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Protecive effect of *Moringa Oleifera* extract on oxidative stress through ischemia/ reperfusion in rat brain

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Abstract

Worldwide, one of the major causes of mortality and morbidity is ischemic strokes. There is considerable memory deficits and cognitive learning among the impairments indicated in the survivors. After ischemia event, the neuroprotective strategies were examined for minimizing these deficits. This study is investigating the protective effect and toxicity of M. oleifera extract on potential oxidative stress in animal model related to ischemia-reperfusion. Also, there are thirty-six male Wistar rats (350–430 g) have been divided into 3 groups, in treatment group, the *M. oleifera* leaves extracts at 400 mg/kg doses has been orally and daily given 3 times a day in two weeks prior to the occlusion regarding cerebral 2 arteries for 25 mins. Later, blood was drawn from the heart for biochemical analyses regarding potential oxidative stress effects. Ischemia–reperfusion impaired showing *M.oleifera* extracts supplementation the capability to protect from the oxidative effect; Show the extract have the ability of maintaining the functional levels related to antioxidant enzyme GSH-Px, SOD as well as the vital signs glutathione MDA and GSH.

Keywords: Moringa oleifera; oxidative stress; ischemia–reperfusion; brain

1. Introduction

The central nervous system is considered as the organ with high vulnerability to hypoxia. In the case when the flow of blood isn't enough for providing the needed oxygen to brain, then mild symptoms including difficulty to learn tasks and decrease in short term memory is going to be induced, also, the prolonged oxygen deprivations lead to cognitive disturbances as well as decreased motor control, brain death, seizures, long term consciousness's loss, even induce fainting, coma, and cessation related to brain stem reflexes. High blood pressure, traumatic brain injuries, cerebrovascular diseases and other brain diseases might start as whole or limited hypoxia (decreased availability of oxygen to brain tissue), that in the case when not appropriately managed, will result in many complications, some of them are life threatening. [1]. yet, the accumulative lines of evidences in the current decade indicating the importance of oxidative stress. Furthermore, it was indicated that the decrease of cerebral blood flow as well as reperfusion period inducing the increase in lipid peroxidation and oxidative stress [2–4].The brain in humans is constituting just 2.3% of the body mass, yet consuming approximately 18% of the cardiac output as well as about 20% of total body oxygen. These elevated metabolic and vascular demands and the absence of considerable energy reserve render it distinctively vulnerable to the blood supply alterations [5]. Thus generating more free radicals than other devices. In addition, brain tissue contains large amounts of fat with unsaturated fatty acids and high concentrations of iron, so the brain is more prone to free radical damage [6]. It has been indicated that the spinal cord and brain tissue have high sensitivity to ischemia in comparison to tissues like kidney and heart. The blockage regarding the blood flow to brain for just five minutes might cause the death of neurons, whereas the death of cells in kidney or cardiac tissues happens following experiencing ischemia for a period between 20 and 40 mins [7, 8].

M.oleifera is a tree growing in the north region of India, yet it is now majorly found in Asia, Africa and Americas [9]. From many plants recognized in bioprospective researches, the M. oleifera (Lam) and, in a few regions worldwide, as horseradish tree or drumstick tree, were considered as alternate medical therapies, exhibiting advantages for controlling many diseases [10,11]. Plant extracts showed high efficacy with regard to fight oxidative stress [12]. The biological

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significance of the M. oleifera plant lies in effective anti-oxidants (which have been molecules against the free radicals with the ability to secure or deactivate free radicals prior to damaging the cells). Where the results showed containment the extract M. oleifera a number of important vitamins from antioxidants (C, E and A) [13]. There have been a lot of antioxidant systems which are synergistically working with each other for protecting the organs of body as well as the organ systems from free radical damages. M.oleifera contains many important chemical compounds, such as vitamins, also secondary metabolites including vanillin, flavonoids, ferulic acids, gallic acids, ellagic acids, phenolic acids, chlorogenic acids, glucosinolates, quercetin, also kaempferol, that have nutritional, antimicrobial and/or pharmaceutical properties [14,15]. Yet, the amount of such metabolites in the extracts of M. oleifera is changing on the basis of climatic conditions, exposure to sun, soil, and location [16]. Also, the approach, also the utilized solvents for extraction might be modifying the contents related to compounds acquired from plant, majorly flavonoids and phenols [17]. More and more epidemiological and experimental evidences are suggesting that M. oleifera have antioxidant impacts against the damage of oxidative stress [18–20], while the antioxidants existing in plant leaves, act in cooperation with the antioxidant system existing in the body. Many researches are currently indicating the increase in many antioxidant and detoxication enzymes due to the treatment with M. oleifera or phytochemicals that are isolated from the M. oleifera [21]. A lot of studies indicated the possible therapeutic values related to M. oleifera such as anti- microbial, , anti -diabetes, anti- rheumatoid arthritis [22], anti-atherosclerotic[23],anti-fertility, anti-fungal depressant and pain relief [24], diuretic as well as thyroid regulation effects [25]. Recently, some studies indicating that the extracts of leaves are showing antioxidant effects and might be protecting against the oxidative damage [26, 27].

