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Evaluation of the Hepatoprotective Efficacy of *Moringa oleifera* on Tramal-Induced Liver Toxicity in Animal Modules.

Ashraf Y. Albarakai[1*] and Hassan Mohamed Abd Elrhman Alsbery[2].

[1]Human Anatomy department, College of Medicine, Taif University, Saudi Arabia.
[2]Basic Medical Science, Histopathology Dep. National Organization for Drugs Control and Research, Cairo, Egypt.

ABSTRACT

The present work studied the effect of *Moringa oleifera* on liver injury induced by the tramal in albino mice. Animals were divided into 4 groups. Group I: control, group II: given *Moringa oleifera* only (20 mg/kg b.w) for two weeks, group III**:** given tramal (3 mg/kg) only for two weeks and group IV: given tramal and *Moringa oleifera* for two weeks. Histopathological liver of tramal -treated mice showed many alterations of lymphocytic aggregation and congested in the blood vessels. Degeneration of hepatocytes with karyolitic nuclei and elongated Kuffer cells were also seen. Biochemical results showed that tramal alone, caused significant increase in liver enzyme, Aspartate transaminase (AST), Alanine transaminase (ALT). Treating animals with tramal and *Moringa oleifera* led to an improvement in the histological structure of the liver together with significant decrease in levels of liver enzymes (AST and ALT). The present results indicated that *Moringa oleifera* has ameliorative effect against liver damage induced by tramal and this may be mediated by its antioxidant activity.

Keywords: Tramal, *Moringa oleifera*, hepatotoxicity, mice

Corresponding author

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INTRODUCTION

Tramadol is a centrally acting synthetic opioid analgesic commonly prescribed for moderate to severe pain [1]. Tramadol has a dual mode of action; weak binding to μ opiate receptors and reuptake inhibition of serotonin and noradrenaline neurotransmitters. As with all other analgesic drugs, the dose of tramadol should be adjusted according to the severity of the pain and the individual sensitivity of the patient. Tramadol is widely prescribed to relieve pain and low efficacy the addictiveness [2]. Every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Hepatic metabolism is, first and foremost, a mechanism that converts drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the parent compound [3]. Every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Hepatic metabolism is, first and foremost, a mechanism that converts drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the parent compound [3, 4].

Tramadol abuse, dependence as well as acute overdose–related deaths have been increasingly reported especially in young male adults [5]. The liver is responsible for the tramadol metabolism and excretion and the high risk of hepatotoxicity [6]. The mechanism of its analgesic action is complex. Most reports suggest that the analgesic activity and other clinical effects of tramadol are result of opioid and non-opioid mechanisms. Tramadol binds to the μ-opioid receptor, although much more weakly than morphine. It also inhibits the neuronal reuptake of norepinephrine and serotonin as do the antidepressant drugs such as amitriptyline and desimpramine [7]. Tramadol is metabolized in the liver by two principal pathways: O-demethylation to O-desmethyltramadol (M1) by CYP2D6 and N-desmethylation to N-desmethyltramadol (M2) by CYP2B6 and CYP3A4. Only one of tramadol metabolites, M1, is pharmacologically active. Its selectivity for μ-receptors has recently been demonstrated, showing a higher affinity for opioid receptors than the parent drug [8]. It is transformed in the liver to O-desmethyl-tramadol, which itself is an active substance and 2-4 times more effective and potent than tramadol [9].

A number of studies were seen to find out new effective mechanism without side effects, for treating liver diseases are still on-going. Natural remedies, mainly from traditional plants are found to be both effective & safe alternatives for the treatment of hepatotoxicity. Extracts from plant sources have also been investigated for hepatic-protective & antioxidant effects against liver damage [10]. *Maringa oleifera* is one of the herbal plants with a wide range of medicinal [11].

The leaves are source of protein, β-carotene, vitamins (A, B, C, E, and riboflavin), nicotinic acid, folic acid, pyridoxine, amino acids, minerals, various phenolic, with a known powerful antioxidant property [12] Previous studies on rats have been demonstrated that the hepato-protective effects of extracts from different parts of *Moringa oleifera* against liver toxicity [13]. But, the anti-hepatotoxic nature of *Moringa oleifera* leaves against tramal induced liver toxicity in mice has not yet been demonstrated. Therefore, the present study was designed to determine the effect of leaf extract of *Moringa oleifera* on liver toxicity caused by tramal toxicity, in the experimental animals.

