Original Article

Moringa Oleifera Extract Could Ameliorate Aging-Related Structural and Functional Hepatic Changes by Regulating Autophagy Signaling Pathway

Heba Osama Mohammed[1], Rania Saad Ramadan[1,2], Amal Fawzy[3], Aliaa Talaat[3], Eman Ahmed Alaa El-Din[4] and Maha Ahmed Abdulrahman[1]

Department of Human Anatomy and Embryology, Faculty of Medicine, [1]Zagazig University, Zagazig, Egypt, [2]Al-Baha University, Al-Baha, Saudi Arabia

[3]Department of Medical Biochemistry and Molecular Biology, [4]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

Introduction: Aging is a biological process that increases oxidative damage in the cell. Moringa oleifera (MO) plant has a hepatoprotective effect by inducing the antioxidant defense mechanism.

Aim of the Study: To evaluate aging-related impacts on the hepatic structure and function and the possible mitigating role of MO extract, with clarifying the mechanistic role of autophagy in aged rat models.

Material and Methods: Twenty four rats, aged 3 months, were assigned into 4 groups as following, group I: adult control, group II: administered MO leaf aqueous extract (50 mg/kg body weight) by nasogastric tube for 4 months, group III: received no treatment till age 20 months, and group IV: received no treatment till age 16 months, and then received MO leaf aqueous extract for 4 months. At the time of sacrificing, serum liver enzymes (aspartate aminotransferase and alanine aminotransferase) were measured and the hepatic specimens were processed for light and electron-microscopic assessment and analysis of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and mammalian target of rapamycin (mTOR) gene expression.

Results: The aged rats revealed significant elevation of liver enzymes and hepatic malondialdehyde as well as reduction of hepatic glutathione content. Aging also inhibited autophagy via upregulation of autophagy inhibitory genes; PI3K, Akt, and mTOR. Immuno-histopathological evaluation of the hepatic tissue of aged rats displayed deteriorated cytoarchitecture in terms of hydropic degeneration, congested veins, and inflammatory infiltrates in addition to increased glial fibrillary acidic protein expression and decreased microtubule-associated protein 1A/1B-light chain 3 (LC3) expression. MO administration to the aged rats promoted significant amelioration of aging-induced hepatic dysfunction and tissue alterations, counteracted oxidative stress and enhanced autophagy via downregulation of PI3K, Akt and mTOR genes

Conclusion: MO can reduce aging-induced structural and functional liver damage by combating oxidative stress, and stimulation of autophagy through downregulation of the PI3K/Akt/mTOR signaling pathway.

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Key Words: Aging; autophagy; glial fibrillary acidic protein; moringa oleifera extract; hepatocytes; PI3K/Akt/mTOR pathway.

Corresponding Author: Heba Osama Mohammed, MD, Department of Human Anatomy and Embryology, Faculty of Medicine, 1Zagazig University, Zagazig, Egypt, **Tel.**: +20 11 1999 1616, **E-mail:** yassin_mekkawy@yahoo.com

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INTRODUCTION

The aging process has a complex and multifactorial progression, featured by marked loss of physiological integrity and disturbed function[1]. It was believed that aging does not change the liver cells significantly[2], although it is clear now that the liver shows considerable structural and functional alterations in old age. Aging is one of the risk factors of chronic liver diseases including mainly steatohepatitis, portal hypertension and cirrhosis[3,4]. The mortality rate of liver diseases is higher three to five folds in old aged than in young aged humans[5].

Autophagy is a crucial cellular recycling process[6] essential for sustaining liver metabolism. Autophagy provides hepatocytes with the necessary nutrients to maintain their basal metabolic function[7]. Aging decreases the autophagosomes' number and function and leads to accumulation of lipofuscin that decreases the activity of autophagy enzymes, causing substantial inhibition of autophagy[8-13].

The mammalian target of rapamycin (mTOR) pathway is considered a vital autophagy-related pathway. Recently, phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt) and mTOR are suggested to be associated with hepatic cell aging[14-16]. Inhibition of mTOR activity can induce autophagy[16].

Moringa oleifera (MO) is considered as Moringaceae family member[17]. It is widely recognized in Indonesia as kelor, also called a magical plant because of its wide

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use as a medicinal plant, cosmetic, and food ingredient[14]. Moringa is also proven to have a hepatoprotective effect against carbon tetrachloride and acetaminophen induced liver toxicity, and to decrease total cholesterol and triglycerides levels[15,16]. Many studies revealed that MO extract could ameliorate hepatic injury by inducing the antioxidant defense mechanism[16,18]. However, few studies paid attention to not for the effects of MO on the aging-induced injury of the hepatic tissue and the mechanism by which these extracts ameliorate liver damage.