2. Experimental

Plant Extracts

The dried leaves have been grinded using an electric mill, the leaves were soaked in an alcoholic aqueous solvent (50% methanol 50% water), and 50 g of treated plant are extracted in solvent of 250 ml and kept in shaker incubator for 24 hours at (45°C). After that, a gauze is used to filter the extract and succeeded via filter paper, after that the solvent was evaporated at (65°C) and the resulting crude extract is separated by Separation device (Spray dryer). The dried extract was collected, weighed, the extract's percent yield is 16.4% and stored at low temperature (0°C), in dark bottle till using [28].

Ethical clearance.

With regard to using laboratory animals, the protocol utilized in this work is accepted via Research Ethics Committee at Anbar University. The rats have been kept in cages dedicated as well as fed on commercial rat pellets and enabled for freely accessing tap water in the bottles. Also, they experienced 12 hours of light and same hours of darkness where shelter and care were carried out according to the conditions and use of the animal in research institutions and accordance with internationally recognized guidelines [29].

Evaluation of toxicity extract M. oleifera

The test was conducted on 18 male Swiss white mice, with age between 6 and 8 weeks, weight between 15 and 20 g, have been utilized for determining the percentage of animal deaths 24 hours following taking oral dose (0,2ml) for 3 days. In addition, all dosing materials have been prepared fresh every day and oral extracts (via gastrointestinal tube) were given from three doses per day with concentrations (100, 200, 400, 600, 800 and 1000) mg/kg to 6 groups (test groups). Animal behaviors were monitored for 14 days In life clinical signs within observations involved appearance changes (mucous membranes, fur, skin, eyes), excretions and secretions, autonomic activity (unusual respiratory pattern), gait, posture, response to handling, and behavior (self-mutilation, repetitive circling, excessive grooming, walking backwards). This test was conducted in the toxicity unit / Veterinary Drug Center (Ibn Bitar) Ministry of Industry and Minerals.

Experimental Design

The experiment has been developed for evaluating the impact of dose 4mL per 400 mg/kg of and by three doses a day for 14 days from the extract of *M. oleifera* on oxidative stress. Therefore, animals have been divided into 3 groups (each one of the groups contain 12 rats).

Group A (Control): In control animals anesthesia is, without the occlusion of carotid arteries.

Group B (I/R): Rats exposed cerebral ischemia are induced for a period of 25 mins via bilateral common carotid artery occlusion by means of aneurysm clips figure. (1), after that clips are removed, while the carotid arteries are inspected visually for reperfusion.

Group C (*M. oleifera* treatment + I/R): The extract has been dissolved in distilled water for facilitating its oral administration to rats, the treated group have received *M. oleifera* extract at 400 mg/kg dose and via 3 doses daily for two weeks via orogastric bottle. In addition, the extract dose has been chosen since it is providing optimum protection against the focal ischemic strokes which has been induced via cerebral artery occlusion [30], and after the expiration of the dosing period then the rats were subjected to oxidative stress conditions and the same way as the group(B).

Determination and Evaluation of Oxidative Stress Markers.