MATERIALS AND METHODS

Plant materials:

Moringa oleifera leaves were obtained from plant biochemistry department, National Research Centre, Cairo, Egypt.

Preparation of extract:

The leaves of Moringa oleifera were cleaned thoroughly, and then dried in room temperature & crushed into coarse powder. About 20 gm of powder was taken and soaked separately in 100 ml of water & chloroform by keeping it in a Shaker for 3 days. It was filtered through cheese cloth and reduced to 10% of its original volume (organic solvent). Then, using a rotary evaporator, the filtrate was concentrated in vacuum, while aqueous extract was dried using water bath.

Therapeutic dose of tramal:

The the rapeutic dose of tramal (3 mg/kg/bw. orally for two weeks) was calculated by extrapolating the therapeutic dose of humans to mice by referring to the table reported by Paget and Barnes and is equivalent to human daily therapeutic dose used in clinical fields [14].

Experimental animals:

Twenty *albino mice*, weighing between (40-60 gm) were obtained from the animal house of animal reproduction research institute, Cairo, Egypt. The animals were housed and maintained at an air-conditioned room and allowed free access to water and food.

Experimental design:

The animals in this experiment were divided into four groups, five animals in each group, as follows:

Group I: Five mice used as control group, received normal saline only.
Group II: Five mice, received dally oral dose of *Moringa oleifera* leaf extract, (9 mg/kg/bw) for two weeks.
Group III: Five mice, received dally oral dose of tramal (3 mg/kg/bw) for two weeks.
Group IV: Five mice, received dally oral dose of tramal (3 mg/kg/bw) for two weeks, followed by *Moringa oleifera* extract (20 mg/kg/bw) for two weeks.

Biochemical studies:

The blood sample (2 ml) was collected directly via cardiac puncture to detect the levels of liver enzymes (AST & ALT) in the serum. Spectrophotometer was used to assay levels of AST and ALT of mice serum [15].

Statistical analysis:

The data were expressed as means ±SD from 5 animals per group. The differences between the groups were compared for statistical significance using the student '*t*' test, $p<0.05$ was taken as significant.

Histological studies:

Animals from control and treated groups were sacrificed by cervical dislocation then they were dissected and small pieces of the livers were quickly removed and fixed in10% neutral buffered formalin fixative fluid. Following fixation, specimens were dehydrated, embedded then sectioned with thickness of 10 microns and then mounted on the clean slides without using any adhesives medium. For histopathological examination sections were stained with Ehrlich Hematoxylin and Eosin (Culling, 1974). The cytoplasm appeared pink and nuclei acquire a blue color.

RESULTS

Histopathological results

The histological examination of liver section of the treated animals with Moringa Oleifera only for two weeks (group II), showed that the central vein and hepatocytes nearly normal, (Figs. 2&3). While liver section of the treated animals with tram tramal for two weeks (group III), showed aggregation of lymphocytic in the central vein, degeneration of hepatocytes with karyolitic nuclei and elongated Kuffer cells (Figs. 4&5). In contrast the histological examination of liver section of the treated animals with tramal and then treated with *Moringa Oleifera* for two weeks in the group IV, showed that moderate reduction of lymphocytic aggregation and retrieval of the normal architecture of the liver tissue with partially improvement in blood sinusoidal (Figs. 6&7).

