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Effect of *Moringa oleifera* on some physiological parameters in diclofenac treated rats

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Abstract

The present work aims to study the potential effect of aqueous leaves extract *Moringa oleifera* (MO) on male albino rats treated orally with diclofenac sodium (DcNa). Forty-five male albino rats were used and randomly divided into 5 groups as follows: group I "normal group", group II "DcNa group", group III "MO group", group IV "DcNa then MO group" and group V "MO then DcNa group". Treatment with DcNa showed a significant decrease in body weight while caused significant increases in relative organs weight (liver, kidney, spleen and brain). *Moringa oleifera* caused a significant increase in body weight while showed non-significant decreases in relative organs weight (liver, kidney and spleen). DcNa exhibited significant decreases in WBCs count, lymphocytes % and neutrophil % while caused a significant increase in monocyte %. Also, there were

non-significant increase and decrease in eosinophil % and basophil % respectively. Treatment with MO showed non-significant increases in white blood cell count, monocyte % and neutrophil % while showed non-significant decrease in lymphocyte %. Eosinophil % and basophil % were increased significantly. Administration of DcNa caused significant decreases in serum total protein, albumin and globulin levels. Treatment with *Moringa oleifera* showed a significant increase in serum albumin level while showed non-significant increases in serum total protein and globulin levels.

The present study suggests that *Moringa oleifera* is useful in maintaining the harmful effect of diclofenac sodium in male albino rats.

Key-words: *Moringa oleifera*, Diclofenac sodium, body weight, organ weight, white blood cell and protein.

Introduction

Diclofenac is phenylacetic acid with strong analgesic, antipyretic, antibacterial and anti-inflammatory effects so it is widely used for treatment pain, inflammation of rheumatic and non-rheumatic origin **[1].** It causes problems in gastrointestinal tract and severe toxicity in liver and kidney at higher toxic doses for long period **[2].**

The mechanism of action of DcNa which considered as unique member of the NSAID family operates by the way of cyclooxygenase (COX) inhibition, therefore it blocks prostaglandins synthesis **[3].** In the mechanism of COX enzymes, the phospholipids of the membrane are hydrolysed and the activation of the phospholipase A2 enzyme lead to release arachidonic acid into the cytoplasm. Arachidonic acid transform by COX pathway into some prostaglandins (PGs) such as prostacyclins (PGI_2), PGD_2, PGE_2 and thro mboxane A_2 (TXA_2), and by the lipoxygenase pathway into leukotrienes and lipoxins **[4].** Cyclooxygenase enzyme-1 induces the production of TXA_2 in the platelets and leads to platelet aggregation, proliferation in the

smooth muscle cells and vasoconstriction. Contrarily, COX-2 induces the production of prostacyclin in the endothelial cells and causes the relaxation in the vascular smooth cells and vasodilatation **[5].** The gastric mucosa is protected from erosive effects of stomach acid by several prostanoids such as PGE_2 and prostacyclin **[3,6].**

Natural medicinal plants are widely used globally and played a vital role in healthcare. This is because they are cheap, readily available, easily ingested, relatively non-toxic and effective **[7,8].** *Moringa oleifera* is one of the most important natural plants which used as food and medicine because they are good sources of bioactive compounds including nutrient and anti-nutrient substances **[9].** These nutrients are used to combat malnutrition and supplement vitamin and minerals deficiencies **[10]** such as protein, fats, fibers, carbohydrates, minerals and vitamins **[11].** *Moringa oleifera* is a rich in non-nutritive chemicals which known as phytochemicals. These phytochemicals include phenolic compounds glucosinolates, isothiocyanates, sterols, Alkaloids and others **[12]**. Several of these products in *Moringa oleifera* have antihypertensive, antioxidant, anti-inflammatory, antitumor and antibacterial properties **[13].** In the present study, aqueous leaves extract of *Moringa oleifera* was evaluated for its protective properties against diclofenac sodium induced hepato-renal toxicity.

Material and methods

Analgesic agent

Diclofenac sodium (DcNa) is the effective material in the commercial drug (voltaren®) used as anti-inflammatory drugs. It was induced in rats by an oral dose (10 mg/Kg. wt. dissolved in 0.5 ml water) for 7 days according to **[14]**.

Preparation of Moringa oleifera leaves extract

Moringa oleifera leaves were collected from green pharmacy company, Egypt. *Moringa oleifera* leaves were air-dried (20°C in the dark), then were milled.

