lam. **Fig.1** had an average change and **Fig. 2** had a change below average and **Fig. 3** Simple, **Fig. 4** have slight pathological changes , The effects are due to the active MO extract in liver cell repair by activating antioxidant enzymes.

The pods extract was found to be more effective than the leaves extract and the effect was found to be concentration dependent.

5. Effect of *Moringa Oleifera lam* (MO) leaves and pods ethanolic extracts on the kidney function (serum urea and creatinine) in APAP induced toxicity

Data presented in **Table (5)** showed that, urea and creatinine (CR) were significantly elevated after oral administration of (APAP 4g/kg b.w) as compared with normal control rats after 3 weeks of treatment, urea was decreased in hepatotoxicity rats treated with *Moringa Oleifera lam* leaves and pods extracts at the two doses 300 and 600 mg/kg.b.w. Similarly, creatinine (CR) was increased after treatment with (APAP 4g/kg b.w) by (0.702 mg/dl) as compared with normal control rats (3.738 mg/dl) after 3 weeks. After 3 weeks treatment with *Moringa Oleifera lam* extracts of leaves and pods at the two doses 300 and 600 mg/kg.b.w. (CR) was decreased significantly in hepatotoxic induced rats. Reduction in CR in serum was noticed to be corelated with the extract concentration used. our findings are in agreement with those of **(Govindarajan Karthivashan., et al 2016).** Relationship of the liver to the kidney when paracetamol poisoning (acetaminophen). The occurrence of renal failure before liver damage, is indicating that the dose of paracetamol in the kidneys is lower than the liver dose. Liver damage results not from paracetamol itself, but from one of its metabolites, *N*-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI

Table 3. Effect of ethanolic extract of MO leaves and pods on serum T. Bil, and T. protein

Treatment	T.Bil mg/dl		T. protein g/dl	
	Zero time	Week 3	Zero time	Week 3
Normal Control	4.956±0.328[ab]	3.738±0.848[b]	5.898±0.132[a]	6.137±0.095[bc]
Negative Control (DMSO)	5.070±0.172[a]	2.967±0.390[b]	5.833±0.113[a]	5.542±0.368[c]
Positive Control (APAP) 4g/kg b.w	5.005±0.314[ab]	6.001±1.068[a]	6.112±0.250[a]	2.769±0.094[d]
MOLE 300mg/kg b.w	4.172±0.254[abc]	0.207±0.074[c]	5.783±0.113[ab]	6.475±0.248[b]
MOLE 600mg/kg b.w	4.293±0.450[bc]	0.129±0.053[c]	5.967±0.175[a]	7.640±0.366[a]
MOPE 300mg/kg b.w	3.570±0.269[c]	0.099±0.025[c]	5.243±0.177[b]	8.223±0.107[a]
MOPE 600mg/kg b.w	3.747±0.423c	0.054±0.015[c]	5.795±0.137[ab]	8.230±0.187[a]

a,b,c,d ,e Values of 6 rats means ± S.E., Means with the letter are not significant different, , Number in the same column followed by the same letters are not significant at P<0.05.

Table 4. Effect of ethanolic extract of *Moringa Oleifera lam* (MO) leaves and pods on serum malondialdehyde (MDA), serum superoxide dismutase (SOD) and serum catalase (CAT) activity

