Moringa Oleifera versus Simvastatin on Liver Steatosis in an Adult Male Rat Model of High Fat Diet; Histological and Immunohistochemical Study

Original Article

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ABSTRACT

Introduction: Obesity is a global major health concern. Fatty diet is a significant factor leading to obesity and likewise results in steatosis and steatohepatitis. Moringa oleifera (MO) leaves are commonly used for both nutritional and medicinal purposes for curing of several diseases. They had also revealed hepatoprotective activities.

Aim of the Work: To study the impact of high fat diet (HFD) on the livers of rats and to assess the potential protective role of MO leaves extract, comparing it with that of simvastatin.

Materials and Methods: Forty adult male albino rats were used in this study. Animals were separated into four groups (ten rats per group): Group 1: Control group: received standard diet for eight weeks; Group 2: HFD group: received HFD for eight weeks; Group 3: Simvastatin treated group: received Simvastatin with the HFD; Group 4: MO leaves extract treated group: received MO leaves extract in addition to HFD. By the end of the 8th week rats were weighed then sacrificed. Liver was removed, weighed and examined histopahologically using hematoxylin and eosin (H & E) as well as Masson's trichrome stain. Immunohistochemical staining using the avidin-biotin peroxidase complex technique for Anti-Glial fibrillary acidic protein (GFAP) antibody and Anti-Beclin1 antibody were also done.

Results: Body weight and liver weight of rats on HFD (Group 2) were increased and the liver architecture of this group was noticeably affected. Serum liver enzymes as well as serum lipids were markedly elevated. In Group 3, simvastatin attenuated all these studied parameters. The protective role MO leaves extract demonstrated in Group 4 was obvious and even exceeded that of simvastatin.

Conclusion: The protective effect of MO leaves extract was evident in liver steatosis. We recommend increased awareness of people about the beneficial effect of MO especially in overweight and obese people.

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Key Words: Beclin-1, GFAP, liver steatosis, moringa oleifera, simvastatin.

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INTRODUCTION

Obesity is a global major health concern[1]. Its prevalence is growing due to the living of sedentary lifestyle resulting in high dietary energy consumption together with low energy expenditure[2,3].

Even though the etiology of obesity is compound, fatty diet is a chief factor leading to obesity[4]. Fatty diet, as well, is a leading cause of metabolic syndrome which beside obesity, comprise dyslipidemia and insulin resistance[5,6]. Fatty diet and obesity are frequently associated with many complications as coronary heart disease, hypertension, atherosclerosis, strokes, diabetes mellitus and many others[7-10].

Furthermore, fatty diet and obesity lead to hepatic fat accumulation, resulting in oxidative stress as well as pro-inflammatory cytokines liberation. This results in a diverse liver damages which range from simple steatosis to steatohepatitis named nonalcoholic steatohepatitis or NASH, that may end in fibrosis and cirrhosis[11].

Simvastatin is an inhibitor of the 3 hydroxy 3 methylglutaryl coenzyme A reductase enzyme (HMG-CoA reductase), with subsequent lipid-lowering properties. It is extensively used for the management of disorders of lipid metabolism as hyperlipidemia[12-14]. Moreover, it similarly displays additional promising benefits as being anti-oxidant, anti-inflammatory, steadying atherosclerotic plaques and ameliorating endothelial malfunction[15,16]. Modern studies confirmed that simvastatin not only reduced serum lipids but diminished lipid deposition in the liver as well. Consequently, simvastatin can be considered a potential treatment in steatohepatitis[17].

Moringa oleifera (MO) related to the Moringaceae family, which cultivates in the world tropical and

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subtropical areas[18,19]. MO was suggested to be "most nutrient rich plant so far discovered" by Khawaja *et al*[20]. MO is rich in numerous essential nutrients such as amino acids, vitamins, minerals, beta-carotene, omega 3 and 6 fatty acids[21,22]. Moreover, MO contain phytochemicals chiefly polyphenols that was linked to its anti-oxidant & anti-inflammatory properties[23]. MO leaves were worldwide used for nutritive and also for medicinal purposes. They are habitually used in curing of headache, fever, inflammation, infections, diabetes, hyperlipidemia in addition to many other diseases[22,24,25]. Newly, MO has also revealed hepatoprotective actions. It has demonstrated anti-fibrotic effects on rat liver fibrosis[26].

This work aimed to study moringa oleifera versus simvastatin effects on liver steatosis in a rat model of HFD, monitored by serological, histological, immunohistochemical and morphometric studies.

MATERIALS AND METHODS

Animals

In this study, forty Sprague-Dawley adult (weighing 180–200 g) male albino rats were used. They were obtained from Animal House of Faculty of Medicine, Cairo University, They were maintained in a temperature of 20–25◦C in an air-conditioned room. They were exposed to a day light/darkness cycle 12:12h, as well as free food and water access. Animal handling ethical protocols were followed.

Simvastatin: Zocor tablets 20 mg from MSD pharma

Moringa oleifera (MO) leaves extract preparation: The fresh MO leaves were washed by running water. They were then air dried, crushed to powder and lastly preserved in a dry air-tight bottle. Plant derived aqueous extract was obtained by dissolving 1 g dried leaves powder in 10 ml boiling water for 15 min, then filtering through filter paper twice into a sterile cylinder and lastly left for cooling.

Study design

Animals were randomly divided into four groups (n = 10 per group):

Group 1: Control group: rats obtained standard diet for eight weeks.

Group 2: HFD group: rats obtained HFD for eight weeks. HFD contained 20 g of fat/100 g of diet (19g of butter oil in addition to 1g of soybean oil that provides essential fatty acids)[27].

Group 3: Simvastatin treated group: rats obtained in addition to HFD, Simvastatin at a dose of 20 mg/kg daily for eight weeks via gastric intubation[28].

Group 4: Moringa oleiefera (MO) leaves extract treated group: rats obtained in addition to HFD aqueous extract of MO leaves at a dose of 200 mg/kg daily for eight weeks. The extract was ingested via gastric intubation[29,30].

By the end of the 8th week, the body weight (BW) all rats were measured. In addition, tail vein blood samples were collected for measurement of liver enzymes alanine aminotransferase (ALT), and aspartate transaminase (AST), as well as lipid profile Triglyceride (TG) and Cholesterol (TC) at the Biochemistry department, Faculty of Medicine, Cairo University. Then, under lethal dose of general anesthesia, rats were sacrificed by intraperitoneal (ip) injection of 100 mg/kg ketamine-xylazine[31].

Histological study

At the end of the experiment, livers were dissected and weighed. Liver index was calculated as liver weight/ body weight × 100%. Then liver specimens were fixed in a solution of formol saline 10% and embedded in paraffin. Using a microtome (Leica RM 2025, Germany), serial sections (5-7 µm thickness) were mounted on glass slides, rehydrated in descending grades of alcohol then stained with the following stains at Histology Department, Faculty of Medicine, Cairo University:

a. Hematoxylin and Eosin (H & E) stain[32].

b. Masson's trichrome stain[33] to demonstrate collagen fibers.

c. cImmunohistochemical staining using the avidin-biotin peroxidase complex technique[34] for:

   1. Anti-Glial fibrillary acidic protein (GFAP) antibody; is a class III intermediate filament. It is a ready-to-use rabbit monoclonal antibody (1:2000, cat no. 14-9892-82, eBioscience, ThermoFisher, San Diego, CA). GFAP positive cells showed cytoplasmic and membranous brown reaction.

   2. Anti-Beclin1 antibody; Belongs to the beclin family. It is a ready-to-use rabbit monoclonal antibody (1:1000, ab210498; Abcam, Egypt). Beclin 1 positive cells showed cytoplasmic brown reaction.

Application of the primary (1ry) antibodies was followed by incubation in a humid chamber at room temperature for 60 min. Rat brains were used as $^{+}$ve control specimens for both GFAP and beclin 1. On the other hand, one of the liver sections was used as –ve control by passing the step of applying the 1ry antibody.

Morphometric Study

The "Leica Qwin 500 C" image analysis computer system Ltd, (Cambridge, UK) was used at Histology Department, Faculty of Medicine, Cairo University. For each group, 10 non-overlapping fields were examined at a magnification of x100. The following parameters were assessed:

a. Area % of collagen fibers in Masson's trichrome stained sections.

b. Number of GFAP & Beclin 1 immunopositive cells.

Statistical Analysis

The obtained data was analyzed using SPSS version 22 statistical package. Data was summarized using mean and standard deviation. Groups comparisons were done by means of analysis of variance (ANOVA) with multiple comparisons post hoc test, when more than 2 groups were compared. The difference was considered statistically significant when *p* (probability) value < 0.05[35].

RESULTS

Body weight (BW) &Liver index Results

Group II showed a significant increase in the mean values of BW & liver index compared to the other groups. Meanwhile, Group III revealed a statistically significant increase compared to the control & Group IV. Group IV presented a statistically significant increase in the mean value BW compared to the control (Table.1).

Biochemical Results

The mean values of TG, TC, ALT and AST showed a statistically significant increase in HFD group (Group II) in comparison to the remaining groups. In addition, simvastatin-treated group (Group III) showed a statistically significant increase compared to the control and moringa-treated group (Group IV). No significant difference was noticed between the control and moringa-treated group except in the mean value of AST that displayed a statistically significant increase in comparison to the control (Table. 2).

