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Original Article

Metformin Versus Losartan: Prevention of Non-Alcoholic Fatty Liver Disease in Adult Albino Rats, An Immunohistochemical Study

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is considered a worldwide epidemic defined as the accumulation of excessive fat in the liver in the absence of excessive drinking of alcohol. Its prevalence in the Middle East ranges between 20 and 30 %.

Aim of the work: We designed this study to evaluate the preventive effect of both metformin and losartan on NAFLD in rats.

Material and Methods: We induced NAFLD by high fat diet and fructose in drinking water for 8 weeks. Twenty four rats were used in this study and randomly divided into 4 groups; control group, high fat diet group, high fat diet with metformin group and high fat diet with losartan group. Blood samples were used for detection of liver enzymes and lipid profile assessment. Paraffin and frozen liver sections were done.

Results: Metformin and losartan significantly prevented the increase in the serum levels of liver enzymes and the lipid profile induced by NAFLD in rats. Also, they partially preserved the hepatic architecture.

Recommendation: Combination of both drugs (metformin and losartan) should be tested for better biochemical and histopathological results.

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Key Words: Fatty liver, losartan, metformin.

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INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is a metabolic disorder that includes a wide range of clinicopathological conditions starting from hepatic steatosis to steatohepatitis, then progress to fibrosis and cirrhosis[1]. Obesity is one of the essential risk factors for NAFLD development, which may cause insulin resistance and metabolic syndrome[2].

Metformin is considered the first drug treating type 2 diabetes, as it rasises insulin sensitivity by upregulating AMP activated protein kinase (AMPK) which in turn reduce production of hepatic glucose. It also suppresses glucose-6-phosphatase expression and inhibits mitochondrial oxidative phosphorylation, thus it has a major role in metabolism of glucose and lipid in the liver[3,4].

In vitro studies suggest that AMPK inhibit proliferation and activation of hepatic stellate cells by supressing Akt, inducing antioxidant enzymes, and blocking the cell cycle[5,6].

Losartan is selective Angiotensin II receptor blocker (ARBs) type 1. As an antihypertensive agent, it has been expected to be effective for treatment of NAFLD. As it suppresses the renin-angiotensin system (RAS) which play a role in the mechanisms of insulin resistance and is involved in the pathways of liver damage[7]. Moreover, losartan has been reported to decrease the number of activated hepatic stellate cells, which play

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a essential role in the progression of hepatic fibrosis[8].

MATERIAL AND METHODS

Animals used:

Twenty four Sprague-Dawley rats, weighing 150 – 250 gm were used in this study. They were obtained from Mansoura University Research Center, Mansoura, Egypt. They were housed in meshed stainless-steel cages under control conditions of temperature (23°C±3), and relative humidity. The rats were permitted free access to standard commercial diet and water ad libitum along with fixed 12h light/dark cycle throughout acclimatization and experimental periods. All rats were kept under specific pathogen-free conditions. All the experiments were done according to the rules and regulations lay down by the committee on animals' experimentation of Mansoura University.

Experimental design

Two weeks after acclimatization, the animals were divided into 4 groups; Group 1 (control group) (6 rats): were fed on a basal diet only and sacrificed after 8 weeks. Group 2 (NAFLD model) (6 rats): were fed on high-fat diet (20 mg butter/100 gm diet) and significant amount of fructose in drinking water[9], and were sacrificed after 8 weeks. Group 3 (Metformin prevention) (Met-P) (6 rats): were fed on high fat diet (50% fat) and significant amounts of fructose in drinking water. Metformin (October Pharma, Egypt) (300 mg/kg/day via gastric tube)[10] was administered from the first day. They were sacrificed after 8 weeks. Group 4 (Losartan prevention) (Los-P) (6 rats): were fed on high-fat diet (50% fat) and significant amounts of fructose in drinking water. Losartan (Pharaonia, Egypt) (10 mg/kg/day via gastric tube)[11] was administered from the first day. They were sacrificed after 8 weeks. All the 24 rats were weighed at time of scarification and subjected to laboratory, histopathological and immuno-histochemical study.