Based on the previous data, the present work was designed to evaluate the potential influence of MO extract on aging-related hepatic alterations at the biochemical, morphological, and ultrastructural levels, with special emphasis on the role of autophagy PI3K/Akt/mTOR signaling pathway.

MATERIALS AND METHODS

Materials

Experimental animals

Adult male albino rats (3 months old, 200-250 g) were got from the animal house of Zagazig Faculty of Medicine, Egypt. The rats were maintained in a standard environment with an adjusted temperature at 25 ± 2oC and a 12h/12h light/dark cycle. The rats were kept for 7 days before the experimental procedures for acclimatization, and given a chow diet and water ad libitum during the experiment. Animal care was according to the guidelines of the Zagazig University Ethical Committee with approval no ZU-IACUC/3/F/131/2022.

Plant extract preparation

MO leaves were obtained from the Agricultural Research Center in Egypt. The leaves underwent sun drying. The dried leaves (10 g) were macerated, for 24 hours, in 100ml of absolute methanol at the room temperature. Centrifugation of the extract was performed for 25 min at 4500 rpm, followed by filtration using Whatman Millipore filter paper. The extract was then dried at the room temperature and weighed to determine the extract yield of MO leaves. This dried extract was used for determination and identification of polyphenols and flavonoid. Then, the aqueous extract was prepared by soaking 1 g of dried extract in 10 ml of cold water, after boiling this mixture, it was filtered and collected in a sterile tube. The collected extract was 2.5 ml (400mg/ml). Each 1ml of this extract was diluted 8 folds with distilled water to obtain 50mg/ml[19]

Plant extract characterization (total composition of phenolic and flavonoid compounds)

Total composition of phenolic and flavonoid compounds was measured at the Biochemistry Department, Faculty of Medicine, Zagazig University, using the method of Folin–Ciocalteu. Gallic acid was used as a control and a calibration curve was created. The total composition of phenol was assessed in mg gallic acid equivalent (GAE) per gram of dry extract[20]; it was 278.4 ± 2.5 mg GAE /g extract. The aluminum chloride colorimetric assay was used to estimate the total flavonoid content, with rutin serving as a standard[21]. The total flavonoids were measured in mg rutin equivalent (RE) per gram of dry extract. Flavonoid total content was 53.25 ± 1.8 mg RE/g of extract. This characterization was performed in order to detect phenolic and flavonoid content of each gram dried extract.

Methods

Experimental design

Twenty-four rats (3 months old) were randomly separated into 4 groups (n = 6 each). The Open epi program was used for sample size calculation[22]. The mean ± standard deviation of alanine aminotransferase (ALT) levels was supposed to be 10.68 ± 0.93 in the adult group but 15.85 ± 5.92 in the aged group[21]. The estimated sample will be at least 22 male albino rats, but 24 rats were used to avoid lost animals.

Group I (the adult control group): the rats were kept untreated for 4 months, then sacrificed.

Group II (MO control positive group): the adult rats received MO leaf aqueous extract (50 mg/kg body weight) by nasogastric tube for 4 months[23], then the rats were sacrificed.

Group III (aged group): the rats were kept untreated till age 20 months[24], then sacrificed.

Group IV (aged + MO group): the rats were kept untreated till age 16 months and then received MO leaf aqueous extract (50 mg/kg) for 4 months. The rats were then sacrificed.

At the time of sacrificing, each rat was anesthetized with 60 mg/kg sodium thiopental injected intraperitoneally[25]. Samples of venous blood were immediately got from the caudal vein and liver specimens were obtained by careful laparotomy. Each isolated liver was sectioned into right and left lobes. The right lobes were prepared for light and electron microscopic inspection, while the left lobes were kept at −80°C for studying gene expression.

Biochemical study

The obtained blood samples were incubated at 37°C until blood clotting and then centrifuged at 2000 rpm to separate sera and detect the ALT and aspartate aminotransferase (AST) levels[26].

Oxidative enzyme and lipid peroxidation estimation

The supernatant from the homogenized liver specimen was centrifuged for 20min at 1000 rpm at 4oC for separation and analysis of:

- Malondialdehyde (MDA), a lipid peroxidation marker, was measured in the hepatic homogenate[27] using a commercially accessible kit (Biodiagnostic, Cairo, Egypt).

- Reduced glutathione (GSH), an antioxidant marker, was detected in the hepatic homogenate[28] using an available kit (Biodiagnostic).