Treatment	MDA (nmol/ml)		SOD U/ml		Catalase Activity(mmol/min/μL)	
	Zero time	Week 3	Zero time	Week 3	Zero time	Week 3
Normal Control	0.067±0.013[a]	0.058±0.011[b]	153.960±13.8[a]	179.703±19.391[c]	3.835±0.365[ab]	4.013±0.265[c]
Negative Control (DMSO)	0.071±0.027[a]	0.049±0.002[b]	149.340±15.947[a]	189.604±17.670[c]	3.533±0.076[ab]	4.113±0.204[c]
Positive Control (APAP)4g/kg b.w	0.075±0.014[a]	0.341±0.085[a]	154.291±19.206[a]	63.201±8.410[d]	3.919±0.171[a]	2.302±0.317[c]
MOLE 300mg/kg b.w	0.098±0.029[a]	0.035±0.004[b]	159.571±19.634[a]	477.393±16.854[b]	3.811±0.238[ab]	20.552±1.051[b]
MOLE 600mg/kg b.w	0.091±0.013[a]	0.034±0.005[b]	99.505±12.121[b]	489.934±42.167[b]	3.215±0.191[b]	24.356±0.778[b]
MOPE 300mg/kg b.w	0.131±0.020[a]	0.027±0.008[b]	151.650±19.864[a]	503.135±12.073[b]	3.216±0.257[b]	67.807±7.456[a]
MOPE 600mg/kg b.w	0.127±0.035[a]	0.022±0.003[b]	93.069±4.532[b]	596.370±16.815[a]	3.362±0.284[ab]	71.426±11.041[a]

a,b,c,d Values of 6 rats means ± S.E., Means with the letter are not significant different, , Number in the same column followed by the same letters are not significant at P<0.05.

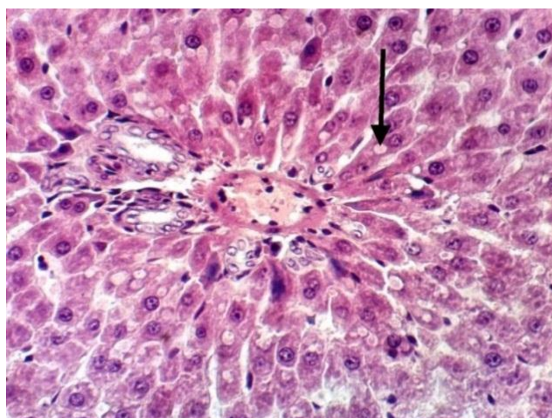

Fig. 1. Liver of rat from group leaves extract 300 showing vacuolar degeneration of hepatocytes (H & E X 400).

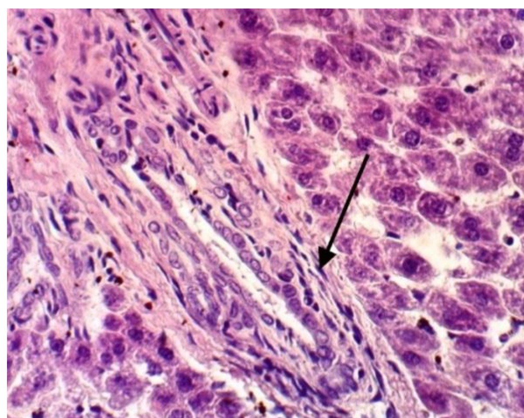

Fig. 2. Liver of rat from group leaves extract 600 showing fibroplasia in the portal triad (H & E X 400).

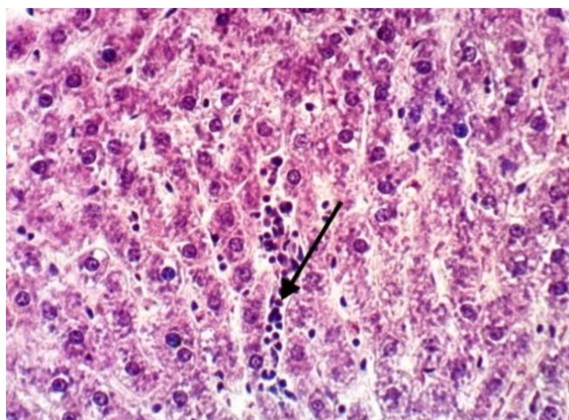

Fig. 3. Liver of rat from group pods extract 300 showing sinusoidal leucocytosis (H & E X 400)