Dried leaf powder (100 g) was extracted with 1000 ml distilled water for 60 min and then filtered. Water extracted leaves were evaporated under vacuum (rotary evaporator Büchi R-110) to give a crude residue (yield: 50%). The extract was stored at –10ºC until used.

Experimental animals

Fourty five male albino rats; *Rattus norvegicus* weighting 200 ± 10 were purchased from Helwan farm of Egyptian Organization for Vaccines and Biological Preparations. Animals were housed in clean cages under laboratory condition (temperature 25 ± 2°C with dark/light cycle 12/12h), fed on a standard diet and supplied with water ad libitum throughout the study. All animals were acclimatized for 1week before the beginning of the experiment. Animals were humanely treated according to the ethical guidelines of the Faculty of Science, Benha University Bufs-REC-2024-115 Zoo.

Experimental design

The rats under study were classified into five groups (9 rats each) as follows:

Group I (normal untreated control rats).

Group II (DcNa group) that treated orally with DcNa (10 mg/kg b.wt. daily) dissolved in 0.5 ml water for 7 days.

Group III (*Moringa oleifera* group) that treated with an oral dose of *Moringa oleifera* leaves extract (350 mg/kg b.wt. daily) for 21 days according to **[15].**

Group IV (DcNa then MO treated group) that treated orally with DcNa for 7 days then treated orally with *Moringa oleifera* leaves extract for 21 days.

Group V (MO then DcNa treated group) that treated orally with *Moringa oleifera* leaves extract for 21 days then treated with DcNa for 7 days.

Blood sampling

At the end of the experimental periods, the animals were fasted overnight. Rats of each group were weighed and anaesthetized by di-ethyl ether inhalation**.** Blood samples were collected from post caval vein divided into 2 groups of tubes, one of them containing Ethylene di amine tetra acetic acid (EDTA) as anticoagulant for hematological parameters determination and the other blood samples were collected in dry centrifuge tube and

centrifuged at 3000 rpm for 15 min then sera were separated and frozen at -20°C for measuring physiological parameters. Organs (liver, kidney, spleen and brain) were removed at the end of the experimental period and weighted.

Hematological parameters

White blood cells (WBCs) count and their differential count (lymphocytes %, monocytes %, neutrophil %, eosinophil % and basophils %) were performed by using automated hematology cell counter (MS4e Automatic Hematology Analyzer).

Physiological parameters

Serum protein was determined by using SPECTRUM kit **[16].** Serum albumin was determined by using BEACON kit **[17]**. Serum globulin was estimated according to the following equation: Serum Globulin = Total proteins – Albumin

Statistical Analysis

The value of the measured and calculated parameters was expressed as the mean of 7 individual values ± standard deviation "SD". Statistical analysis **[18].** Statistical analysis was performed using Statistical Package for social science (SPSS) computer program, Version 20.00 produced by IBM software, Inc. Chicago, USA **[19].** All figures were drawn using Sigma plot (version 10) program produced by Systat software, Inc. Chicago, USA **[20]**.

Results

Effect of Moringa oleifera "MO" leaves extract on body weight change and relative organs weight of rats treated with Diclofenac sodium "DcNa"

Body weight:

Table (1) showed that DcNa treatment caused a significant decrease in body weight compared to those of control group and all other treated groups. Rats treated with MO leaves extract showed a significant increase in body weight compared to control group, DcNa, MO leaves extract then DcNa treated groups. There was a significant increase in body weight in rats treated with DcNa then MO leaves extract and rats treated with MO leaves extract then DcNa compared to DcNa treated group.

Table (1): Effect of *Moringa oleifera* "MO" leaves extract (350 mg/kg b.wt. daily for 21 days) on body weight (wt) change and relative organ weights of rats treated with Diclofenac sodium "DcNa" (10mg/kg b.wt. daily for 7 days).