Analysis of hepatic expression of PI3K, Akt and mTOR genes

Total RNA extraction was performed using QIAamp RNA Tissue Mini Kit as recommended by the manufacturer. The extracted RNA underwent for reversed transcription using the QuantiTect Reverse Transcription Kit as instructed by the manufacturer. Specific RNA amplification was carried out in a 20µl reaction mixture including 5µl cDNA template, 10µl Eva Green mix (Jena Bioscience), and 100pmol/µl primers. The primers are shown in (Table 1). Amplification was achieved using real-time polymerase chain reaction (PCR, Strata gene Mx3005P-qPCR System). Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was considered a housekeeping gene. The PCR cycling parameters were as follows: initial extension at 95oC for 30s then 95oC for 5s, 61oC for 1s, and 72oC for 30s (40 cycles) and finally at 72oC for 10 min. For mTOR: 95C for 3min, then 35 cycles of 30s at 95oC, 30s at 59oC, and 45s at 72oC, after cycles at 72oC for 10min. The 2-ΔΔCT method was used for determining relative gene expression changes.

Table 1: Primer sequences of mTOR, Akt, PI3K, and GAPDH

Gene	Forward primer	Reverse primer
mTOR	F: 5'- CACATCACTCCCTTCACCA-3	R: 5'-GCAACAACGGCTTTCCAC-3'
PI3K	F: 5'-AACACAGAAGACCAATACTC-3	R: 5'-TTCGCCATCTACCACTAC-3
Akt	F:5'- ATC CCC TCA ACA ACT TCT CAG T'-3	R: 5'- CTT CCG TCC ACT CTT CTC TTT C'-3
GAPDH	F: 5′ GTCGGTGTGAACGGATTTG3	R: 5′ CTTGCCGTGGGTAGAGTCAT3′.

Light microscopic examination

The hepatic specimens were preserved in buffered formalin10% as a fixator and embedded in paraffin. Sections of 5µm-thickness were placed on the slides, deparaffinized in xylene, and then processed for hematoxylin and eosin (H&E) staining[29]. Each slide was examined by light microscopy (Leica ICC50W) at the Anatomy Department Imaging Unit, Faculty of Medicine, Zagazig University.

Immunohistochemical staining for glial fibrillary acidic protein (GFAP) and microtubule-associated protein 1A/1B-light chain 3 (LC3)

Hydrogen peroxide (0.6%) was used as endogenous peroxidase inhibitor. The slides were incubated at 4°C for 20 h with anti-mouse anti-GFAP antibody, a marker for activated hepatic stellate cells,[30] at 1:500 dilution of the antibody, and rabbit anti-LC3 antibody, an autophagy marker,[31] at 1:1000 dilution of the antibody. Next, the slides were rinsed and incubated with biotinylated secondary antibodies followed by the avidin-biotin complex. Hematoxylin stain was used as a counterstain. GFAP- and LC3-positive cells displayed brown cytoplasm and blue nuclei. Negative control was obtained by omitting the primary antibody. Positive control was done on cerebellar tissue and mouse colon carcinoma CT26 cells respectively[32,33].

Transmission electron microscopic examination

The hepatic samples were sliced into 1mm3 pieces, fixed in buffered glutaraldehyde 4% solution (pH 7.4) at 4oC, then rinsed twice in cacodylate buffer solution for 15 min each. The samples were further postfixed in 1% buffered osmium tetraoxide for two hours at room temperature, and rinsed again to eliminate the extra fixative. Ultrathin sections were mounted on copper grids[34], inspected and photographed at Faculty of Science, Ain Shams University by a JEOL transmission electron microscopy (JEM-1200 EX-II, Japan), operated at 60-70 kV.

Morphometrical analysis

The morphometric study was carried out by the ImageJ plug at the Anatomy Department, Zagazig Faculty of Medicine. H&E, GFAP and LC3 stained slides from each animal were investigated to detect the area percentages of hydropic degeneration as well as GFAP and LC3 positive expressions. For each slide, 5 nonoverlapped fields, at 400 magnification, were randomly assessed. The grade of hydropic degeneration was evaluated according to Reel *et al*.[35]: 0 for no hydropic degeneration, one for <25%, two for 25-50%, and three for >50%.

Statistical analysis

The statistical tests were achieved using GraphPad Prism version 5.0 (GraphPad Software, CA, USA). The variables were represented as the means ± standard error, as the data displayed normal distribution. The normality of different variable readings were tested by the Kolmogorov-Smirnov test. For detection of significant differences ($P < 0.05$) between groups, ANOVA (one-way analysis of variance) test was used. Whereas, for multiple comparisons among different groups, A post hoc Tukey's test was used.

RESULTS

Biochemical results

Serum levels of AST and ALT were significantly increased in group III relative to both groups I and II. These enzymes were significantly reduced in group IV, despite being significantly higher compared to both groups I and II (Table 2).

Oxidative enzyme assay and lipid peroxidation results

Lipid peroxidation (MDA level) was significantly

elevated in group III relative to groups I and II. In group IV, MDA level was considerably reduced compared to group III; however, it was still significantly higher relative to both groups I and II (Table 2).

The GSH level was markedly reduced in group III in comparison to groups I and II. Although its level was significantly increased in group IV, it was still significantly less compared to both groups I and II (Table 2).