Histological Results

a) H&E Results

Examination of H&E-stained histological sections revealed apparently normal histological structure of the hepatic tissue formed of normal central veins and hepatocytes cords radiating from them. The hepatocytes appeared polyhedral cells with deeply acidophilic cytoplasm and vesicular nuclei with prominent nucleoli. Blood sinusoids appeared normally separating hepatocytes cords. Portal tract appeared normal containing a branch of the portal vein, a branch of the hepatic artery as well a bile duct (Figure 1 A,B,C). Group II showed marked disruption in the hepatic tissue structure as most of central veins appeared dilated and congested. Most of hepatocytes exhibited vacuolated cytoplasm with pyknotic nuclei. Blood sinusoids appeared slightly dilated. Portal tract showed dilated and congested portal vein with interrupted wall. Meanwhile, the hepatic artery and the bile duct appeared normal (Figure 2 A,B,C). Group III showed moderate improvement in the histological structure of the hepatic tissue, but some central veins still dilated and congested. Some hepatocytes appeared vacuolated and with pyknotic nuclei. Blood sinusoids appeared normally separating hepatocytes cords. Portal tract exhibited dilated portal vein and normal appearance of hepatic artery and bile duct (Figure 3 A,B,C). Group IV revealed marked improvement in the histological structure of the hepatic tissue with apparently normal central veins. Most of hepatocytes appeared normal with deeply acidophilic cytoplasm and vesicular nuclei with prominent nucleoli, yet few cells exhibited pyknotic nuclei. Blood sinusoids appeared normally separating hepatocytes cords. Note apparent normal contents of the portal tract (Figure 4 A,B,C).

b) Masson's Trichrome Results

Masson's Trichrome stain demonstrated collagen fibers which were normally distributed around central veins and in portal area in the control group (group I) (Figure 5 A,B). Group II showed noticeable collagen fibers deposition around central veins and in portal area (Figure 6 A,B). Group III showed less collagen fibers deposition around central veins and in portal area (Figure 7 A,B). Group IV showed apparently normal distribution of collagen fibers around central veins and in portal area (Figure 8 A,B).

c) Immunohistochemical Results

1. GFAP Results: Examination of liver sections immuno-stained with glial fibrillar acidic protein (GFAP) antibody showed few hepatic stellate cells (HSCs) which were flattened with mild cytoplasmic expression of GFAP in control rats (group I) (Figure 9A). Group II showed many enlarged HSCs with long processes exhibiting strong cytoplasmic expression of GFAP (Figure 9B). Group III showed some enlarged HSCs with strong cytoplasmic expression of GFAP (Figure 10A). Meanwhile, group IV showed few flattened HSCs with mild cytoplasmic expression of GFAP (Figure 10B).

2. Beclin 1 Results: Examination of liver sections immuno-stained with beclin 1antibody showed many hepatocytes with strong brown cytoplasmic reaction in control rats (group I) (Figure 11A). Group II showed weak cytoplasmic reaction in few hepatocytes (Figure 11B). Group III showed moderate cytoplasmic reaction in some hepatocytes (Figure 12A). Meanwhile, a strong cytoplasmic reaction was noticed in many hepatocytes of group IV (Figure 12B).

Morphometric Results

In group II (HFD group), the mean area % of collagen fibers and number of GFAP immune-positive cells showed a statistically significant increase in comparison with all other groups. In addition, a statistically significant increase was detected in simvastatin-treated group (group III) when compared to the control & moringa-treated group (group IV). Moreover, the mean number of beclin-1immune-positive cells revealed a statistically significant decrease in group II compared to all other groups and also in group III compared to groups I &IV. However, there was no statistically significant difference between the control & moringa-treated group (group IV) (Table. 3).

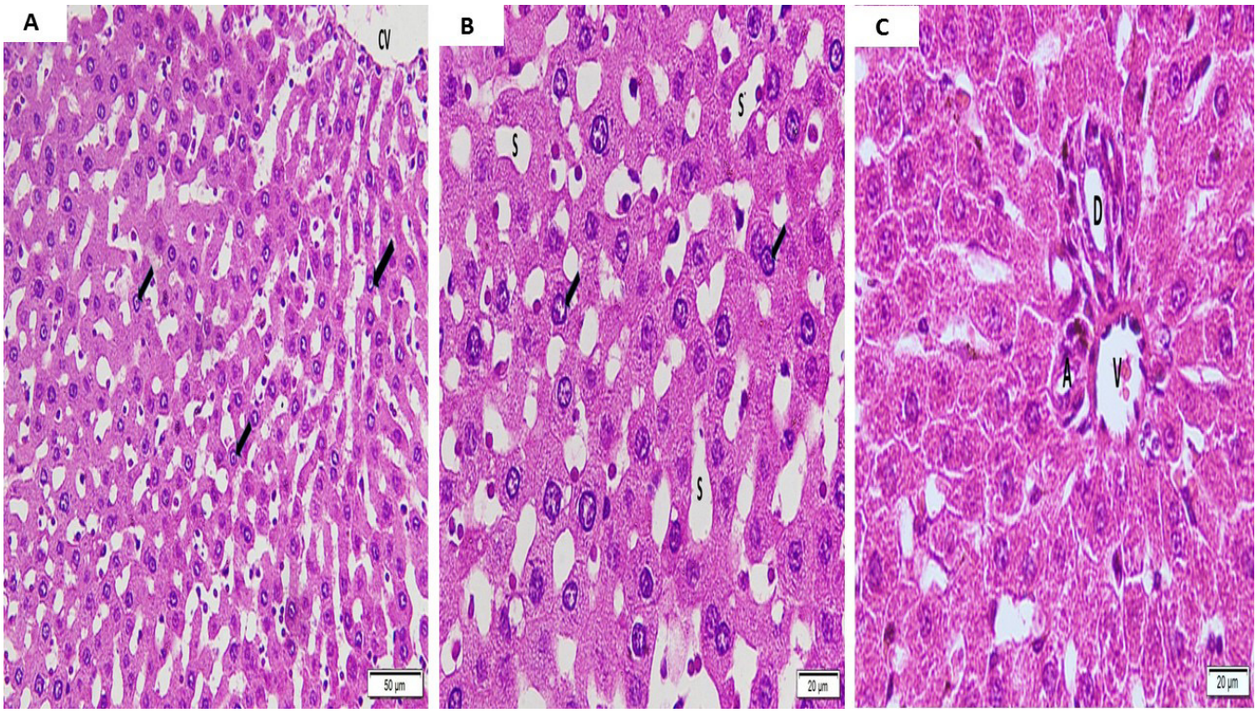

Fig. 1: Photomicrographs of liver tissue from group I show normal structure of hepatocytes with vesicular nuclei (arrows) around apparently normal central vein (CV). Blood sinusoids (S) appear normally separating hepatocytes cords. Portal tract containing a branch of portal vein (V), branch of hepatic artery (A) and bile duct (D) (H&E; Ax200, B&Cx400).

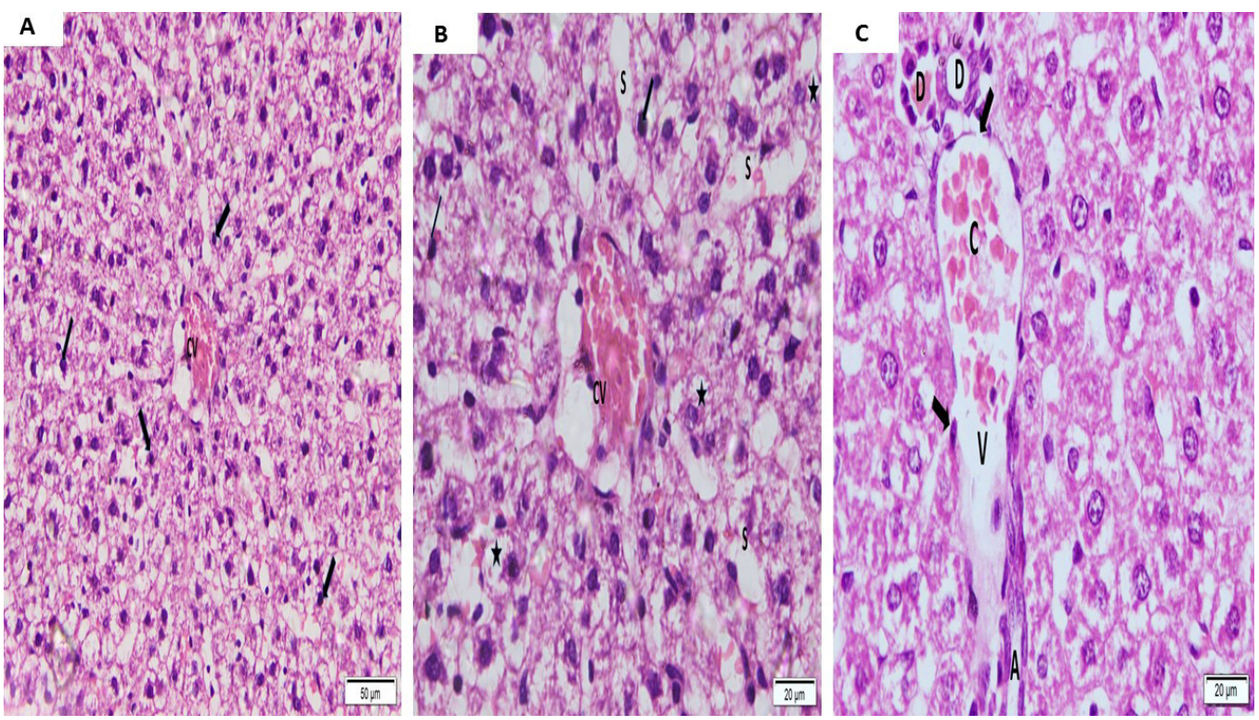

Fig. 2: Photomicrographs of liver tissue from group II show dilated and congested central vein (CV). Hepatocytes appear with vacuolated cytoplasm (stars) and pyknotic nuclei (arrows). Blood sinusoids (S) appear slightly dilated. Portal tract shows dilated portal vein (V) with congestion (C) and interrupted wall (arrows). Note normal bile duct (D) and hepatic artery (A) (H&E; Ax200, B&Cx400).