Sacrifice of rats and specimens collection

At the exact times, all rats (fasting for eight hours) were anaesthetized and blood samples were collected for assessment of liver enzymes, lipid profile, and serum glucose and serum insulin. Then, scarification of rats was done. The livers were rapidly removed and its absolute weight was recorded. Parts of the livers were processed as tissue homogenate for quantitative analysis of malondialdhyde and reduced glutathione. Other parts were dissected and kept frozen for oil red o staining and the remaining parts were fixed for histopathology and processed for light microscopic examination.

Biochemical studies

The serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) were assayed according to the routine biochemical analysis system using clinical test kits (Elitech, UK) spectrophotometrically. Serum lipids; triglycerides (TG) and total cholesterol (TC) were measured by an automated analyzer (Dade Behring RXL, Deerfield, IL, USA)[12]. Xtra Plus test strips and an Optium Xceed device (Abbott Diabetes Care, Ltd., Maidenhead, UK)[13] was used to measure fasting blood Glucose levels with Precision. Hepatic malondialdehyde (MDA) was determined using a colorimetric assay for lipid peroxidation (Bioxytech, Portland)[14]. Glutathione (GSH) activity was assessed by commercial kit from Jiancheng Biological Engineering Institute following the manufacturer protocol. The reaction of GSH was read at 420 nm and the activity of enzyme was calculated as mg/g protein[15].

Histological techniques

a) Paraffin sections

The specimens were prepared for paraffin sectioning then cut using microtome at thickness of 5-6 μm pieces and mounted on the slide for staining. Then tissues were stained with H&E[16] and Sirius red[17] stains for histopathological examination & immunostaining for tumour necrosis factor alpha (TNF α) antibody[18].

b) Frozen sections

Immediately after sacrification, the liver was removed and a part of it was immediately frozen. Frozen sections were cut at 8 to10 μm, air dried then fixed in formalin and stained with oil red O stain[19].

Morphometric analysis

Analysis of the images stained with oil red O[20] (fat area), sirius red (collagen area) and TNF-α[21] were done by Computer Assisted digital image analysis (Digital morphometric study) using Image-Pro Plus 4.5 for Windows with a specific built-in routine stain quantification and area measurement. Six slides from each group were prepared, 10 consecutive microscopic fields (×100 magnification) from each slide were analyzed.

Statistical analysis

Data were analyzed using the computer program SPSS (Statistical package for social science) version 22.0. The significance of difference between the different groups was tested using analysis of variance (ANOVA) to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey for multiple comparisons.

RESULTS

Assessment of the body weight and liver weight (Table 1)

There was significant progressive increase in both body and the liver weight 8 weeks after HFD compared with the control group. Administration of metformin or losartan with the HFD from the start (Met-P & Los-P) significantly prevented the marked increase in both body and liver weight compared to NAFLD group. However, the liver weight of both Met-P & Los-P groups was significantly increased compared to control group. No significant difference was observed in the liver weight between Met-p & Los-P groups. However, there was significant difference in the body weight between the both groups.

Table 1: Body and liver weight in different groups sacrificed at 8 weeks

	CONTOL	NAFLD	Met-P	Los-P
Body weight	198.49± 11.25	258.43 ± 15.43*	205.71± 7.86#	222.31± 7.5*'#'$
Liver weight	7.33 ± 0.47	11.08 ± 1.27*	8.26 ± 0.48*'#	8.05 ± 0.68*'#

The data is presented as mean ± standard deviation.
P > 0.05 = non-significant, *P < 0.05* = significant
- * = significant as compared to control
- # = significant as compared to NAFLD
- $ = significant as compared to Met-P

Biochemical assay

Assessment of the serum levels of liver enzymes (ALT & AST) and lipid profile (TC & TG) (Table 2)

Administration of HFD for 8 weeks significantly increased the serum levels of ALT, AST, TC and TG when compared with control animals. (Met-P & Los-P) significantly prevented the increase in the serum levels of liver enzymes (AST & ALT) and the lipid profile (TG & TC) compared to NAFLD group. Metformin succeeded to normalize the serum level of ALT and AST while losartan succeeded to normalize the serum level of TG. And both drugs succeeded to normalize TC.