Hepatic expression of PI3K, Akt and mTOR genes in different studied groups

Insignificant differences were recorded in the autophagy-related gene expression: PI3K, Akt, and mTOR mRNA levels among groups I, II and IV, whereas group III displayed significantly upregulated PI3K, Akt, and mTOR expression (Table 3).

Histological findings

H&E

Examination of groups I and II showed no histological differences. Liver sections of both groups displayed thin-walled portal and central veins as well as bile ducts lined by cuboidal cells. Acidophilic hepatocytes appeared with vesicular nuclei and were arranged in cords separated by thin sinusoids (Figures 1a,b). Regarding group III, the hepatic tissue revealed vacuolated hepatocytes, inflammatory cell infiltrates in the portal areas and congested central veins with detached lining (Figures 1c,d). The liver architecture was partially restored in group IV, as some hepatocytes exhibited acidophilic cytoplasm, whereas others showed cytoplasmic vacuolations. Additionally, few inflammatory cell infiltrates and congested central veins were still noted (Figures 1e,f).

Immunohistochemical findings

GFAP Immunohistochemical analysis

Both groups I and II demonstrated minimal GFAP expression. Inversely, GFAP expression was increased in group III. Group IV revealed a noticeable decrease compared to group III (Figures 2a–c).

LC3 Immunohistochemical analysis

Positive LC3 expression was detected in groups I and II, whereas groupIII exhibited relatively decreased LC3 expression. LC3 reactivity was elevated in group IV in comparison with group III (Figures 2d–f).

Electron microscopic findings

Liver specimens of groups I and II revealed hepatocytes with regular euchromatic nuclei, multiple mitochondria, parallel tubules of rough endoplasmic reticulum (RER) and scattered cytoplasmic glycogen granules. The hepatocytes of group III showed cytoplasmic vacuolations and rarefaction, mitochondria with destructed cristae, and irregular nuclear envelopes. Group IV hepatocytes displayed fewer vacuolations and less rarefaction within their cytoplasm with few lipid droplets. Their nuclei appeared rounded with regular envelopes (Figure 3).

Morphometric results

The area percentage of hepatic hydropic degeneration in group III showed significantly higher scoring compared to both groups I and II. The scoring decreased significantly in group IV relative to group III and was nonsignificant compared to groups I and II (Table 4).

Regarding GFAP immunostaining, group III revealed significantly increased area percentage of positive expression compared to groups I and II. Conversely, GFAP reactivity was significantly reduced in group IV relative to group III (Table 4).

Concerning LC3 immunostaining, group III exhibited a significant decrease of LC3 area percentage compared to groups I and II. This percent was relatively increased in group IV (Table 4).

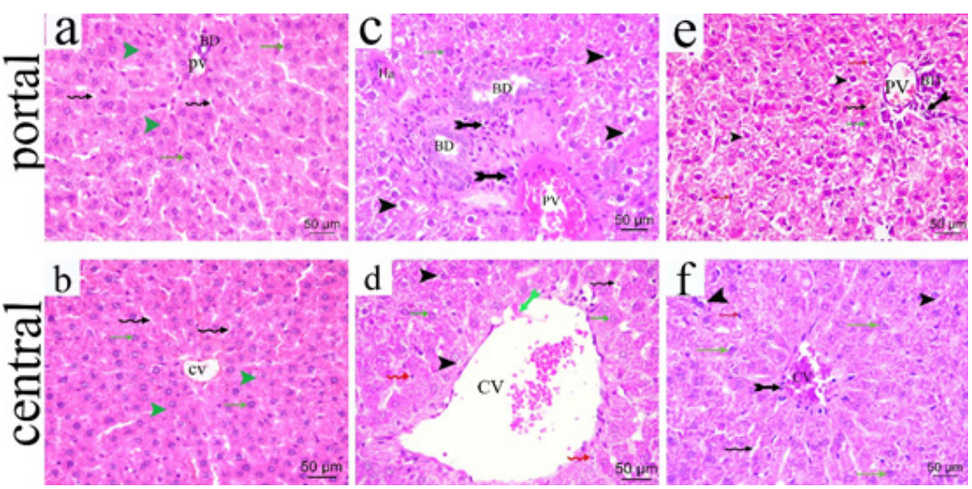

Fig. 1: A representative photomicrograph of liver sections in different groups; (a) portal area of control groups, (b) central area of control groups, (c) group III portal area, (d) group III central area, (e) group IV portal area, (f) group IV central area. Portal vein (PV), bile duct (BD), central vein (CV), hepatic artery (Ha), acidophilic hepatocyte (green arrowhead), sinusoids (zigzag arrow), Kupffer cell (red zigzag arrow), vacuolated hepatocyte (black arrowhead), vesicular nucleus (green arrow), inflammatory cells (black tailed arrow), detached lining (green tailed arrow). (H&EX400)