Groups Parameter	Control	DcNa	MO leaves extract	DcNa then MO leaves extract	MO leaves extract then DcNa
Body wt (g)	261.00± 17.48^{b}	193.93± 3.92^{c}	290.60± 8.21^{a}	274.36± 2.08^{ab}	256.93± 8.70^{b}
Liver wt (g)	7.15±0.35^{c}	9.35±0.21^{a}	6.53±0.45^{c}	8.32±0.24^{b}	6.73±0.55^{c}
Kidney wt (g)	1.07± 0.06^{b}	1.35± 0.05^{a}	1.06± 0.11^{b}	1.33± 0.05^{a}	1.13± 0.11^{b}
Spleen wt (g)	1.25± 0.05^{b}	1.55± 0.05^{a}	1.23± 0.11^{b}	1.06± 0.25^{b}	1.13± 0.05^{b}
Brain wt (g)	1.56± 0.05^{a}	1.56± 0.05^{a}	1.46± 0.05^{a}	1.63± 0.05^{a}	1.46± 0.15^{a}

All data expressed as mean ±SD for 8 rats.

abcd = values with different letters are significantly different ($P < 0.05$)

Organs weight:

Table (1) showed data for relative weight of organs (liver, kidney, spleen and brain) in control and treated groups. Treatment with DcNa caused significant increases in liver, kidney and spleen relative weight compared to control group. Rats treated with MO leaves extract showed non-significant decreases in all measured relative organs weight compared to control group. Treatment with DcNa then MO leaves extract showed significant decreases in liver and spleen relative weight while the decrease

in kidney relative weight was non-significant compared to DcNa treated group. There was non-significant increase in relative weight of brain in DcNa then MO leaves extract treated group compared to control group and other treated groups. Rats administrated with MO leaves extract then DcNa showed significant decreases in relative organs weight except brain in which the decrease was non-significant compared to DcNa treated group.

Effect of Moringa oleifera "MO" leaves extract on hematological parameters in rats treated with Diclofenac sodium "DcNa"

White blood cells (WBCs) count:

Rats treated with DcNa showed a significant decrease in WBCs count compared to control group and all other treated groups. The WBCs count in rat treated with MO leaves extract increased non-significantly and significantly compared to control group and DcNa treated group respectively. Treatment with DcNa then MO leaves extract caused a significant elevation in WBCs count compared to control group and other treated groups except MO leaves extract then DcNa treated group in which the increase was non-significant. In rats treated with MO leaves extract then DcNa, WBCs count increased significantly compared to control group, DcNa and MO leaves extract treated groups but decreased non-significantly compared to DcNa then MO leaves extract treated group (Table 2).

Table (2): Effect of *Moringa oleifera* "MO" leaves extract (350 mg/kg b.wt. daily for 21 days) on hematological parameters of rats treated with Diclofenac sodium "DcNa" (10 mg/kg b.wt. daily for 7 days).

Groups / Parameter	Control	DcNa	MO Leaves extract	DcNa then MO Leaves extract	MO Leaves extract then DcNa
WBCs (th/mm^3)	4.50± 0.46^{b}	3.65± 0.18^{c}	4.65± 0.50^{b}	5.93± 0.28^{a}	5.77± 0.42^{a}
Lymphocyte (%)	84.48± 5.52^{a}	71.25± 7.61^{b}	78± 3.10^{ab}	74.66± 2.30^{b}	76.66± 0.57^{b}
Monocyte (%)	7.20±0.28^{c}	9.90±0.56^{a}	7.25±0.35^{c}	8.50± 0.50^{b}	6.25±0.25^{d}
Neutrophil (%)	19.97±1.66^{a}	13.20± 0.98^{b}	21.50± 1.27^{a}	19.50± 1.50^{a}	20.75± 1.06^{a}
Eosinophil (%)	1.61± 0.38^{c}	1.26± 0.16^{c}	4.49± 0.38^{a}	2.52± 0.37^{b}	1.57± 0.35^{c}
Basophil (%)	0.75± 0.25^{c}	0.96± 0.15^{cb}	1.73± 0.21^{a}	1.13± 0.10^{b}	1.50± 0.25^{a}

All data expressed as mean ±SD for 8 rats.

abcd = values with different letters are significantly different ($P < 0.05$)

Differential of white blood cells:

Lymphocyte %:

The results in table (2) revealed that DcNa treatment caused reduction in lymphocyte % significantly and non-significantly compared to control group and all other treated groups respectively. Rats treated with MO leaves extract showed non-significant decrease in lymphocyte % compared to control group but showed non-significant increase compared to all other treated groups. Lymphocyte % in rat groups which treated with DcNa then MO leaves extract and MO leaves extract

then DcNa decreased significantly and non-significantly compared to control group and MO leaves extract treated group respectively but when compared to DcNa treated group increased non-significantly.