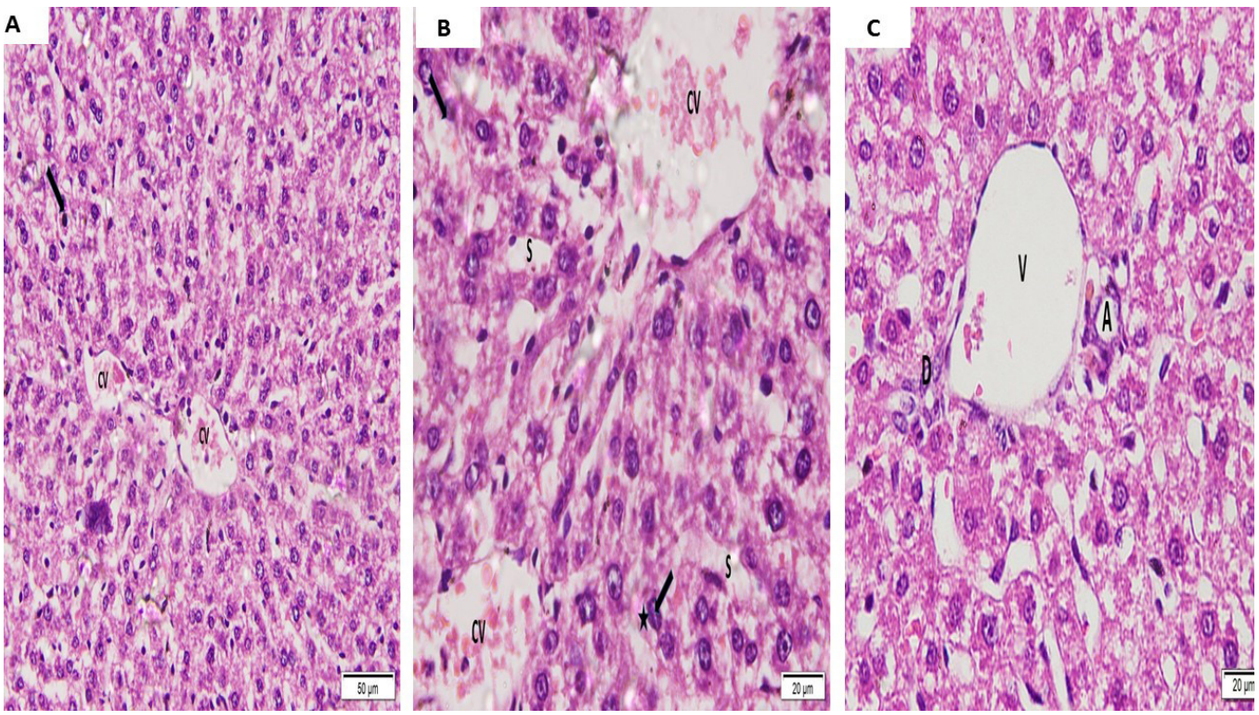

Fig. 3: Photomicrographs of liver tissue from group III show congested and dilated central vein (CV). Some hepatocytes appear with vacuolated cytoplasm (stars) and pyknotic nuclei (arrows). Blood sinusoids (S) appear normally separating hepatocytes cords. Portal tract exhibits dilated portal vein (V). Note normal bile duct (D) and hepatic artery (A) (H&E; Ax200, B&Cx400).

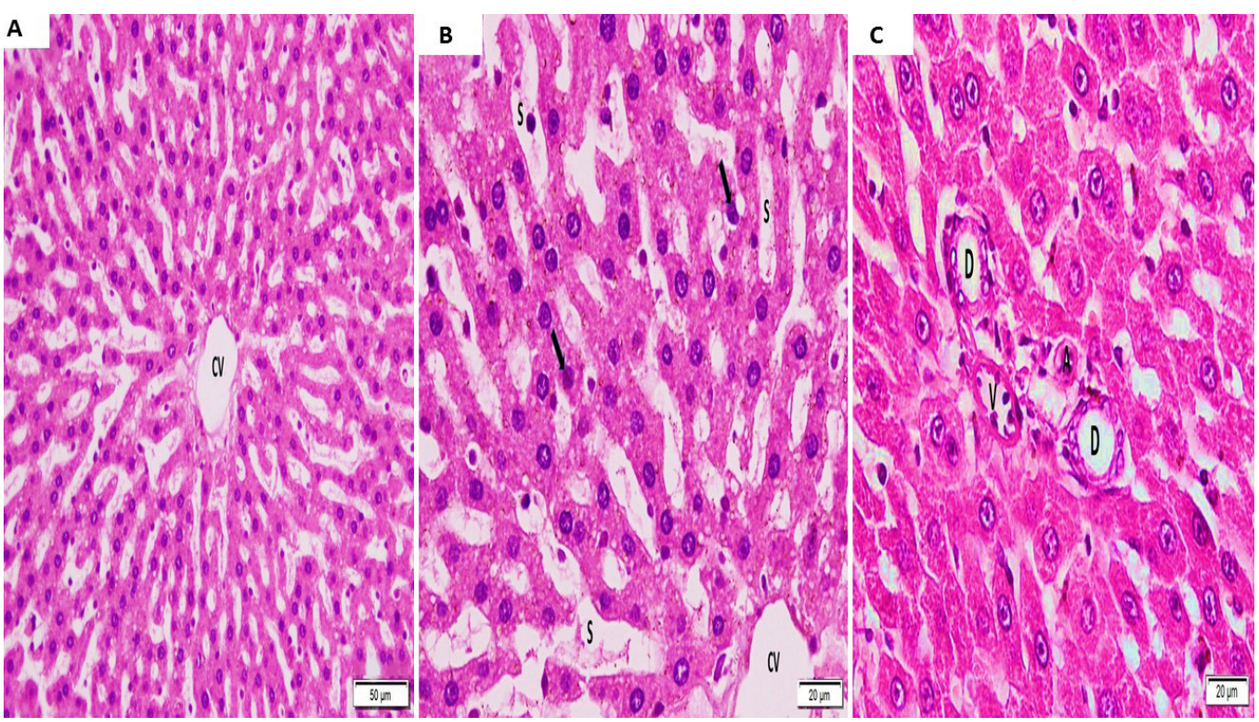

Fig. 4: Photomicrographs of liver tissue from group IV show apparently normal central vein (CV). Few hepatocytes appear with pyknotic nuclei (arrows). Blood sinusoids (S) appear normally separating hepatocytes cords. Portal tract contains apparently normal portal vein (V), hepatic artery (A) and bile duct (D) (H&E; Ax200, B&Cx400).

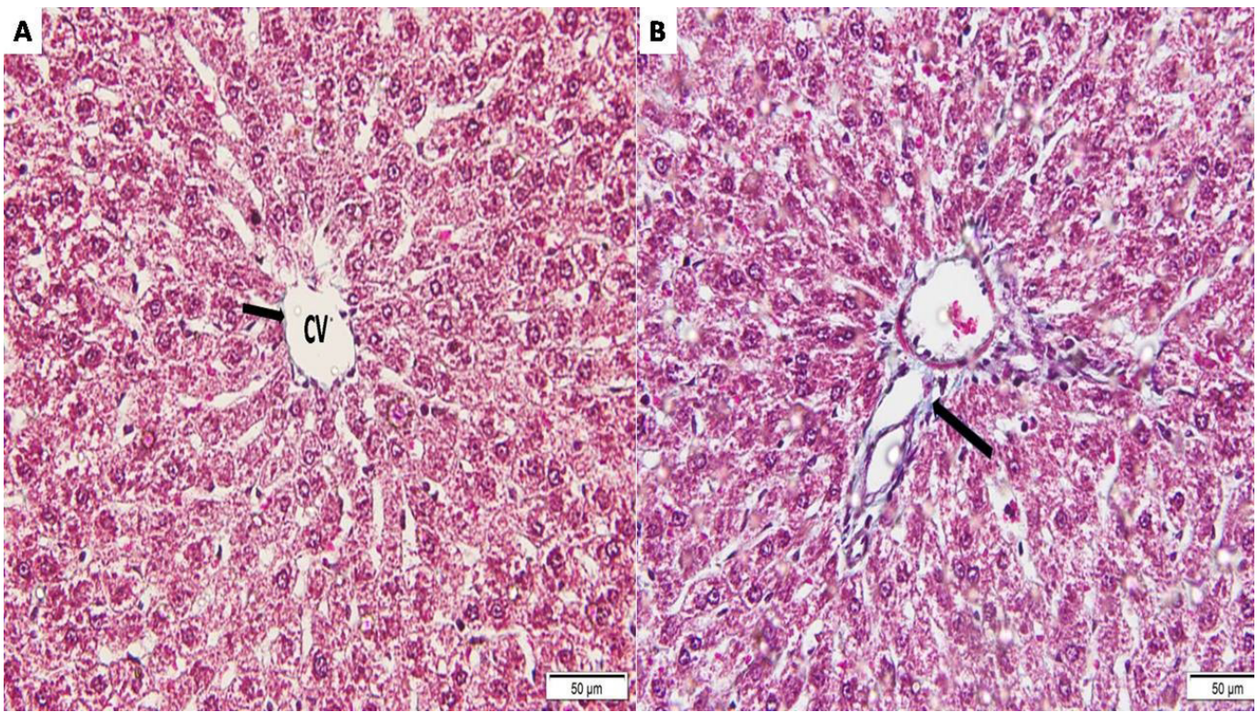

Fig. 5: Photomicrographs of liver tissue from group I show normal distribution of collagen fibers around central vein (CV) and in the portal area (arrow) (Masson's trichrome; A&B x200).