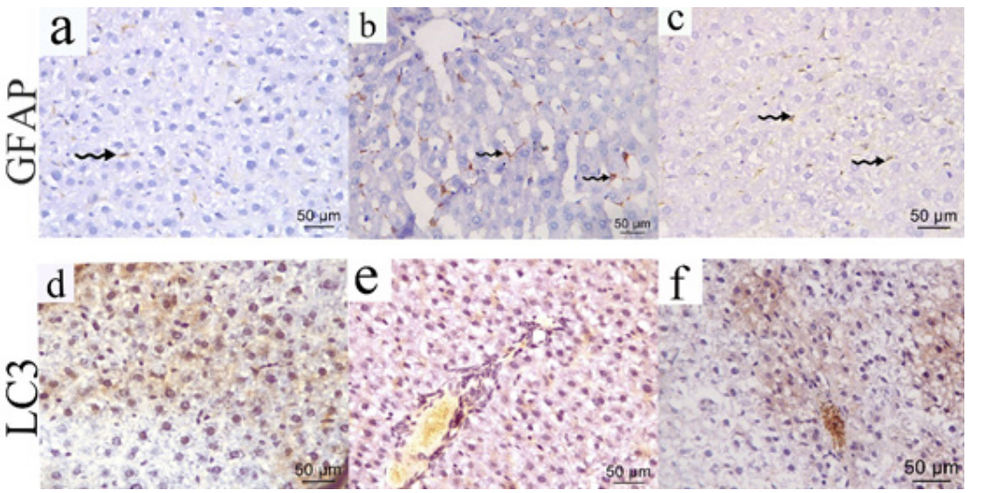

Fig. 2: A representative photomicrograph of liver specimens; (a) control groups, (b) group III, (c) group IV. (zigzag arrow) GFAP positive cells. (GFAP immunostaining x400). (d) control groups with marked positive LC3 expression, (e) group III with less positive expression, (f) group IV revealing marked positive expression. (LC3 immunostaining x400)

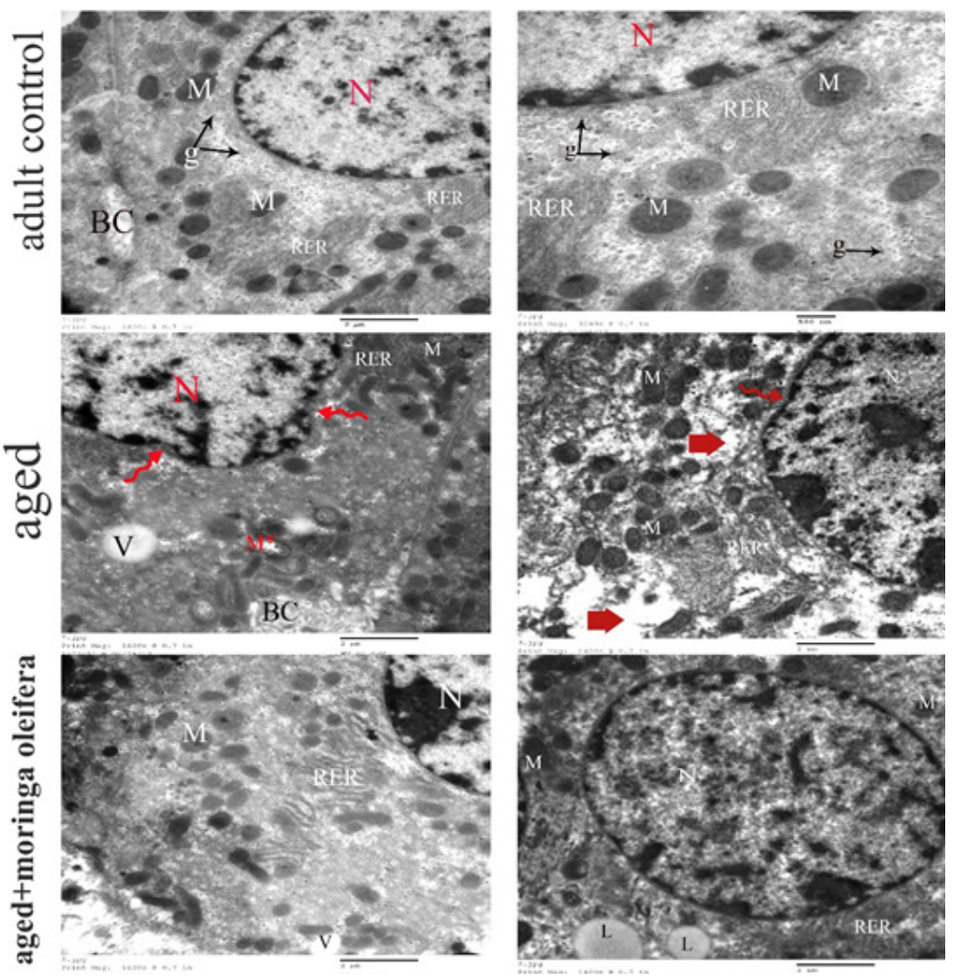

Fig. 3: A representative electron micrograph of different groups; hepatocyte nucleus (N), mitochondria (M), scattered glycogen granules (g), rough endoplasmic reticulum (RER), bile canaliculus (BC), irregular nuclear envelope (red zigzag arrow), mitochondria with destructed cristae (M*), rarefaction of cytoplasm (thick red arrow), vacuolation (V), lipid droplets (L). (scale bar=2µm) (scale bar=500nm).