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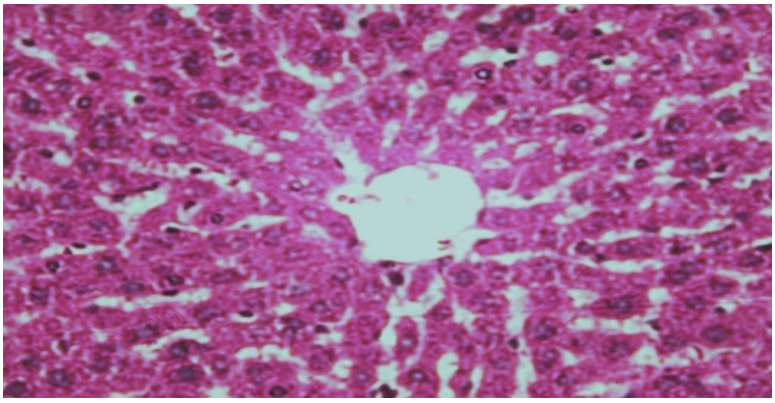
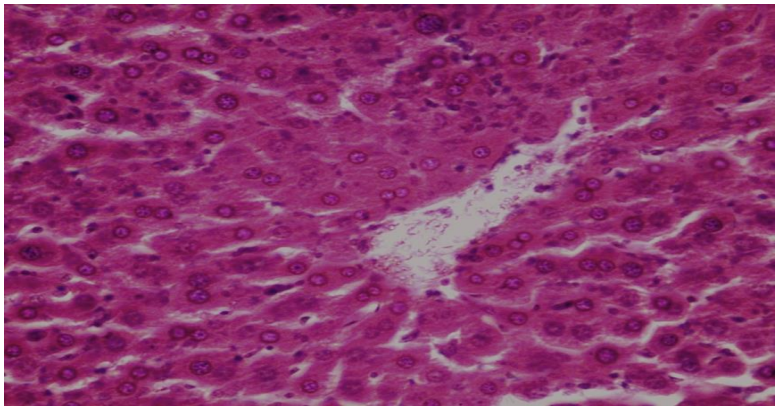
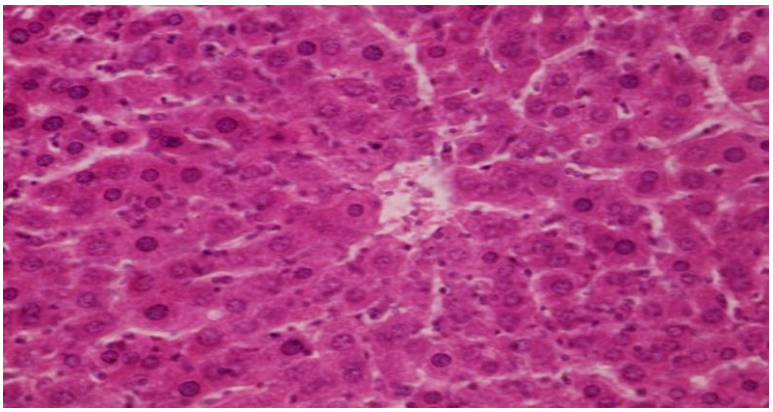
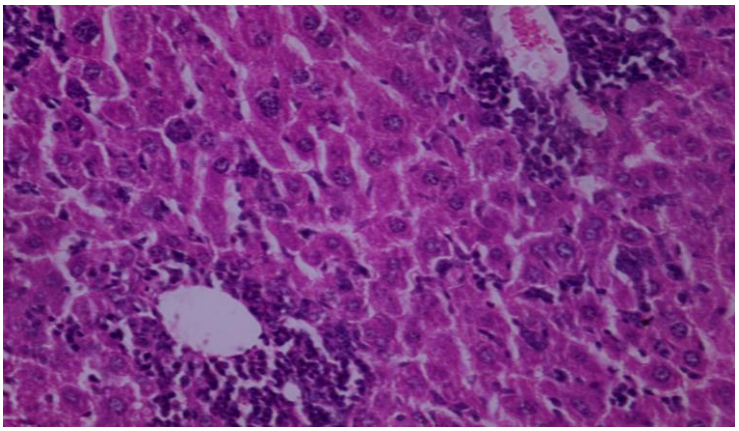


Fig.1: Photomicrograph of cross liver section of control mice showed, normal central vein with hepatocytes, (H&E 400).





Figs.2&3: Photomicrograph of cross liver section received *Moringa Olefera* for two weeks showed, central vein and hepatocytes as well as previous group, (H&E 400).