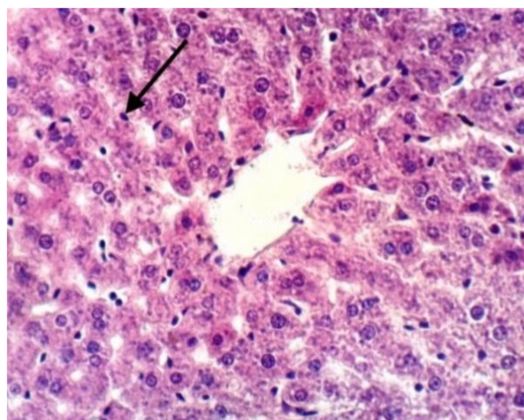

Fig. 4. Liver of rat from group pods extract 600 showing slight activation of Kupffer cells (H & E X 400).

decreases the liver's glutathione and directly damages cells in the liver according to **Webb**, **Andrew, et al (2016).** The end- product of creatine metabolism in muscles is creatinine. In renal failure, creatinine is retained with other non-protein nitrogen of the blood. hence, serum creatinine level increases in cases of kidney diseases. Consequently, the determination of creatinine level in serum can be considered as a good index for renal impairment rather than serum urea (**Baron, 1987)**. Therefore, kidney function was analyzed. Paracetamol overdose cause a marked proteinuria and glucosuria with a significant increase creatinine and urea plasma levels **(Trumper et al 1998)**. High dose Paracetamol administration caused, marked depletion of GSH levels and antioxidant enzyme activity **(Cekmen et al 2009),** and is often associated with a wide range of metabolic disorders including serum electrolytes, urea and creatinine. The renal functions with the increased serum urea and creatinine levels are considered one of the most documented parameters for investigating drug induced nephrotoxicity in animals and man **(Adelman et al 1981).** High dose of Paracetamol caused renal insufficiency is consistent with acute tubular necrosis, an increase in the plasma creatinine level and a decrease in the glomerular filtration rate (GFR). The development of ARF in rats within 24hrs by oral administration of a single dose of acetaminophen (750mg/kg) was reported (**Pelani et al 2009).**

Table 5. Effect of ethanolic extract of *Moringa Oleifera lam* (MO) leaves and pods on kidney function parameters in serum (urea and creatinine)

Treatment	Urea(mg/dl)		creatinine g/dl	
	Zero time	Week 3	Zero time	Week 3
Normal Control	44.770±4.214[a]	44.563±1.706[c]	1.040±0.076[ab]	0.702±0.143[b]
% Change	98.1%	22.7%	97.5%	14.5%
Negative Control (DMSO)	49.455±3.364[a]	56.107±3.802[bc]	1.128±0.245[ab]	0.728±0.102[b]
% Change	108.4%	28.6%	105.7%	15.0%
Positive Control (APAP)4g/kg b.w	45.643±2.677[a]	196.123±13.094[a]	1.067±0.181[ab]	4.842±0.816[a]
% Change	100%	100%	100%	100%
MOLE 300mg/kg b.w	54.678±5.860[a]	81.417±10.729[b]	0.963±0.047[b]	0.408±0.078[b]
%Change	119.8	41.5%	90.3%	8.4%
MOLE 600mg/kg b.w	44.830±2.006[a]	65.107±15.852[bc]	1.337±0.139[ab]	0.380±0.090[b]
%Change	98.2%	33.2%	125.3%	7.8%
MOPE 300mg/kg b.w	54.367±1.508[a]	59.407±2.523[bc]	1.603±0.451[a]	0.345±0.028[b]
%Change	119.1%	30.3%	150.2%	7.1%
MOPE 600mg/kg b.w	47.550±7.454[a]	40.730±3.397[c]	1.153±0.096[ab]	0.242±0.032[b]
%Change	104.2%	20.8%	108.1	5.0%

a,b,c,d ,e Values of 6 rats means ± S.E., Means with the letter are not significant different, %change from positive control (100% toxicity), Number in the same column followed by the same letters are not significant at $P<0.05$.

CONCLUSION

Ethanolic extract of MO leaves and pods is an active in treating liver toxicity caused by drugs like Acetaminophen. The effect on liver and kidney was found to be a concentration dependent means that the active ingredients in both leaves and pods are similar and high level was correlated with the protective effect against hepatotoxicity.

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