Table 2: Comparison among different experimental groups regarding mean values of AST, ALT, MDA and GSH.

Parameter Mean±SE	Group I	Group II	Group III	Group IV
AST(IU/l)	35.10±0.3642	34.50± 0.4374^{NSa}	58.57± 0.5673 P < 0.05$^{a\&b}$	44.35± 0.4372 *P* < 0.05$^{a,b\&c}$
ALT(IU/l)	17.29± 0.2177	16.70± 0.3006^{NSa}	33.87± 0.5788 P < 0.05$^{a\&b}$	22.42± 0.3329 *P* < 0.05$^{a,b\&c}$
MDA (nmol/mg)	4.947± 0.2524	4.955± 0.3452^{NSa}	14.93± 0.2191 P < 0.05$^{a\&b}$	9.380± 0.2136 *P* < 0.05$^{a,b\&c}$
GSH mg/dl	3.337± 0.1669	3.917± 0.1870^{NSa}	1.627± 0.1200 P < 0.05$^{a\&b}$	2.355± 0.1543 *P* < 0.05$^{a,b\&c}$

NS = non-significant (*P* > 0.05), *P* < 0.05 =statistically significant, a = versus group I, b = versus group II, c=versus group III

Table 3: Comparison among different experimental groups regarding mean values of mTOR , PI3K and Akt mRNA expression

Parameter Mean±SE	Group I	Group II	Group III	Group IV	*P value*
mTOR	1.09±0.13	1.12±0.11	2.51±0.31^{a,b}	1.35±0.19^{c}	0.0000
PI3k	1.11±0.15	1.03±0.21	1.82±0.35^{a,b}	1.33±0.23^{c}	0.0001
Akt	1.19±0.09	0.98±0.19	1.56±0.22^{a,b}	1.24±0.23^{c}	0.0001

NS = non-significant (*P* > 0.05), *P* < 0.05 =statistically significant, a = versus group I, b = versus group II, c=versus group III

Table 4: Comparison among different groups regarding hydropic degeneration scoring, GFAP area percentage (%) and LC3 area %

Parameter Mean±SE	Group I	Group II	Group III	Group IV
Hydropic degeneration scoring	0.3333±0.2108	0.5000±0.2236^{NSa}	1.667± 0.2108 *P* < 0.05 $^{a\&b}$	0.8333±0.3073 *P* < 0.05$^{cNSa\&b}$
GFAP area %	0.8927± 0.03591	0.8566± 0.04005^{NSa}	11.05± 0.4808 *P* < 0.05 $^{a\&b}$	5.883± 0.3676 *P* < 0.05$^{a,b\&c}$
LC3 area %	49.75± 0.7492	49.18± 0.7418^{NSa}	15.49± 0.4227 *P* < 0.05$^{a\&b}$	21.42± 0.7814 *P* < 0.05$^{a,b\&c}$

NS = non-significant (*P* > 0.05), *P* < 0.05 =statistically significant, a = versus group I, b = versus group II, c=versus group III. Score of hydropic degeneration was (0) for no changes, (1) for < 25% affected cells, (2) for changes 25% - 50%, and (3) for > 50%

DISCUSSION

Aging process leads to progressive decline of cellular functions due to loss of capability to respond perfectly to any injury[36]. The rate of hepatic diseases increases throughout the aging process[3,4]. Many theories have emerged trying to elucidate the aging process; however, none seems entirely adequate. These theories include free radical theory, DNA injury theory, and autophagy theory[37]. Autophagy is an essential process that gets rid of damaged organelles and subcellular elements, promoting cellular homeostasis[38]. Many studies have been interested in medicinal plants that have therapeutic effects on tissue injury during aging[39,40]. Recently, MO has gained interest as an anti-aging agent via combating oxidative stress and improving nutrient metabolism[41]. Taken together, our research explored the potential effects of MO extract on aging-related structural and functional liver alterations, with reference to the possible role of the autophagy PI3K/Akt/mTOR signaling pathway.

Owing to their short life span and relatively small size, experimental rats are considered one of the models of choice for aging research[42]. To achieve our aim, aged rats 20 month-old (comparable to 65 years in humans[43]) were employed in the present study. It is worth mentioning that the adult rats that received MO extract, in our research, did not show any sign of hepatic damage, implying safety of MO extract treatment.