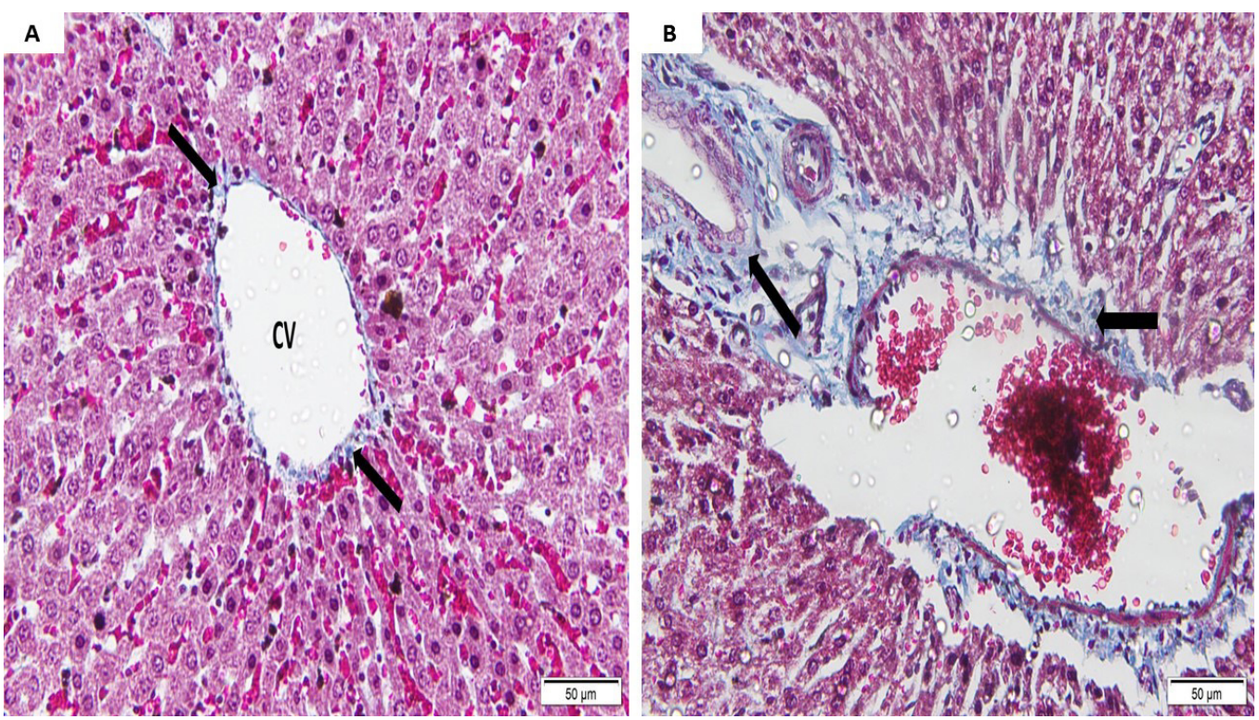

Fig. 6: Photomicrographs of liver tissue from group II show marked increase in collagen fibers around central vein (CV) and in the portal area (black arrows). (Masson's trichrome; A&B x200).

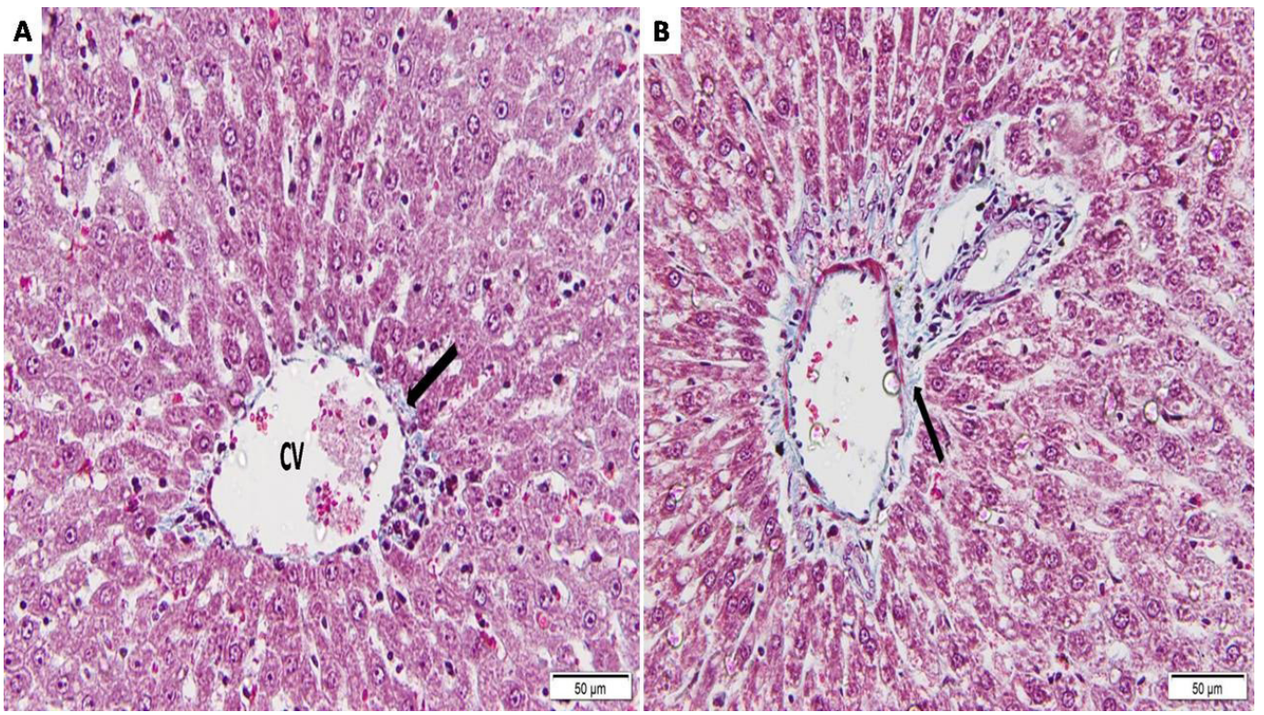

Fig. 7: Photomicrographs of liver tissue from group III show mild increase in collagen fibers around central vein (CV) and in the portal area (arrow) (Masson's trichrome; A&B x200).

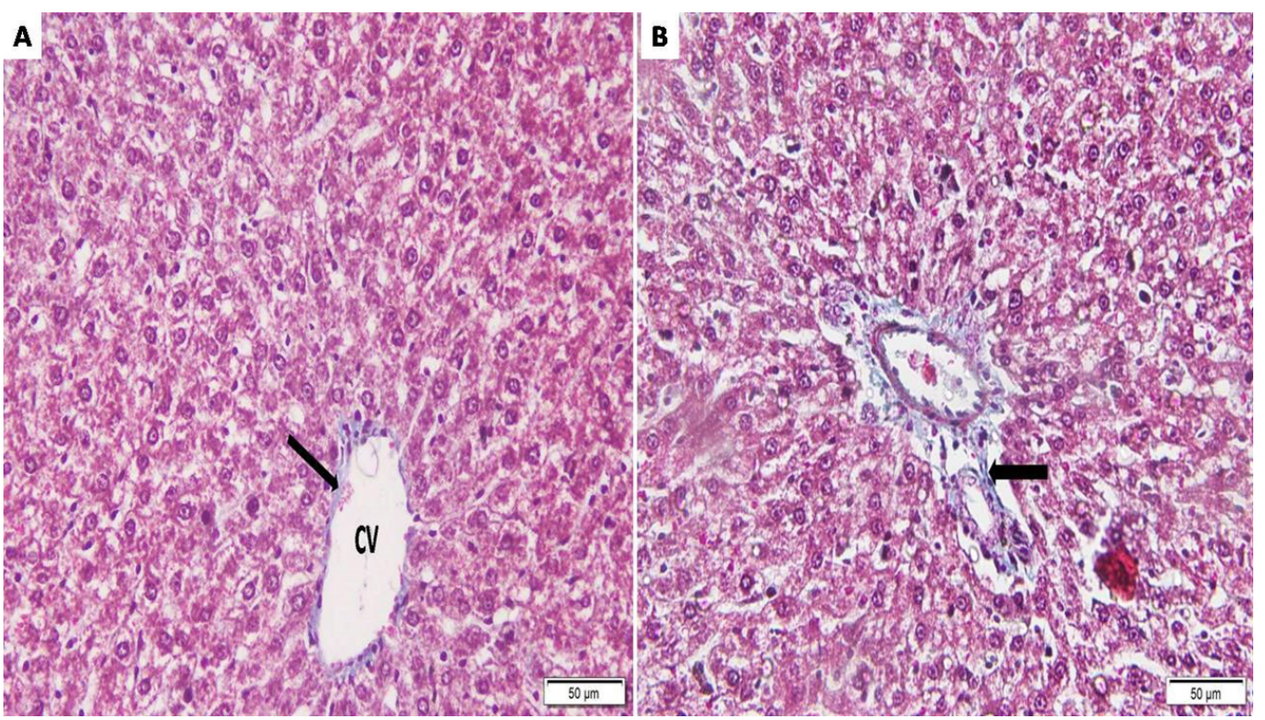

Fig. 8: Photomicrographs of liver tissue from group IV show apparent normal collagen fibers around central vein (CV) and in the portal area (arrow) (Masson's trichrome; A&B x200).

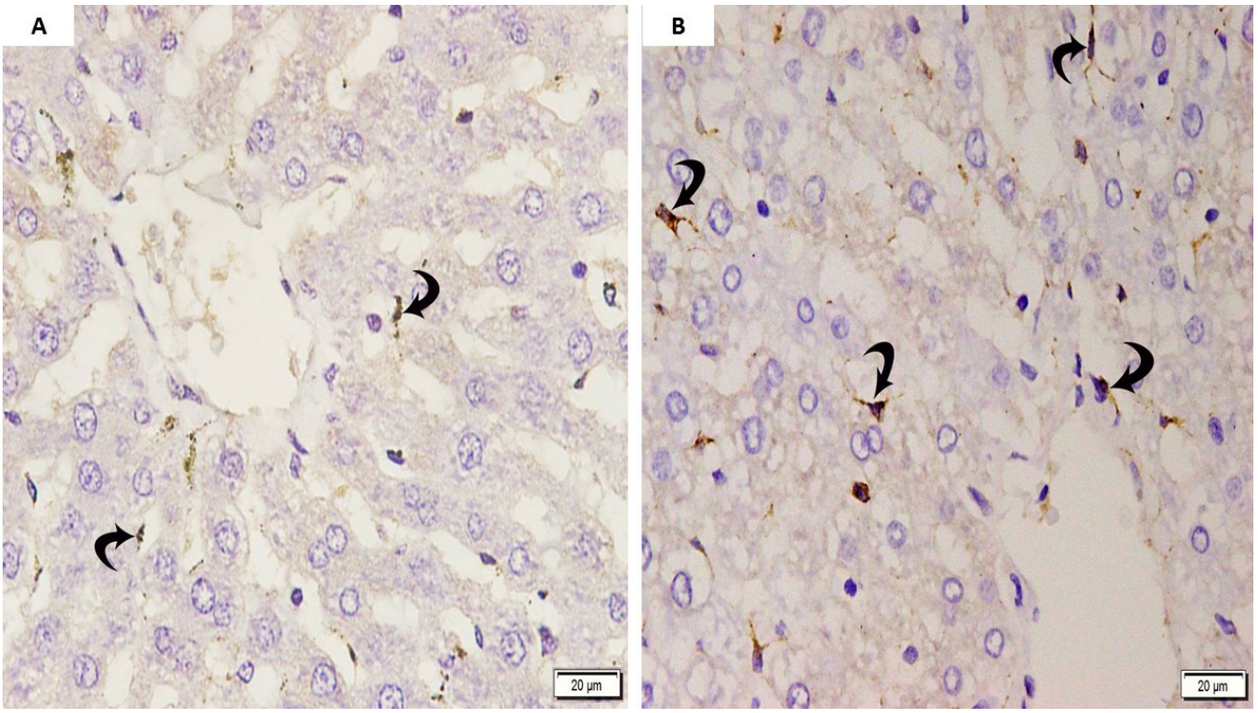

Fig. 9: Photomicrographs of liver sections; A: Control group exhibiting few flattened HSCs with mild immunoreactivity (curved arrows). B: Group II shows strong immunoreactivity in many enlarged HSCs with long processes (curved arrows) (anti-GFAP X400).