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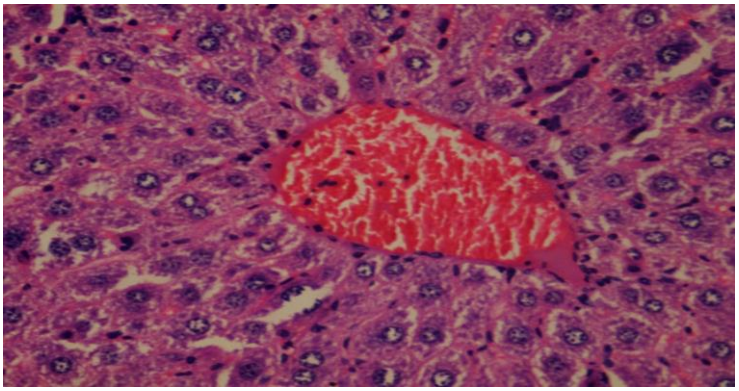
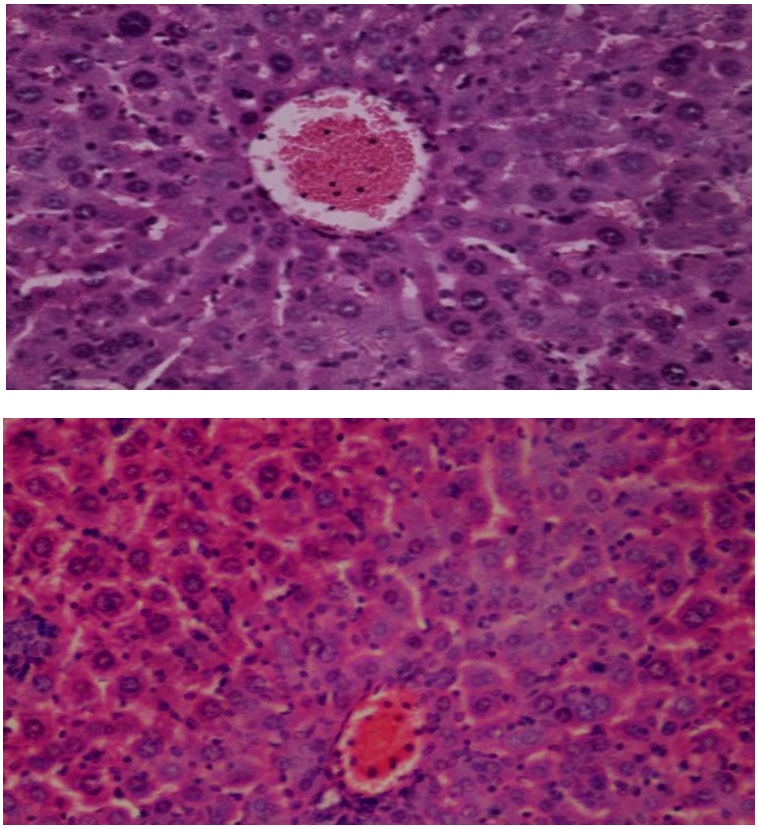


Figs. 4&5: Photomicrograph of cross liver section received tramal for two weeks showed lymphocytic aggregation and congested blood vessels. Degeneration of hepatocytes with karyolitic nuclei and elongated Kuffer cells, (H&E 400).



Figs. 6&7: Photomicrograph of cross liver section received tramal then treated with *Moringa Olefera* for two weeks showed that, moderate reduction of lymphocytic aggregation and retrieval of the normal architecture with partially improvement in blood sinusoidal, (H&E 400).

Biochemical Results

The levels activities of aspartate transaminase (AST) and alanine transaminase (ALT) were taken as indices for hepatotoxicity induced by tramal, and the hepato-protective efficacy of *Moringa oleifera* leaves. Serum levels of the enzyme activities were therefore analyzed in the different groups and the results are presented in Table (1).

According to the statistical analysis of AST and ALT values, there were no significant values were seen at compared the treated animals with *Moringa oleifera* leaves alone (56±6.2 & 48.8±9.1), respectively, (group II) to the control group I (Figs 8&9). In contrast the statistical analysis of AST and ALT levels in mice serum were significantly increased ($p<0.05$) in group III (135.4 ± 9.7 & 97±6.5), respectively, when compared to group I, (Figs 8&9).

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The statistical analysis of the group IV showed non-significant result ($p < 0.05$) of AST (70.8 ± 6.8) and ALT (52 ± 8.6) levels when compared to tramal treated group (IV), (Figs 8&9).

To gather, these results confirmed that the liver enzymes AST and ALT level were reduced significantly in the tramal treated (group III) as compared to the control group I ($p<0.05$). However on treatment with *Moringa oleifera* leaves the AST and ALT level was found to be enhanced significantly ($p<0.05$) (Figs 8&9). The treatment with tramal for two consecutive weeks caused elevation of the activities of the sera AST and ALT levels, while supplementation of *Moringa oleifera* extract exhibited a significant decrease in the levels of these hepatic marker enzymes and restored it to the control values as shown in Table 1.