Reactive oxygen species (ROS) have been proven to negatively impact the liver functions and are closely associated with many aging-related diseases[44,45]. In the same context, the present research revealed significantly elevated AST and ALT serum levels and hepatic MDA content, in addition to diminished GSH levels in the aged rats compared to the controls. In agreement with these findings, increased liver enzymes, lipid peroxidation levels and oxidation markers were previously detected in the livers and kidneys of aged rats[46,47], proving aging-related alterations of the oxidant status. On the other hand,

the aged rats received MO revealed significant decline in the liver enzymes and MDA levels and increased GSH levels. Consistent with these results, previous studies stated that MO extract has a preserving effect on the hepatocyte membrane structure and prevents the leakage of enzymes[23,48]. The antioxidant properties of MO extract were ascribed to the high amount of polyphenolics and other antioxidants, such as ascorbic acid, flavonoids and tocopherol[49].

This oxidative status of aged rats was confirmed by the histological findings of the liver. The hepatic tissue of aged rats revealed inflammatory infiltrates in the portal areas and congested central veins with detached endothelial lining. In the same line, Jin *et al*.[46] detected increased neutrophilic infiltration in the liver of old mice. Furthermore, a close association between aging and vascular endothelial dysfunction was established[50]. In fact, the observed inflammation and endothelial injury are pathophysiologically linked to oxidative stress[51]. The aged hepatocytes in the present study appeared clearly vacuolated. In association with these results, Hamden *et al*.[52] observed increased lipid deposition in the livers of aged rats. Moreover, Mohammed *et al*.[53] reported vesicular bodies within the hepatic cytoplasm in the aged rat liver.

The current investigation demonstrated significantly increased GFAP expression in the aged rats relative to the adults. Conversely, GFAP expression was significantly decreased on MO administration compared to the aged group. In accordance, GFAP expression was observed in myofibroblasts and HSCs in the hepatic fibrotic areas and its expression was increased with further development of fibrosis[54]. These results supposed that GFAP immunoreactivity is linked to fibrotic activity and disease progression.

Ultrastructural findings emphasized the microscopic results, as represented by vacuolated rarefied cytoplasm and destructed mitochondrial cristae of aged hepatocytes. In agreement with our observations, Mahmoud and Hegazy[55] detected cytoplasmic vacuolization and mitochondria with few cristae in the hepatic cells of aged rats. Brandt *et al*.[56] mentioned that the mitochondria of aged mice hepatocytes appeared wider with less interconnected cristae to the inner membrane. Deloncle *et al*.[57] considered cytoplasmic vacuolization as the initial sign of cell death. These changes were significantly ameliorated in the aged rats administrated MO. Our histological findings were confirmed by the scoring of hydropic degeneration of hepatic cells, as the scoring of the liver in the aged group administrated MO extract was significantly less than in the aged group. In the same line, Fattah *et al*.[58] detected significantly less scoring of tissue damage in the group co-administrated MO extract with lead acetate compared to the lead acetate-treated group. Likewise, Aly *et al*.[48] mentioned that MO leaf extract had hepatoprotective effect against acetaminophen toxicity, manifested as decreased hepatic fibrosis and minimal inflammatory infiltrate. The phenolic compounds found in MO leaves as gallic acid and protocatechuic acid were previously known to have antioxidant effects that improve hepatic toxicity[59,60].

Autophagy is a crucial aging target in the liver[61]. The triggering of autophagy is the main anti-aging process for the clearance of accumulated damaged macromolecules and organelles. PI3K/Akt/mTOR pathway is considered the main signaling pathway implicated in autophagy inhibition, which makes it responsible in the aging process[62-64]. The current research illustrated a significant increase of mRNA PI3K/Akt/mTOR expression in the aged rats compared to the adults, consistent with many studies that reported suppressed autophagy activity in the aged than in the young liver[65,66]. Therefore, activating autophagy via pharmacological interference appears to be effective. In our study, there was significantly decreased mRNA PI3K/Akt/mTOR expression in the aged group treated with MO extract, supporting the idea that MO extract may induce autophagy. The mTOR pathway is considered a pivotal autophagy-related pathway. O'Neill[67] suggested that when mTOR activity is downregulated, it permits autophagy activation, leading to the preservation of organelle and biomolecular health essential for healthy cells. In agreement with our data, mTOR inhibition with rapamycin has been established to delay aging and increase lifespan[68]. LC3 is one of the most important proteins and is now considered the only reliable autophagosome marker. Upregulation of LC3 expression is associated with induction of autophagy. Accordingly, autophagy activation or inhibition can be easily assessed by evaluating LC3 levels[69]. This study revealed a weak LC3 immunoreaction in the aged group, whereas the aged group treated with MO extract had noticeably increased LC3 immunoreactivity, supporting the role of MO extract as an autophagy inducer. In accordance with this study, Chauhan *et al*.[70] concluded that the dichloromethane extract of MO leaves extended the lifespan and stress tolerance in Caenorhabditis elegans. Additionally, Gunadi *et al*.[71] proved autophagy induction by the decreased gene expression of LC3 and p62 in MO-treated rats than in the controls.