Enzymatic assay for Glutathione peroxidase (GSH-Px), SOD (super-oxide dismutase) activities, Glutathione (GSH) Concentration and MDA (malondialdehyde) and have been conducted in Serum blood of rats utilizing spectrophotometric approaches. SOD and GSH-PX assay kits are procured from

Elabscience® and cohesionbio, USA. Also, the blood has been drawn directly from the heart via stab the heart (utilizing syringe) for getting the largest amount of blood and collected into micro centrifuge tubes. Blood is left to clot for 15-20 minutes. Serum is obtained by centrifugation at 4000 rpm for 15 minutes. Collecting the serum is pooled and frozen at -20 ºC. GSH activities were assayed according using the method [31], MDA [32], also, SOD and GSH-PX activities are assayed based on the instructions of manufacturer in assay kit pack.

Data analysis

GENSTAT software V-12) is used to analyze the obtained data, while values have been plotted in ANOVA with the use of multiple comparisons test. In addition, the acquired data have been provided as mean ± standard error of mean and significance level placed atp values not more than 0.001 or 0.005. The acquired results have been specified in bar charts with error bars for showing the mean as well as standard error of mean.

3. Results and Discussion

Evaluation of toxicity M. oleifera extract.

M.oleifera, in many forms, was consumed as medicine and food for a lot of time in many cultures worldwide. More and more interests in moringa containing dietary supplements are requiring significant toxicological evaluations regarding standardized moringa preparation entering the marketplace. A short-term oral toxicity study of 14- day was evaluated in mice to verify the safety of hydro-alcoholic M. oleifera extract leaves. According short-term toxicity studies with rodents [33]. Miller and Tainter method were used to assess the LD_{50} of M. oleifera leaves extract in mice.

Oral dose performed in different concentrations (100,200,400,600,800 and 1000 mg·kg−1) of the extract by three doses per day for a period of three days as shown in table (1). The results related to acute toxicity study tests with M. oleifera leaves extracts showing safe range, while the animals receiving extracts of M. oleifera didn't show marked behavior change. The movement behaviors and animal deaths were monitored after the 14-day dose. Furthermore, the results were in accordance with those presented via Adedapo et al. [34], indicating that plant leaves are fairly safe for medicine and nutritional uses.

Table (1) Evaluation of toxicity content in *M.oleifera* extract.

Concentration (µg/ml)	The number of times PPM	The movement Behaviors the dose	The deaths
100	3	Normal	0
400	3	Normal	0
600	3	Normal	0
800	3	Normal	0
1000	3	Normal	0

Evaluation antioxidant activity of M. oleifera leaves.

I showed the results and data represented in **Fig**. (2,3,4) a considerable decrease ($p \leq 0.001$) in the activity of GSH-PX, SOD, and the GSH level in ischemia group (B) when put to comparison with the control group (DW) or *M.oleifera* treatment group (C) .The group rats (C) receiving the extract dose 14 days and were exposed was subjected to the same conditions of oxidative stress showed increased activity the SOD, GSH-PX , and the level of GSH compared with group B, but at a lower level than the control group with p-value ≤0.001. Respectively, indicating the protective activity of the extract against oxidative stress when the brain is exposed to oxidative stress conditions. Based on the results provided in this work, there was an imbalance between antioxidant and oxidant biomarkers in serum following hypoxia's induction, such imbalance was ensured via significant increase (p-values ≤0.001) in MDA concentration compared to that of control group.

In fact, an obvious enhancement in the level of MDA was shown in the group that treated with *M. oleifera* extract leaves doses and were exposed was subjected to the same conditions of oxidative stress compared to the I/R group. in this study, the level of MDA was significantly reduced compared with group B **Fig.** (5), but not at a lower level the control group, with p-value ≤0.001, respectively. The results indicating the possible protection of *M. oleifera extract* in the case when it has been administered prior to the hypoxia's induction. Overall, the results of the present investigation support the hypothesis that the of *M. oleifera* protects rat brain and blood from oxidative stress by its antioxidant properties.

The consumption of *M. oleifera* has extremely wide safety range in which its toxicity was extremely low, LD_{50} related to the alcoholic extracts of *M. oleifera* leaves in range of (2800-5000mg·kg−1 BW) [35]. Thus, even in the case when it has been consumed in high quantities, it is showing non-toxic events [36]. The results of this work indicating that the dosed concentrations are not showing a concern for toxicity, and such information suggesting that it was safe even in the case when being consumed in high quantities because of its high LD_{50}. On the basis of vital role of oxidative stress on the pathophysiology related to cerebral ischemia as well as the antioxidant impact of *M. oleifera* leaves, the impact of *M. oleifera* leaves extracts against the focal ischemic stroke was focused. Furthermore, the *M. oleifera* leaves extract when taken alone hasn't histologically obvious disorderly or damaging impacts on cerebral cortex [37].