Monocytes %:

Analysis of variance indicated that Monocyte % showed a significant increase in rats treated with DcNa compared to those of control group and all other treated groups. In group which treated with MO leaves extract, monocyte % decreased significantly compared to DcNa treated group and DcNa then MO leaves extract treated group. Treatment with DcNa then MO leaves extract caused a significant decrease in Monocyte % compared to DcNa treated group. Rats treated with MO leaves extract then DcNa showed a significant decrease in Monocyte % compared to control group and all other treated groups (Table 2).

Neutrophil %:

Rats treated with DcNa showed a significant decrease in neutrophil % compared to control group and all other treated groups. Neutrophil % increased non-significantly in rats treated with MO leaves extract compared to control group and other treated groups except DcNa treated group in which the increase was significant. Treatment with DcNa then MO leaves extract helps to return neutrophil near to control value. Rats treated with MO leaves extract then DcNa showed a significant elevation in neutrophil % compared to DcNa treated group only (Table 2).

Eosinophil %:

Table (2) showed that there was non-significant decrease in eosinophil % compared to those of control group and MO leaves extract then DcNa treated group. Rats treated with MO leaves extract showed a significant elevation in eosinophil % compared to control group and all treated groups. Eosinophil % of rat's group which treated with DcNa then MO leaves extract was a significant increase compared to control group, DcNa and MO leaves extract then DcNa treated groups. Treatment with MO

leaves extract then DcNa helps to return eosinophil % near to control value.

Basophil %:

Treatment of rats with DcNa caused non-significant elevation in basophil % compared to control group. Rats group treated with MO leaves extract showed a significant increase in basophil % compared to control group and other treated groups except MO leaves extract then DcNa treated group in which the increase was non-significant. There was non-significant increase in basophil % in rat group which treated with DcNa then MO leaves extract compared to DcNa treated group. Treatment with MO leaves extract then DcNa showed a significant increase in basophil % compared to control group, DcNa and DcNa then MO leaves extract treated groups (Table 2).

Effect of Moringa oleifera "MO" leaves extract on serum protein levels in rats treated with Diclofenac sodium "DcNa"

Total protein:

Analysis of the variance and data in table (3) indicated that DcNa treatment caused a significant decrease in serum total protein level when compared to control group and all other treated groups. Serum total protein level increased non-significantly in rats treated with MO leaves extract when compared with control group. Rats treated with DcNa then MO leaves extract and MO leaves extract then DcNa caused a significant elevation in level of total protein compared to control group and all other treated groups.

Albumin:

Serum albumin level showed a significant reduction in rats treated with DcNa compared to control group and other treated groups (Table 3).. Treatment with MO leaves extract caused a significant elevation in serum albumin level compared to control group and DcNa treated group. Rats treated with DcNa then MO leaves extract showed a significant increase in serum albumin level compared to those of control group and DcNa treated group. There was increased significantly in serum albumin level in rats treated with

Table (3): Effect of *Moringa oleifera* "MO" leaves extract (350 mg/kg b.wt. daily for 21 days) on serum protein levels of rats treated with Diclofenac sodium "DcNa" (10 mg/kg b.wt. daily for 7 days).

Groups Parameter	Control	DcNa	MO Leaves extract	DcNa then MO Leaves extract	MO Leaves extract then DcNa
T.protein (g/dl)	5.24±0.01^{b}	4.53±0.14^{c}	5.41±0.06^{b}	5.96±0.30^{a}	6.19±0.26^{a}
Albumin (g/dl)	3.83±0.15^{c}	3.36±0.05^{d}	4.50±0.17^{b}	4.66±0.01^{ab}	4.73±0.10^{a}
Globulin (g/dl)	1.14±0.04^{c}	0.86±0.06^{d}	1.21±0.06^{c}	1.40±0.04^{b}	2.04±0.11^{a}

All data expressed as mean ±SD for 8 rats.

abcd = values with different letters are significantly different ($P < 0.05$)

MO leaves extract then DcNa compared to control group and other treated groups except DcNa then MO leaves extract treated group in which the increase was non-significant .

Globulin:

Rats treated with DcNa showed significant reduction in serum globulin level compared to control group and all other treated groups. There was non-significant increase in level of globulin in rats treated with MO leaves extract compared to control group. Treatment with DcNa then MO leaves extract caused a significant elevation in serum globulin level compared to control group, DcNa and MO leaves extract treated groups. Serum globulin level increased significantly in rats treated with MO leaves extract then DcNa in relation to control group and all other treated groups (Table 3).