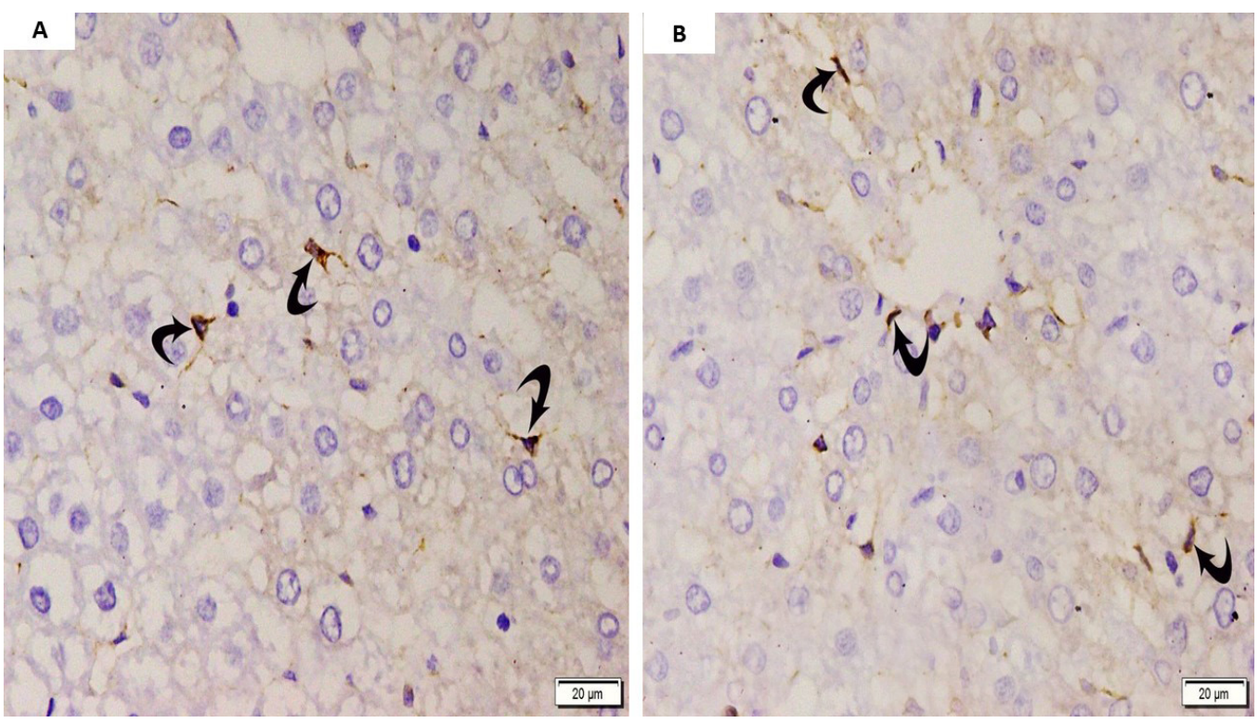

Fig. 10: Photomicrographs of liver sections; A: Group III showing some enlarged HSCs with strong immunoreactivity (curved arrows). B: Group IV shows few flattened HSCs with mild immunoreactivity (curved arrows) (anti-GFAP X400).

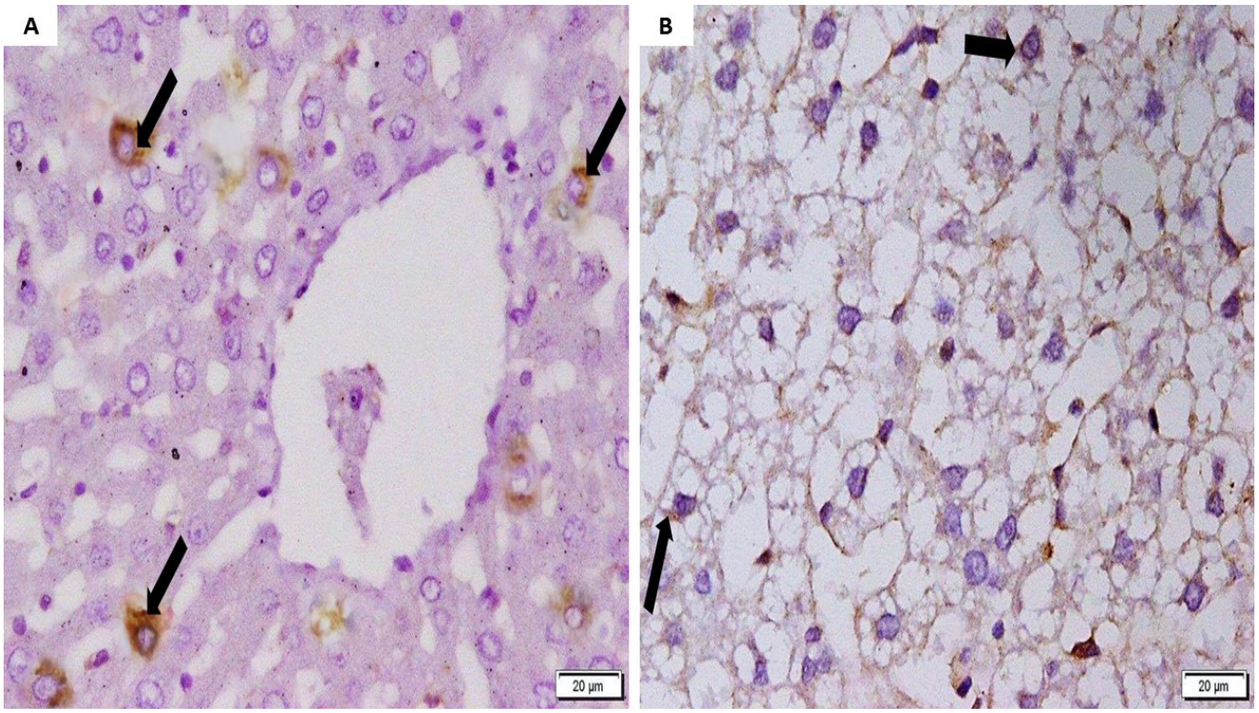

Fig. 11: Photomicrographs of liver sections; A: Control group exhibiting strong immunoreactivity in many hepatocytes (arrows). B: Group II shows weak immunoreactivity in few hepatocytes (arrows) (anti-beclin-1 X400).

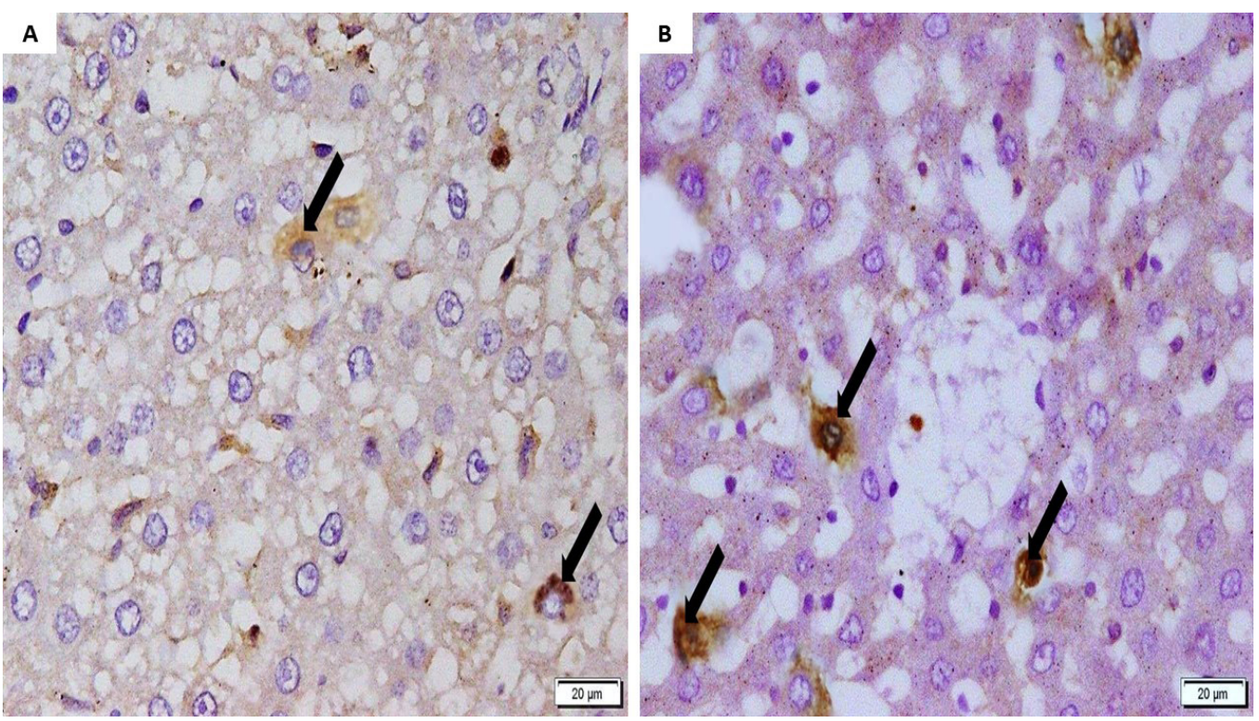

Fig. 12: Photomicrographs of liver sections; A: Group III showing moderate immunoreactivity in some hepatocytes (arrows). B: Group IV shows strong immunoreactivity in many hepatocytes (arrows) (anti-beclin-1 X400).