The results of this work indicated that the leaf extracts of *M. oleifera* have the capability for protecting the brain from the impacts of oxidative stress resulting from experimentally induced hypoxia in the male rats via its ameliorative impact on oxidative stress in treating group (C). Many plant parts, particularly the leaves have dietary and medicinal significance because of elevated content of carotene, ascorbic acid, vitamin E, iron and essential amino acids calcium [38,39]. Former

researches showed that the leaf extracts of *M. oleifera* has elevated antioxidant content and enhance immunity [40,41]. Another research suggested that the *M. oleifera* leaves extract might be exerting protective effects against the oxidative damage which is induced via diabetes [42]. In this work, it has been indicated that *M. oleifera* might be enhancing the activity of antioxidant enzymes (GSH, SOD and GSH-PX) and reducing oxidative stress markers (MDA) the possible explanation. In a study conducted by [43], showing that *M. oleifera* extracts might be considerable enhancing improve spatial memory and reduced MDA levels, yet improved CAT, SOD activities and thus reducing the exhausted levels regarding antioxidant enzyme via hypoxia because of overproduction and accumulation of ROS contributing to brain damage and disturbed the neuronal function [44]. It has been indicated that *M. oleifera* is increasing the neurons density and showing vasodilation effects on brain blood vessels [45,46], as well as moderating the role of monoamines transmitters like dopamine [47]. In addition, the antioxidant capacity showed via *M. oleifera* in this work was because of the existence regarding many types of antioxidant compounds including carotenoids, flavonoids, ascorbic acid and phenolics [48,49]. Previous researches suggesting that the protective effect related to *M. oleifera* extracts is due to the existence of phytoconstituents which is scavenging free radicals as well as activate endogenous antioxidant enzymes [50,51]. Comparably, at adequate dose, it was indicated that Moringa is precenting likely nicotine induced neuronal cell damage through boosting the antioxidant status related to neuronal cells [52].

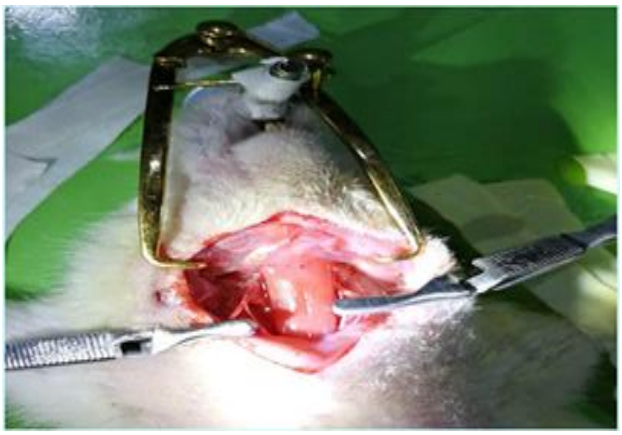

Fig. (1) : Closure of the artery by the clip for 25 min.

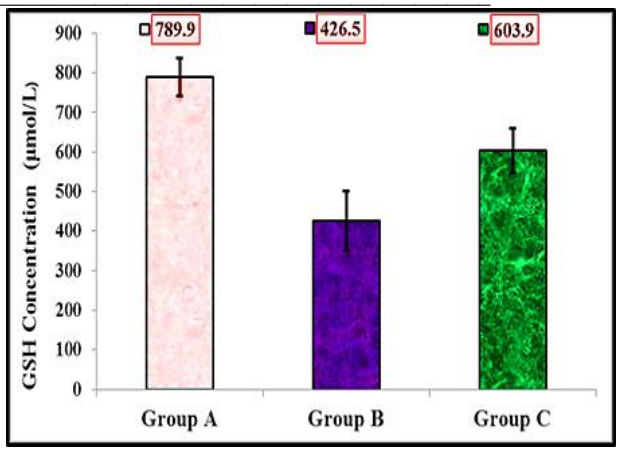

Fig. (2) Glutathione (GSH) Concentration in group.
A = control, B = ischemia I/R, C = M. oleifera + I / R.