CONCLUSIONS

In conclusion, this study suggested that the aging-related hepatic alterations are closely linked to oxidative stress and autophagy. MO extract significantly improved cellular redox homeostasis and induced autophagy by inhibiting the PI3K/Akt/mTOR pathway, thereby mitigated the aging-induced biochemical and morphological liver changes. These results provide a novel approach for amelioration of aged hepatic tissue.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

يمكن لمستخلص المورينجا أوليفيرا تحسين التغيرات التركيبية والوظيفية للكبد المرتبطة بالشيخوخة من خلال تنظيم مسار إشارات الالتهام الذاتي

هبة أسامة محمد[١]، رانيا سعد رمضان[١،٢]، أمل فوزي[٣]، علياء طلعت[٣]، إيمان أحمد علاء الدين[٤]، مها أحمد عبدالرحمن[١]

قسم التشريح والأجنة، كلية الطب، [١]جامعة الزقازيق، [٢]جامعة الباحة، المملكة العربية السعودية

[٣]قسم الكيمياء الحيوية، [٤]قسم الطب الشرعي و السموم، كلية الطب، جامعة الزقازيق

المقدمة: الشيخوخة هي عملية بيولوجية تزيد من الضرر التأكسدي في الخلية. نبات المورينجا أوليفيرا له تأثير وقائي على الكبد عن طريق تحفيز آلية الدفاع المضادة للأكسدة.

الهدف من الدراسة: تقييم التأثيرات المرتبطة بالشيخوخة على بنية ووظيفة الكبد والدور المحسن المحتمل لمستخلص المورينجا أوليفيرا ، مع توضيح تأثيرها على الالتهام الذاتي في نماذج الجرذان المسنة.

المواد والطرق المستخدمة: تم تقسيم أربعة وعشرين جرذاً، بعمر ثلاثة أشهر، إلى أربع مجموعات على النحو التالي: المجموعة الأولى: مجموعة البالغين الضابطة، والمجموعة الثانية: مجموعة البالغين التي تلقت المستخلص المائي لأوراق المورينجا أوليفيرا (٥٠مجم / كجم من وزن الجسم) عن طريق الأنبوب الأنفي المعدي لمدة ٤ أشهر، والمجموعة الثالثة: لم تتلق أي علاج حتى سن ٢٠ شهرًا، والمجموعة الرابعة: لم تتلق أي علاج حتى سن ١٦ شهرًا، ثم تلقوا مستخلص مائي لأوراق نبات المورينجا لمدة ٤ أشهر. عند الذبح، تم قياس إنزيمات الكبد (الأسبارتات أمينوترانسفيريز والألانين أمينوترانسفيريز) في الدم وتمت معالجة العينات الكبدية للفحص بالمجهرين الضوئي والإلكتروني وتحليل جينات فوسفاتيديل اينوسيتول ٣-كيناز (PI٣K)، و بروتين كيناز ب (akt)، و (mTOR)

النتائج: أظهرت الجرذان المسنة ارتفاعًا ذا دلالة إحصائية في مستويات إنزيمات الكبد والملونديالديهيد في مصل الدم، بالإضافة إلى انخفاض محتوى الجلوتاثيون الكبدي. كما قللت الشيخوخة من الالتهام الذاتي عن طريق زيادة الجينات المثبطة للالتهام الذاتي PI3K و Akt و mTOR .وقد أظهر تقييم الأنسجة الكبدية للجرذان المسنة بنية خلوية متدهورة من حيث التنكس المائي واحتقان الأوردة والتسلل الالتهابي بالإضافة إلى زيادة الإبانة المناعية للبروتين الحمضي الليفي الدبقي وانخفاض إبانة البروتين المرتبط بالأنابيب الدقيقة (LC3) . أدى إعطاء المورينجا للجرذان المسنة الى تحسنًا ملحوظًا للخلل الكبدي والتغيرات النسيجية المرتبطة بالشيخوخة ، كما ثبط الإجهاد التأكسدي وحفز الالتهام الذاتي عن طريق تقليل جينات PI3K و Akt و.mTOR.

الخلاصة: يمكن أن يقلل مستخلص نبات المورينجا من تلف الكبد التركيبي والوظيفي الناجم عن تقدم العمر عن طريق مكافحة الإجهاد التأكسدي ، وتحفيز الالتهام الذاتي من خلال تقليل مسار إشارات PI3K / Akt / mTOR