Table 1: Mean values ± SD of body weight (BW) and liver index in control and experimental groups

Group	BW (g)	Liver index (%)
Group I	189.2 ± 12.7	2.5 ± 0.5
Group II	242.9 ± 27.6[a]	3.9 ± 0.7[a]
Group III	232.2 ± 36.1[b]	3.4 ± 0.4[b]
Group IV	219.2 ± 12.7[d]	2.8 ± 0.1[c]

Liver index calculated as liver weight/body weight × 100%.
a significant increase compared to the other groups.
b significant increase compared to both groups I & IV.
c significant decrease compared to both groups II and III but non-significant compared to group I.
d significant increase compared to group I.

Table 2: Mean values ± SD of the TG, TC, ALT and AST in control and experimental groups

Group	TG (mmol/l)	TC (mmol/l)	ALT (U/l)	AST (U/l)
Group I	0.90±0.10	1.21±0.15	36.4 ± 6.3	37.7 ± 0.6
Group II	1.69±0.39[a]	4.16±0.63[a]	67.2 ± 1.3[a]	76.2 ± 2.02a
Group III	1.31±0.11[b]	3.12±0.68[b]	53.6 ± 1.5[b]	57.2 ± 2.25b
Group IV	1.01±0.09[c]	1.44±0.77[c]	39.1 ± 8.2[c]	42.8 ± 5.4d

a significant increase compared to the other groups.
b significant increase compared to both groups I & IV.
c significant decrease compared to both groups II and III but non-significant compared to group I.
d significant increase compared to group I.

Table 3: Mean values ± SD of the area% of collagen, number of GFAP and Beclin-1immunopositive cells in control and experimental groups

Groups	Area% of collagen	Number of GFAP immune-positive cells	Number of Beclin-1 immune-positive cells
Group I	2.48±2.67	34.81±3.44	15.41±2.25
Group II	6.0±1.24[a]	49.22±2.73[a]	3.61±0.66[d]
Group III	4.3±2.34[b]	42.45±1.92[b]	8.11±1.56[e]
Group IV	2.78±0.70[c]	32.44±2.71[c]	14.62±0.03[f]

a significant increase compared to the other groups.
b significant increase compared to groups I &IV.
c significant decrease compared to both groups II and III but non-significant compared to group I.
d significant decrease compared to the other groups.
e significant decrease compared to groups I &IV.
f significant increase compared to both groups II and III but non-significant compared to group I.

DISCUSSION

World Health Organization (WHO) describe obesity as increased fat accumulation which might affect health. The major cause of obesity is imbalance among calories ingestion and calories spent which results from increased consumption of dense energy food and reduction in bodily activity[36]. Due to the obesity prevalence, non-alcoholic fatty liver disease (NAFLD) has turn out to be the most frequent chronic liver disease[37]. Liver steatosis may lead to steatohepatitis, and finally fibrosis and cirrhosis[38,39]. In this work we studied the effect of HFD on rat liver and the probable protective effect of moringa oleifera (MO) versus simvastatin.

The body weight of rats fed on HFD (group II) was significantly higher as compared to other groups. Similarly, the liver index showed similar increase. This is in accordance with Xu *et al*[40] who noticed an increase in the BW and liver weight of rats on HFD compared to control. The elevated body weight was explained by the increase in adipose tissue and the elevated liver weight was explained by fat accumulation together with inflammation, congestion and collagen deposition in the HFD group, which was demonstrated in our work in the examined liver histological sections.

Group II exhibited a significant increase in the mean values of ALT, AST, TG and TC in comparison to the other groups. This is in agreement with Fontana *et al*[41] who assessed hepatic damage by measuring the activity of liver enzymes in HFD fed rats and found them significantly higher. These elevated plasma TG and cholesterol levels with successive fat accumulation in the liver led to degenerative alterations in hepatocyte, as well as to oxidative stress and eventually inflammation[42,43].

Examination of histological sections of rats' liver fed on HFD (group II) showed marked disruption in the hepatic histologic structure, where most of hepatocytes exhibited vacuolated cytoplasm with pyknotic nuclei, sinusoids were dilated, central veins were congested and the portal vein appeared dilated, congested with interruption of its wall. These findings were in agreement with Altunkaynak[44] who observed hepatocellular steatosis, foci of necrosis, marked inflammation, obvious vascular dilatation and fibrosis. Some researchers suggested that inflammation occurs as a result to hepatocytes damage due to fat deposition and oxidative stress in HFD, leading to cytokine release. These inflammatory cytokines include Interleukin-1-beta (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α), Transforming growth factor beta-1 (TGF-β), and nuclear factor (NF)-κB pathway proteins and monocyte chemoattractant protein (MCP)[45]. Catalase, superoxide dismutase (SOD) and reduced glutathione (GSH) are natural anti-oxidant that reduce oxidative stress. Increase oxidative stress due to lipid peroxidation, leads to decrease catalase and SOD activities, indicating reduced tissue anti-oxidant defenses in rats on HFD[46]. Sinusoids and portal veins dilatation resulted from inflammatory changes following HFD. Again, hypertension related to obesity might be a cause[47].

Liver fibrosis is associated with various liver chronic diseases. The chief cell type accountable for fibrogenesis is the hepatic stellate cells (HSCs). As result of inflammation or liver cells death, HSCs go through transformation to myofibroblast-like cells[48]. Liver fibrosis in this study was confirmed by Massons Trichrome stain, where significant increase in collagen mean area %, was observed in group II in comparison to other groups. This hepatic fibrosis was further established by detection of HSCs with GFAP immunohistochemical staining which exhibited a significant rise in GFAP immunopositive cells in group II as compared to other groups. HSCs are found in the space

of Disse (peri-sinusoidal space). They are surrounded by both the hepatocytes and the endothelial cells of the sinusoids[49].

These findings could be clarified by Lee *et al*[46] who stated that the inflammatory cytokines particularly TGF-β is a key stimulator of fibroblasts and a mediator of fibrosis in the liver tissue. Furthermore, Sandulescu *et al*[50] & Carotti *et al*[51] added that cytokines produced by injured hepatocytes HSCs are transformed to myofibroblasts which are fibrogenic. Glial fibrillary acidic protein (GFAP) is a member of intermediate filaments which sustains cell's mechanical structure and strength. It was first considered in astroglial cells. It has been recognized that GFAP could present a valuable marker of early HSCs stimulation than α-SMA GFAP in chronic as well as in post-transplant recurring hepatitis[51].

Autophagy, a lysosomal-degradative pathway which works to encourage survival of cell by energy supplying during the stress or by eliminating damaged proteins and organelles following damage. The contribution of autophagy in the pathogenesis NAFLD was primarily proposed by the finding that this pathway facilitates the catabolism of lipids in liver cells and so might adjust the progress of liver steatosis[52]. Beclin 1 is one of the autophagy linked proteins[53]. In this work autophagy was assessed by beclin 1 immunostaining, revealed a significant decrease in beclin 1 immunopositive cells in group II compared to other groups. This is concomitant with Kwanten *et al*[54] who stated that autophagy has a role in NAFLD pathology, where the autophagic role is diminished in the fatty liver.

In rats given simvastatin with HFD (group III), there was a significant decrease in body weight and liver index as compared to group II. This was explained by the protective role of simvastatin in HFD fed rats which was observed by many authors who found that administration of simvastatin significantly inhibited the excessive weight gain and the increased liver weight caused by HFD[55].

Regarding liver enzymes (ALT and AST), TG and TC, group III showed a significant decrease in their levels as compared to group II. These findings consequence as statins inhibit enzyme responsible for cholesterol synthesis and are extensively prescribed as cholesterol-lowering drugs[56]. However, though this protective role of simvastatin, Nelson *et al*[57] stated that simvastatin monotherapy seems not to be an effective for NASH.

By LM examination, group III showed moderate improvement in the hepatic tissue structure as some hepatocytes appeared vacuolated and with pyknotic nuclei. Also, some blood sinusoids appeared dilated, central veins appeared congested and the portal vein was dilated. This improvement with simvastatin is caused by reduced lipid deposition in hepatocytes, ameliorated the progressive hepatic steatosis, inflammation, and fibrosis induced by high-fat diet[58].

Group III in this study revealed a significant decrease in collagen fibers mean area % and GFAP immunopositive cells as compared to group II. This beneficial role of simvastatin is explained by reducing plasma lipids, decreasing lipid droplets accumulation in hepatocytes. Simvastatin protects damage from oxidative stress from dyslipidemia, inhibits free radicals synthesis and lessens the lipid peroxidation and oxidative stress induced by HFD, with consequent reduction of inflammation and fibrosis[59]. Moreover, Trebicka *et al*[60] added, statins can diminish hepatic inflammatory reactions and avoid hepatic fibrosis by inactivation of HSCs.

Group III revealed a significant increase in beclin 1 immunopositive cells as compared to group II. This was in accordance with Gu *et al*[61] who mentioned that simvastatin brought autophagic pathway in asthma mouse models.

In this study, MO presented a protective effect superior to simvastatin as group IV. It exhibited a significant decrease in body weight and liver index as compared to group III. Our findings were in agreement with Akinduko *et al*[62] who noticed body weight and liver weight were lower in MO group when compared to HFD group.

The liver enzymes (ALT & AST), TG and TC levels were significantly low in group IV as compared to group III. These results signifying the role of MO in avoiding liver damage resulted from HFD[63]. Almatrafi *et al*[64] reported reduction of these liver enzymes and documented a recovery from liver damage with MO intake, showing the protective properties of MO in liver. MO has significant concentrations of quercetin which is a flavonoid shown to reduce TG synthesis[65]. MO significantly diminished total cholesterol and LDL levels and elevate the level of HDL in rats fed with HFD. This indicates that MO have a hypolipidemic effect, resulting in a decrease in hepatic fat deposition[66].