Table 1: The levels of serum ALT and AST in mice treated with tramal and/or *Moringa olieifera* for two weeks.

Groups	AST (IU/L)	ALT(IU/L)
I	35±6.1	31.2±5.8
II	56±6.2	48.8±9.1
III	135.4±9.7*	97±6.5*
IV	70.8±6.8**	52±8.6**

-Values are expressed as (Mean ±SD).
- (*) Significant increase at $P < 0.05$ compared with control group
- (**) Significant decrease at $P < 0.05$ compared with tramal group

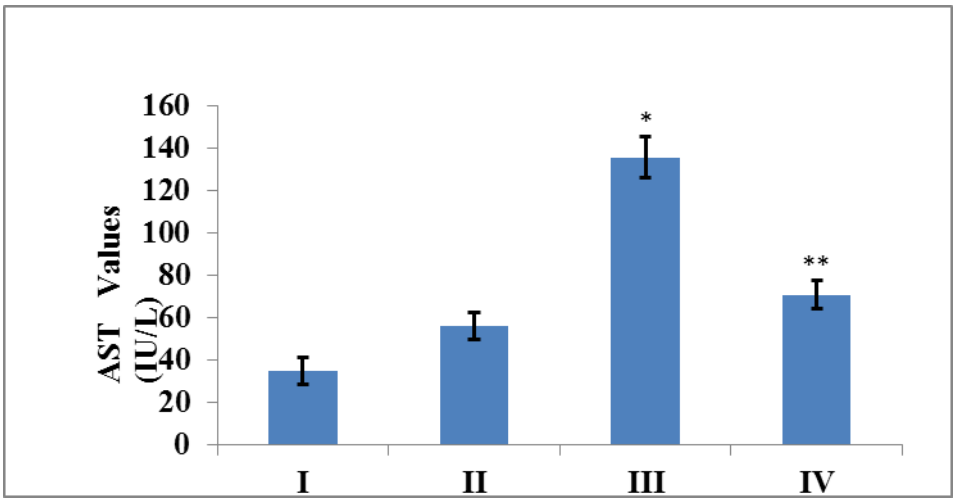

Fig. 8: Effect of leave extract of *Moringa oliefera* on AST levels in mice serum before and after the treatment with tramal.

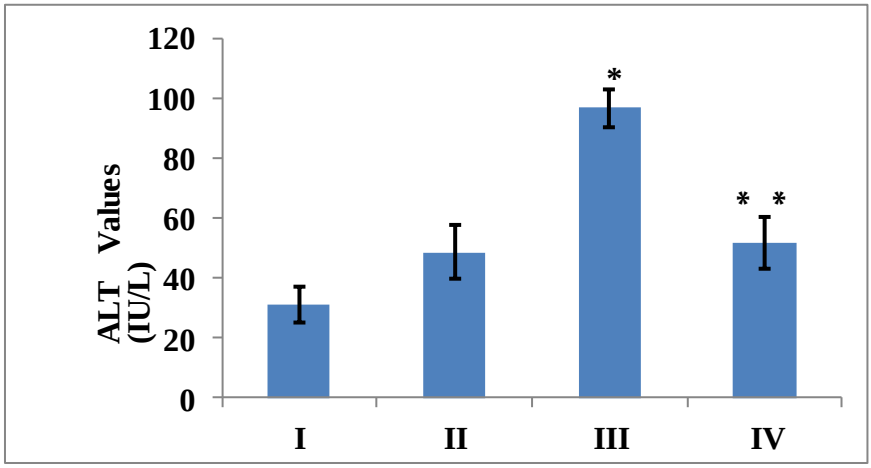

Fig. 9: Effect of leave extract of *Moringa oliefera* on ALT levels in mice serum before and after the treatment with tramadol.

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DISCUSSION

Histological results of group II in this study did not show any hepatic changes after the treatment with *Moringa oleifera* only (Figs. 2&3). This result agreement with [16], who reported that the results of *Moringa oleifera* did not show any toxic effect or disease on animal's models experimental due to the treatment with *Moringa oleifera* at low and/or high doses. The histological results of group II, also, in agreement with [17], who confirmed that, the extract of *Moringa oleifera* did not cause any histopathological lesions to the liver cells.