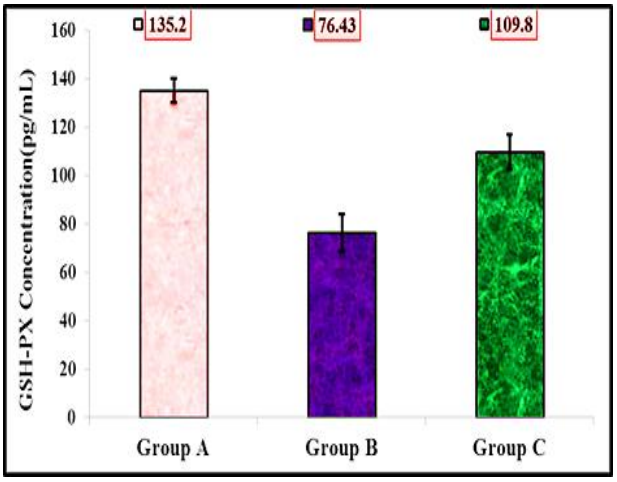

Fig. (3) Glutathione peroxidase (GSH-Px) activity in group
A = control, B = ischemia I/R, C = M. oleifera + I / R.

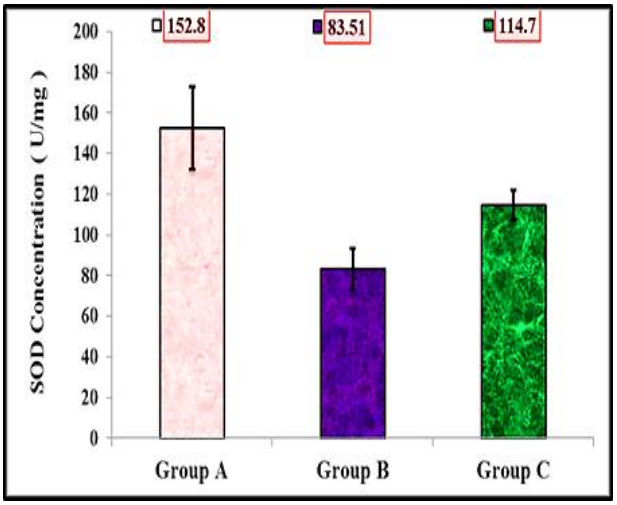

Fig. (4) Superoxide dismutase(SOD) activity in group
A = control, B = ischemia I/R, C = M. oleifera + I / R.

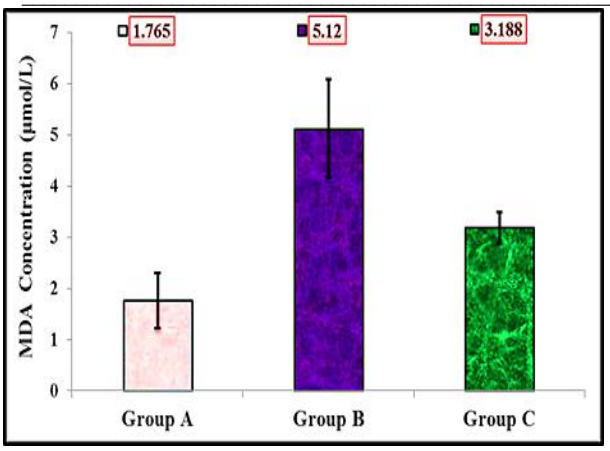

Fig. (5) Malondialdehyde (MDA) Concentration in group
A = control, B = ischemia I/R, C = M. oleifera + I / R.

4. Conclusions

The present economic recession is considered as global issue, particularly in the nations of low-income, thus individuals are more and more using the herbal medicine, and more researches on such plant are needed. This study shows the versatility of plant and this work showed that the leave extracts of *M. oleifera* was potential neuroprotectant that is simple to approach and inexpensive. In addition, the likely underlying approach might partly happen through decreasing the oxidative stress. In this work, the evaluation of antioxidant activity indicating that *M. oleifera* plant with high antioxidants content might be considerable source of natural, even though the parameters utilized in this work have been the quantification of antioxidant properties serving as a guide for using such plant with regard to ROS-associated diseases. More investigations into the identification and isolation regarding responsible antioxidant components as well as their action mechanism was required for better understanding their capability for controlling diseases which has considerable impacts on life quality.

Conflicts of interest

There are no conflicts to declare.

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