Discussion

In the present study, DcNa administration induced a significant decrease in body weight while induced significant increases in relative organs weight (liver, kidney and spleen). These

results are in agreement with **El-Hadary and Ramadan [21]** who reported that DcNa caused a significant decrease in body weight while relative organs weight (liver, kidney and spleen) increased significantly. **El-Baz *et al*. [22]** showed that administration of DcNa caused a significant reduction in body weight that may be attributed to diarrhea. In the present study, the decrease in body weight may be due to reduction in food intake, intestinal nutrient malabsorption and impaired feeding conversion. Also, this may be related to loss of appetite, reduction in water intake resulting from toxic effect of DcNa on the hypothalamus endocrine function which controls appetite and water intake **[23].** The reduction in body weight in DcNa-treated rats may be due to hepatomegaly in response to hepatotoxic effect of DcNa **[14]**. The elevation in relative liver weight was found to be related with concomitant increase of serum levels of AST and ALT that may be due to toxic potential of DcNa **[24]**. These results agreed with **Owumi and Dim [14]** who found that DcNa treatment induced markedly liver and kidney tissues damage which characterized by an increase in liver and kidney weights that may be because of DcNa induces oxidative stress and inflammation. **Taib and Jarrar [25]** reported that administration of DcNa caused splenomegaly. The increase in relative spleen weight may be because of highly toxicity of DcNa resulting from intracellular metabolism by cytochrome P450 and production of ROS by the mitochondrial respiratory chain **[26]**.

Results of the present study revealed that administration of *Moringa oleifera* (MO) leaves extract showed a significant increase in body weight while showed non-significant decreases in relative organs weight (liver, kidney and spleen). These results were in agreement with **Abou-Zeid *et al*. [27]** who reported that MO leaves extract caused a significant increase in body weight. The increase in body weight may be attributed to the high nutritive value of MO leaves that contains amino acids,

vitamins, minerals and fatty acids **[28,29].** Also, MO leaves extract contains an important component such as β-Eudesmol which was found to increase the activity of gastric vagel nerve in rats and therefore stimulates appetite and digestion **[30]. Wahba and Shelbaya [31]** reported that administration of *Moringa* powder or *Moringa* extract or mixture of *Moringa* powder and extract to ulcerated rats caused a significant increase in body weight gain. This may be due to plenty of antioxidants in MO which hinder the oxidative stress **[32].** These results also agreed with **El-Hadary and Ramadan [21]** and **Abubakar *et al*. [33]** who reported that MO leaves showed non-significant difference in relative weights of liver, kidney and spleen. Additionally, **Gupta *et al*. [34]** found that treatment with MO leaves extract showed a significant increase in body weight and non-significant difference in relative liver and kidney weights while showed a significant increase in relative spleen weight.

In the current study DcNa caused significant decreases in WBCs count, lymphocytes % and neutrophil % while caused a significant increase in monocyte %. Also, there were non-significant increase and decrease in eosinophil % and basophil % respectively. These results were in agreement with of **Soussi *et al*. [35]** who reported that DcNa caused a significant decrease in WBCs count. Administration of diclofenac caused significant decreases in WBCs count and lymphocyte % while showed a significant increase neutrophils %. The reduction WBCs count may be attributed to the inflammation effect of DcNa **[1].** Also, the decrease in production immune cells such as WBCs may be due to diclofenac causes bone marrow suppression and thymocyte developmental defects **[2].** The reason for decreased WBCs count might be related to diclofenac decrease the heart rate therefore the oxygen carrying capacity of the blood decrease that leads to decrease of the WBCs, red blood cells

"RBCs", hemoglobin "Hb", and hematocrit "Hct" levels **[35].** In addition, the present results lie in the same line with **Gomaa [2]** who reported that administration of DcNa caused non-significant increase in WBCs count while the differential of WBCs count indicated to relative lymphopenia (decreased lymphocytes), neutropenia (decreased neutrophil), monocytosis (increased monocytes) and basophilia (increased basophil). As well as that NSAIDs have been found to hinder prostaglandins production by inhibition the activities of COX enzymes that leads to reduce proliferation of human T-lymphocytes because of inhibition of IL-2 binding to its receptors **[2]**. The decrease in neutrophil may be due to direct toxicity of diclofenac on myeloid cells mainly neutrophils. This toxicity may be because of either the parent drug or a toxic metabolite **[26].** In addition to that neutropenia may be because of diclofenac induced antibodies against peripheral neutrophils or their bone marrow precursor which is hard to be identified **[36].** Opposite results were reported by **Owumi and Dim [14]** DcNa caused significant increases in WBCs count and lymphocytes % while showed significant decreases in neutrophils % and eosinophils % but eosinophils not significant change when compared with control group. Also, **[37]** mentioned that administration of DcNa caused significant increases in total WBC count and neutrophil while lymphocyte decreased significantly.