The liver is responsible for the tramadol metabolism and excretion and the high risk of hepatotoxicity [6]. However, incubation of adult human hepatocytes with opioids, in therapeutic doses, for 24 hours, is unlikely to produce irreversible damage to these cells in chemically defined culture conditions [18]. The main histopathological results in this study showed that the degeneration of some hepatocytes, loss of architecture and congested blood sinusoids in some areas. Also, degeneration of hepatocytes with karyolitic nuclei and elongated Kuffer cells (Figs. 4&5) after treated the animals with tramal for two weeks (group III). The present data coincide with [19], who reported that liver parenchymal changes in form of sinusoidal dilation, focal necrosis, marked congestion and hemorrhage were seen in during treated the animals with acute dose of tramadol. Also, [20] reported that the tramadol administration in adult male rats for one month was accompanied by hepatic congestion, hemorrhage, and necrosis. In addition, [21], reported a loss of architecture, congested central veins, and expanded portal area with edema and inflammatory reaction in rats treated with tramadol. Also, the histopathological results of the group III are coincided by results of [16], who reported that tramadol treatment induced severe pathological changes in the liver. These changes included degeneration of hepatocytes, apoptotic nuclei and congestion of central veins in the tramadol group. There were also, proliferated bile ducts, cellular infiltration, hemorrhage and fibrosis of blood vessels.

Regarding the effect of *Moringa oleifera*, animals treated with both tramadol and then treated with *Moringa oleifera* were revealed an improvement in histopathological alterations when compared with animals given tramadol alone. Histopathological investigation of the group IV, showed that the protective ability extract of *Moringa oleifera* on hepatic damage due to treatment with tramadol (Figs. 6&7).

The biochemical results in this study, showed that tramadol treatment caused markedly raised serum hepatocellular enzyme (ALT and AST), when compared group III to group I, group II and or IV, (Table 1). These results confirmed that the toxic effects of tramadol on the hepatic tissues after the treatment the animals (group III) for two weeks, significantly increased ($p< 0.05$) of ALT and AST levels, respectively, (135.4 ± 7.5 & 97 ± 6.5), (Figs. 8&9) compared to control group I. These results were partially agreement with [22], who reported that, serum ALT; AST levels were significantly higher in morphine group compared to the control group. A number of publications have supported toxic effects of chronic use of opioids on liver, who reported that increased levels of ALT [23, 24] Specific isoenzymes of AST are present in the liver cell mitochondria and cytoplasm whereas ALT is confined to the cytoplasm [25]. The transaminases are one group of enzymes that are sensitive indicators of liver cell injury [26]. Their serum levels are especially altered in hepatocellular disease particularly in acute diseases and they are often referred to as hepatocellular enzymes [25].

Regarding the effect of *Moringa oleifera* extract on tramadol-induced liver toxicity, animals treated with both tramadol and *Moringa oleifera* extract revealed an improvement in histopathological and biochemical alterations when compared with animals given tramadol alone. The biochemical results of the groups IIV, (Table 1) (Figs. 8&9), which received tramal and then treated with *Moringa oleifera* for two weeks showed moderate reduction of lymphocytic aggregation and retrieval of the normal architecture of the liver tissue, this accompanied improvement in blood sinusoidal. This study agrees with previous studies that *Moringa oleifera* extract has significantly hepato-protective activity. It has been reported that been reported that the *Moringa oleifera* extract has antioxidant and antibacterial products [27]. This could be as a result of anti-inflammatory and anti-arthritic properties of the extract. In addition, [28] reported that the *Moringa olefera* possess a hepatic-protective effects against liver toxicity. Subsequently reduced fibrosis in a dose dependent manner. By using the highest dose, the liver tissue regained its normal structure. The histopathological effects of tramal toxicity on the hepatic tissues in the current study were supported by biochemical study. This study was undertaken to demonstrate the protective ability of Moringa oleifera on tramadol-induced hepatic damage in albino mice.

CONCLUSION

In conclusion, based on the present findings, it is tempting to suggest that the *Moreinga oleifera* extract may possess a strong potential for development antioxidant and anti-inflammatory agents of tramadol-induced hepatic damage. Also, this study suggests that the use of *Moreinga oleifera* extract may be recommended in human nutrition against tramadol-induced hepatic damage.

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