Group IV revealed preservation of the histological structure of the hepatic tissue with apparently normal blood sinusoid and central veins. MO is a rich source of antioxidant, mainly polyphenols. Polyphenols has been recognized to exert potent antioxidant effect. They prevent lipid peroxidation by acting as free radicals scavengers. MO also has anti-inflammatory properties. Treatment with MO demonstrates a decrease in fat deposition, inflammation, congestion and fibrosis. All these findings link with the hepatoprotective, antioxidant and anti-inflammatory properties of MO[67].

Regarding fibrosis, MO shown an anti-fibrotic effect greater to that of simvastatin, as group IV showed a significant reduction in the mean area % of collagen fibers and GFAP immunopositive cells as compared to group III. This was documented by Aly *et al*[68] who reported hepatoprotective effect of MO against inflammation and fibrosis by decreasing TNF-α and TGF-β levels. TNF-α stimulates NF-KB which activates fibrogenic molecule TGF-β and activates the survival and synthesis of stimulated myofibroblasts by differentiation of HSCs.

The stimulation of autophagy is considered a promising approach to prevent lipid accumulation in the liver[69]. Group IV in this study showed a significant rise in beclin 1 immunopositive cells as compared to group III. This was in accordance with Liu *et al*[70] who reported stimulation of autophagy and inhibition of hepatic steatosis in obese animals after exposure to quercetin that is found in MO.

CONCLUSION

This study could suggest potential protective effects of MO and simvastatin which could lead to preservation of the histological and biochemical markers of liver in the HFD-induced liver steatosis in rats. MO protective role was superior to that of simvastatin. We recommend increased awareness of people about the beneficial effects of MO especially in obese individuals.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Popkin BM and Doak CM: The obesity epidemic is a worldwide phenomenon in Nutr. Rev. (1998) 56: 106–114.
2. Spiegelman BM and Flier JSL: Obesity and the regulation of energy balance in Cell. (2001) 104: 531–543.
3. Kopelman PG: Obesity as a medical problem in Nature. (2000) 404 (6778): 635–643.
4. Lichtenstein AH, Kennedy E, Barrier P *et al*: Dietary fat consumption and health in Nutr. Rev. (1998) 56: 3–19.
5. Panico S and Iannuzzi A: Dietary fat composition and the metabolic syndrome in European Journal of Lipid Science and Technology. (2004) 106 (1): 61–67.
6. Grundy SM: Atherogenic dyslipidemia associated with metabolic syndrome and insulin resistance in Clin. Cornerstone. (2006) 8 (1): 21–27.
7. Pi-Sunyer FX: The medical risks of obesity in Obes Surg. (2002) 12: 6-11.
8. Azimi A, Charlot C, Torp-Pedersen *et al*.: Moderate overweight is beneficial and severe obesity detrimental for patients with documented atherosclerotic heart disease in Heart. (2013) 99 (9): 655–660.
9. Nath D, Heemels MT and Anson L: Obesity and diabetes in Nature. (2006) 444 (7121) 839-848.
10. Dorresteijn JAN, Visseren FLJ and Spiering W: Mechanisms linking obesity to hypertension in Obesity Reviews. (2012) 13(1): 17-26.
11. Dowman JK, Tomlinson JW and Newsome PN: Pathogenesis of non-alcoholic fatty liver disease in Q J Med. (2010) 103: 71–83.
12. Miller M, Dolina C, Cromwell W and Otvos JD: Effectiveness of high doses of simvastatin as monotherapy in mixed hyperlipidemia in Am. J. Cardiol. (2001) 87 (2): 232–234.
13. Krysiak R, Żmuda W and Okopień B: The effect of short-term simvastatin trea tment on plasma adipokine levels in patients with isolated hypercholesterolemia: a preliminary report in Pharmacol. Rep. (2014) 66 (5): 880–884.
14. Harisa GI, Alomrani AH and Badran MM: Simvastatin-loaded nanostructured lipid carriers attenuate the atherogenic risk of erythrocytes in hyperlipidemic rats in Eur. J. Pharm. Sci. (2017) 96: 62–71.
15. Khan T, Hamilton MP, Mundy DI, Chua SC and Scherer PE: Impact of simvastatin on adipose tissue: pleiotropic effects in *vivo* in Endocrinology. (2009) 150: 5262-5272.
16. Zafra C, Abraldes JG, Turnes J, Berzigotti A, Fernández M *et al*.: Simvastatin enhances hepatic nitric oxide production and decreases the hepatic vascular tone in patients with cirrhosis in Gastroenterology. (2004) 126: 749-755.
17. Dima A, Marinescu AG and Dima AC: Non-alcoholic fatty liver disease and the statins treatment in Rom J Intern Med. (2012) 50: 19-25.
18. Gopalakrishnanb L, Doriyaa K and Kumara DS: Moringa oleifera: A review on nutritive importance and its medicinal application in Food Science and Human Wellness. (2016) 5: 49–56.
19. Mbikay M: Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review in Front Ethnopharmacol. (2012) 3: 1–12.
20. Abdull Razis AF, Ibrahim MD and Kntayya SB: Health Benefits of Moringa oleifera in Asian Pac J Cancer Prev. (2014) 15 (20): 8571-8576.
21. Fahey JW: Moringa oleifera: A review of the medicinal evidence for its nutritional, therapeutic, and prophylactic properties. Part 1in Trees Life J. (2005). 1, 5.
22. Verma AR, Vijayakumar M, Mathela CS *et al*.: In *vitro* and in *vivo* antioxidant properties of different fraction of Moringa oleifera leaves in Food and Chem Toxicol. (2009) 47: 2196-2201.
23. Ramachandran C, Peter KV, and Gopalakrishnan PK: Drumstick (Moringa oleifera): A multipurpose Indian vegetable in Economic Botany. (1980) 34 (3): 276–283.
24. Kumar SP, Mishra D, Ghosh G, and Panda CS: Medicinal uses and pharmacological properties of Moringa oleifera in International Journal of Phytomedicine. (2010) 2 (3): 210–216.

25. Anwar F, Latif S, Ashraf M and Gilani AH: Moringa oleifera: A food plant with multiple medicinal uses in Phytother Res. (2007) 21:17-25.
26. Hamza AA: Ameliorative effects of Moringa oleifera Lam ameliorative effects of Moringa Oliefera lam ssed extract on liver fibrosis in rats in Food Chem Toxicol. (2010) 48: 345-55.
27. Woods SC, Seeley RJ, Rushing PA, D'Alessio D and Tso P: A Controlled High-Fat Diet Induces an Obese Syndrome in Rats in J. Nutr. (2003) 133: 1081–1087.
28. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A and Lev-Ari S: Moringa Oleifera aqueous leaf extract down-regulates nuclear factor-kappa B and increases cytotoxic effect of chemotherapy in pancreatic cancer cells in Complement Altern Med J. (2013) 13: 212–219.
29. He X, Zheng N, He J, Liu C, Feng J, Jia W and Li H: Gut microbiota modulation attenuated the hypolipidemic effect of simvastatin in high-fat/cholesterol-diet fed mice in J. Proteome Res. (2017) 16 (5): 1900–1910.
30. Jaiswal D, Kumar Rai P, Kumar A, Mehta S and Watal G: Effect of Moringa oleifera Lam. leaves aqueous extract therapy on hyperglycemic rats in J Ethnopharmacol. (2009) 123: 392–396.
31. Wellington D, Mikaelian I and Singer L: Comparison of ketamine-xylazine and ketamine-dexmedetomidine anesthesia and intraperitoneal tolerance in rats in J Am Assoc Lab Anim Sci. (2013) 52(4): 481-487.
32. Kiernan JK (2001): Histological and Histochemical Methods. In: Theory and practice. 3rd edition, Arnold Publisher, London, New York, and New Delhy: 111-162.
33. Bancroft J, Gamble M. 7th ed. Edinburgh, London, Madrid, Melbourne, New York, Tokyo: Churchill Livingstone; 2008. Staining methods. Theory and Practice of Histological Techniques; pp. 121–35. 263-325.
34. Suvarna SK, Layton C, Bancroft JD. 7th ed. New York, USA: Elsevier Health Sciences, Churchill Livingstone; 2012. Bancroft's Theory and Practice of Histological Techniques; pp. 215–39.
35. Emsley R, Dunn G and White IR: Mediation and moderation of treatment effects in randomized controlled trials of complex interventions in Start Methods Med Res. (2010)19(3): 237-270.
36. Hill JO, Wyatt HR and Peters JC: Energy balance and obesity in Circulation. (2012) 126: 126–132.
37. Vajro P, Lenta S, Socha P *et al*.: Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee in J Pediatr Gastroenterol Nutr. (2012) 54 (5): 700- 713.
38. Abu-Abid S, Szold A and Klausner J: Obesity and cancer in J Med. (2002) 33(1-4): 73-86.
39. Adams LA, Sanderson S, Lindor KD and Angulo P: The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies in Hepatol. (2005) 42(1):132-138.
40. Xu JZ, Fan JG, Ding XD, Qiao L, Wang GL.: Characterization of high- fat, diet induced, non-alcoholic steatohepatitis with fibrosis in rats in Dig Dis Sci. (2010) 55(4): 931–940.
41. Fontana L, Zhao E, Amir M, Dong H, Tanaka K and Czaja MJ: Aging promotes the development of diet-induced murine steatohepatitis but not steatosis in Hepatology. (2013) 57: 995-1004.
42. Rezq AA and El-Khamisy AE: Hypolipideimic and hypocholestermic effect of pine nuts in rats fed high fat, cholesterol-diet in World in Appl Sci J. (2011) 15(12):1667–1677.
43. Tapply L, Schneiter Ph, Bortolotti M and Le KA.: Hepatic li¬potoxicity: modulation by nutrients. 18th European Congress on Obesity Proceedings Book, pp:10. 18th European Congress on Obesity, Istanbul, Turkey, 25-28 May 2011.
44. Altunkaynak Z: Effects of high fat diet induced obesity on female rat livers (a histochemical study) in Eur J Gen Med. (2005) 2(3):100-109.
45. Hajiluian GH, Abbasalizad-Farhangi M, Nameni G, Shahabi P and Megari-Abbasi M: Oxidative stress-induced cognitive impairment in obesity can be reversed by vitamin D administration in rats in Nutr Neurosci. (2018) 21(10):744-752.
46. Lee JH, Son CW, Kim MY, Kim MH, Kim HR, Kwak ES, Kim SA and Kim MR: Red beet (Beta vulgaris L.) leaf supplementation improves antioxidant status in C57BL/6J mice fed high fat high cholesterol diet in Nutr. Res. Pract. (2009) 3:114–121.
47. Hassan NF, Soliman GM, Okasha EF and Shalaby AM: Histological, Immunohistochemical, and Biochemical Study of Experimentally Induced Fatty Liver in Adult Male Albino Rat and the Possible Protective Role of Pomegranate in J Microsc Ultrastruct. (2018) 6(1):44-55.
48. Khomich O, Ivanov A and Bartosch B: Metabolic hallmarks of hepatic stellate cells in liver fibrosis in Cells. (2020) 9(1):1- 24.
49. Marrone G, Shah VH and Gracia-Sancho J.: Sinusoidal communication in liver fibrosis and regeneration in J Hepatol. (2016) 65: 608–617.
50. Sandulescu L, Rogoveanu I, Ciurea T, Comănescu MV, Streba C, Ionescu A *et al*.: Immunohistochemical study of stellate cells in patients with chronic viral hepatitis C genotype 1 in Rom J Morphol Embryol. (2011) 52(1):137–143.