In the present study, amelioration (4^{th} group) and protection (5^{th} group) rat groups showed an enhancement in WBCs count and their differential (lymphocytes %, neutrophil %, eosinophil % and basophil) except monocytes % decreased significantly compared to DcNa treated group. These results were in agreement with **Ekundina *et al*. [38]** who reported that MO leaves extract caused a significant increase in WBCs count. The present results also agreed with those of **Lamou *et al*. [39]** who found that administration of leaves extract of MO caused

significant increases in percentage of lymphocytes and granulocytes. This enhancement may be due to the antimicrobial and anti-oxidant properties of MO **[40,41].** Also, **El-Hamalawy *et al*. [42]** found that MO leaves extract caused non-significant increase in WBCs count. In addition, the present results lie in same line with **Usman *et al*. [43]** who found that WBCs count increased significantly in male rats treated with toxic agent then treated with MO leaves extract. On the other hand, **Ajibade *et al*. [44]** found that seed extract of MO caused a significant decrease in WBCs count while caused a significant increase and decrease in lymphocytes and monocytes respectively.

The liver is the main source of most of the serum proteins, in which the parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the α and β globulins **[45]** therefore the level of protein and albumin in the blood is an index of hepatic function **[46].** In the present study, DcNa caused significant decreases in serum total protein, albumin and globulin levels when compared to control group and all other treated groups. These results were in agreement with **El-Hadary and Ramadan [21]** who reported that administration of DcNa caused significant decreases in serum total protein, albumin and globulin levels that may be attributed to the toxicity of DcNa and liver damage through induction of lipid peroxidation which leads to change in mitochondrial function and inhibit of synthesis of protein. The results also agreed with those of **Baravalia *et al*. [24]** who reported that serum total protein and albumin levels were decreased significantly after administration of DcNa that indicate depletion in the protein reserve and therefore reflect hepatic toxicity. In addition, the present results lie in same line with **El-Shopakey and El-Azab [47]** who reported that administration of DcNa showed significant decreases in serum total protein, albumin and globulin levels. On the other hand, **Abiola *et al*.**

[48] who found that treatment with diclofenac showed a significant increase in serum albumin level. **El-Maddawy and El-Ashmawy [49]** found that low dose of DcNa showed non-significant alteration in serum total protein, albumin and globulin levels while high dose of DcNa caused significant decreases in levels of total protein, albumin and globulin.

Amelioration and protection groups in the present study showed significant increases in serum total protein, albumin and globulin levels. These results were in agreement with **Soliman *et al*. [50]** who reported that male rats treated with MO leaves extract then treated with methotrexate (MTX)-induced hepato-renal dysfunction showed significant increases in serum total protein, albumin and globulin levels when compared with MTX treated rats group. This may be attributed to natural antioxidant present in MO leaves extract which alleviate the changes in the protein through the regulation of metabolic activities and protein synthesis **[51].** The present results also agreed with **Singh *et al*. [52]** who reported that the treatment with MO extract and carbon tetrachloride (CCl_4)-induced hepatotoxicity simultaneously stabilized the levels of total protein and albumin. Proteins stabilization might be considered as an indication of enhanced protein synthesis in the liver cells because of inhibition of peroxidation of lipids and scavenging of the free radicals **[52,53].** In addition, the present results lie in the same line with **El-Hadary and Ramadan [21]** who reported that animals treated with DcNa then treated with MO leaves extract showed significant increases in serum total protein, albumin and globulin levels when compared with DcNa-treated rats that may be due to antioxidants contents such as phenolic and flavonoid compounds in MO leaves extract which play an important role in protection against ROS. On the other hand, **El-Hamalawy *et al*. [42]** found that co-administration of Thiamethoxam (TMX) which act as biomarkers of

hepatotoxicity with MO leaves extract caused a significant decrease in serum albumin level.

In conclusion, we conclude that aqueous leaves extract of *Moringa oleifera* are very effective alternatives to improve harm effects of diclofenac sodium.

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