51. Carotti S, Morini S, Corradini SG, Burza MA, Molinaro A, Carpino G, Merli M, De Santis A, Muda AO, Rossi M, Attili AF and Gaudio E: Glial fibrillary acidic protein as an early marker of hepatic stellate cell activation in chronic and posttransplant recurrent hepatitis C in Liver Transpl. (2008) 14(6):806-14.
52. Czaja MJ: Function of Autophagy in Nonalcoholic Fatty Liver Disease in Dig Dis Sci. (2016) 61: 1304-1313.
53. Oh SY, Choi SJ, Kim KH *et al*.: Autophagy-Related Proteins, LC3 and Beclin-1, in Placentas from Pregnancies Complicated by Preeclampsia in Reprod Sci. (2008) 15: 912–920.
54. Kwanten WJ, Martinet W, Michielsen PP and Francque SM: Role of autophagy in the pathophysiology of nonalcoholic fatty liver disease: a controversial issue in World J Gastroenterol. (2014) 20(23):7325-7338.
55. Zhang Q, Fan X, Ye R, Hu Y, Zheng T, Rui Shi R, Cheng W, Lv X, Chen L and Liang P: The Effect of Simvastatin on Gut Microbiota and Lipid Metabolism in Hyperlipidemic Rats Induced by a High-Fat Diet in Front Pharmacol. (2020) 11 (522): 1-9.
56. Park HS, Jang JE, Ko MS, Woo SH, Kim BJ, Kim HS, Park HS, Park IS, Koh EH and Lee KU: Statins Increase Mitochondrial and Peroxisomal Fatty Acid Oxidation in the Liver and Prevent Non-Alcoholic Steatohepatitis in Mice in Diabetes & Metabolism Journal. (2016) 40(5): 376-385.
57. Nelson A, Torres DM, Morgan AE, Fincke C and Harrison SA: A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: A randomized placebo-controlled trial in J Clin Gastroenterol. (2009) 43: 990-994.
58. Zhang B, Zhang C, Zhang X, Li N, Dong Z, Sun G and Sun X: Atorvastatin promotes AMPK signaling to protect against high fat diet induced non alcoholic fatty liver in golden hamsters in Exp Ther Med. (2020) 19(3): 2133-2142.
59. Chen YA, Lin Y J, Lin CL, Lin H, Wu HS, Hsu HY *et al*.: Simvastatin therapy for drug repositioning to reduce the risk of prostate cancer mortality in patients with hyperlipidemia in Front. Pharmacol. (2018) 9 (225): 1-7.
60. Trebicka J, Hennenberg M, Odenthal M, Shir K, Klein S, Granzow M, Vogt A, Dienes HP, Lammert F, Reichen J, Heller J and Sauerbruch T: Atorvastatin attenuates hepatic fibrosis in rats after bile duct ligation via decreased turnover of hepatic stellate cells in J Hepatol. (2010) 53:702–712.
61. Gu W, Cui R, Ding T, Li X, Peng J, Xu W, Guo X: Simvastatin alleviates airway inflammation and remodelling through up-regulation of autophagy in mouse models of asthma in Respirology. (2017) 22 (3): 533-541.
62. Rizvi S Z H, Shah F A, Khan N, Muhammad I, Ali K H, Ansari MM *et al*.: Simvastatin-loaded solid lipid nanoparticles for enhanced antihyperlipidemic activity in hyperlipidemia animal model in Int. J. Pharm. (2019) 560: 136–143.
63. Michael OT, John OO, Maryam MA and Tosin AJ: Hypolipidemic effect of moringa oleifera seed oil on high fat-diet induced hyperlipidemia in liver and heart of albino rats in Mintage Journal of Pharmaceutical & Medical Sciences. (2018) Vol 7, Suppl 1.
64. Almatrafi MM, Vergara-Jimenez M, Murillo AG, Norris GH, Blesso CN and Fernandez ML: Moringa Leaves Prevent Hepatic Lipid Accumulation and Inflammation in Guinea Pigs by Reducing the Expression of Genes Involved in Lipid Metabolism in Int J Mol Sci. (2017) 18(7):1330-1339.
65. Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, Bartsch H and Owen RW: Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of Moringa oleifera Lam in J Med Food. (2010) 13(3):710-716.
66. Asgari-Kafrani A, Fazilati M and Nazem H: New R Hepatoprotective and antioxidant activity of aerial parts of Moringa oleifera in prevention on non-alcoholic fatty liver diseases in Wistar rats in S. Afr. J. Bot. (2020) 129: 82-90.
67. Akinduko D S, Olatosin T M, Uche CZ and Bardi J: Effects of Moringa oleifera Seed Oil on Acetaminophen-Induced Oxidative Stress and Liver Damage in Wistar Albino Rats in IOSR Journal of Pharmacy and Biological Sciences. (2014) 9(2): 53-59.
68. Aly O, Abouelfadl, D.M., Shaker, O.G. *et al*.: Hepatoprotective effect of Moringa oleifera extract on TNF-α and TGF-β expression in acetaminophen-induced liver fibrosis in rats in Egypt J Med Hum Genet. (2020) 21: 69-78.
69. Huang Q, Wang T, Yang L, Wang HY.: Ginsenoside Rb2 alleviates hepatic lipid accumulation by restoring autophagy via induction of Sirt1 and activation of AMPK in Int J Mol Sci. (2017) 18(5): E1063.
70. Liu L, Gao C, Yao P and Gong Z.: Quercetin alleviates high-fat diet-induced oxidized low-density lipoprotein accumulation in the liver: implication for autophagy regulation in Biomed Res Int. (2015) 607531.

الملخص العربى

المورينجا أوليفيرا مقابل السيمفاستاتين على التنكس الدهني للكبد في نموذج الفئران الذكور البالغين للنظام الغذائي عالي الدهون ؛ دراسة هستولوجية وكيميائية مناعية

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مقدمة البحث: السمنة مشكلة صحية كبرى في جميع أنحاء العالم. النظام الغذائي الدهني هو عامل مهم يسبب السمنة و يؤدي أيضًا إلى حدوث تنكس دهني بالكبد والتهاب الكبد الدهني. كثيرا ما تستخدم أوراق المورينجا أوليفيرا للأغراض الغذائية والطبية لعلاج العديد من الأمراض و كذلك أظهرت أيضًا حماية للكبد.

الهدف من البحث: دراسة آثار النظام الغذائي الغني بالدهون على كبد الفئران وتقييم الدور الوقائي المحتمل لمستخلص أوراق المورينجا أوليفيرا ، ومقارنتها بالدور الوقائي لسيمفاستاتين.

مواد و طرق البحث: تم استخدام أربعين ذكور فأر بالغ في هذه الدراسة. تم تقسيم الحيوانات بشكل عشوائي إلى أربع مجموعات (١٠ لكل مجموعة): المجموعة ١: المجموعة الضابطة: تلقت نظامًا غذائيًا قياسيًا لمدة ثمانية أسابيع ؛ المجموعة ٢: مجموعة الغذاء الغني بالدهون: تلقت غذاء غني بالدهون لمدة ثمانية أسابيع ؛ المجموعة ٣: المجموعة المعالجة بـالسيمفاستاتين: تلقت سيمفاستاتين مع غذاء الغني بالدهون؛ المجموعة ٤: المجموعة المعالجة بمستخلص أوراق المورينجا أوليفيرا: تلقت مستخلص أوراق المورينجا أوليفيرا بالإضافة إلى غذاء غني بالدهون. بحلول نهاية الأسبوع الثامن تم وزن الفئران ثم التضحية بها. تمت إزالة الكبد ووزنه وفحصه من الناحية النسيجية باستخدام صبغة الهيماتوكسيلين والأيوزين وكذلك صبغة ماسون ثلاثية الألوان. تم أيضًا إجراء صبغة كيميائية مناعية باستخدام تقنية أفيدين-بيوتين بيروكسيديز للأجسام المضادة للبروتين الليفي الحمضي (GFAP) والجسم المضاد المضاد لـ Beclin1.

نتائج البحث: تمت زيادة وزن الجسم ووزن الكبد للفئران التي تلقت غذاء غني بالدهون (المجموعة ٢). كذلك تأثرت البنية الهستولوجية للكبد و إرتفع مستوى إنزيمات الكبد و نسبة الدهون في الدم بشكل ملحوظ. سيمفاستاتين في المجموعة ٣ خفف جميع المتغيرات المدروسة. الدور الوقائي لمستخلص أوراق المورينجا أوليفيرا في المجموعة ٤ كان واضحا، بل إنه تجاوز الدور الوقائي للسيمفاستاتين.

الخلاصة: كان التأثير الوقائي لمستخلص أوراق المورينجا أوليفيرا واضح و لذلك نوصي بزيادة وعي الناس حول تأثيره المفيد خاصة عند الأشخاص الذين يعانون من زيادة الوزن والسمنة.