Table 2: serum ALT, AST TC and TG levels in different groups sacrificed at 8 weeks

	CONTOL	NAFLD	Met-P	Los-P
ALT	54.15 ± 7.53	105.17 ± 11.02*	59.60 ± 3.87#	62.26 ± 4.93*'#
AST	86.45± 5.92	141.6± 11.77*	88.22± 2.69#	90.88± 4.88*'#
TC	82.10 ± 2.96	139.45 ± 9.57*	91.98 ± 6.73#	87.29 ± 6.93#
TG	58.14 ± 2.26	83.28 ± 9.17*	70.34 ± 6.33*'#	63.53 ± 8.65#

The data is presented as mean ± standard deviation.
P > 0.05 = non-significant, *P < 0.05* = significant
- * = significant as compared to control
- # = significant as compared to NAFLD
- $ = significant as compared to Met-P

Assessment of the levels of FBG (Table 3)

Administration of HFD for 8 weeks significantly increased the serum level of FBG when compared to control animals. Administration of each of metformin or losartan (Met-P & Los-P) significantly decreased the level of FBG compared to NAFLD group. However, there was significant elevation in Los-P group compared to control group. Notably, metformin significantly prevented the elevation compared to Los-P group.

Table 3: Fasting Blood Glucose concentration in different groups sacrificed at 8 weeks

	CONTOL	NAFLD	Met-P	Los-P
FBG	100.13 ± 9.07	139.16 ± 13.58*	105.80 ± 4.01#	113.81± 7.71*'#'$

The data is presented as mean ± standard deviation.
P > 0.05 = non-significant, *P < 0.05* = significant
- * = significant as compared to control
- # = significant as compared to NAFLD
- $ = significant as compared to Met-P

Assessment of the oxidative stress markers (MDA & GSH) (Table 4)

Administration of HFD for 8 weeks significantly increased hepatic MDA; significantly declined the hepatic GSH compared to the control rats. Administration of metformin or losartan with the HFD from the start (Met-P & Los-P) significantly decreased the hepatic level of MDA and significantly increased the hepatic level of GSH compared to NAFLD group. On the other hand, both drugs failed to normalize the hepatic levels of MDA and GSH compared to control group. Nevertheless, no significant difference between both drugs on the hepatic levels of both oxidative stress markers was observed.

Table 4: Hepatic MDA and GSH concentration in different groups sacrificed at 8 weeks

	CONTOL	NAFLD	Met-P	Los-P
MDA	75.66 ± 4.28	160.95 ± 11.92*	84.36 ± 4.02*'#	83.99 ± 6.44*'#
GSH	162.05 ± 10.90	89.87 ± 10.05*	140.43 ± 7.05*'#	142.60 ± 5.60*'#

The data is presented as mean ± standard deviation.
P > 0.05 = non-significant, *P < 0.05* = significant
- * = significant as compared to control
- # = significant as compared to NAFLD
- $ = significant as compared to Met-P

Histopathological study

a) Haematoxylin and Eosin (H & E) stained sections (Fig. 1)

In the control group, the light microscopic examination of the liver of control group showed the normal liver architecture with no detected abnormalities. The hepatocytes were arranged into cords forming flat, anastomosing plates radiating from the central vein and separated by hepatic sinusoids. Hepatocytes were polyhedral with eosinophilic cytoplasm and open face vesicular nuclei with prominent nucleoli. The portal tracts contained a branch of hepatic artery, portal vein and bile duct. Minimal amount of fibrous tissue was seen around the portal tracts. Administration of HFD for 8 weeks (NAFLD) caused distortion of the hepatic architecture. Signet ring appearance of some hepatocytes was observed. Hepatocellular ballooning was observed in the cytoplasm of many hepatocytes. Thin few fibrous tissue septa were observed between portal tracts. Administration of metformin or losartan with the HFD (Met-P, Los-P) partially preserved the hepatic architecture, however limited number of hepatocytes showed micro and macro-vesicular steatosis with little amount of fibrous tissue around the portal tracts.

b) Oil Red O stained sections (Fig. 2, Table 5)

The frozen sections of control group revealed scanty small sized lipid droplets scattered in the hepatocytes. In NAFLD group, the frozen sections showed large number of variable sized fat droplets. By image analysis, the area occupied by fat in NAFLD group was significantly increased compared with control group. Administration of metformin or losartan with HFD (Met-P, Los-P) revealed different sizes of lipid droplets in some of the hepatocytes. By image analysis, each drug significantly decreased the area occupied by fat compared with NAFLD group. However, neither of them succeeded to eliminate the fat as there was significant increase in the area occupied by fat compared with control group. Moreover, No significant difference was observed between either drugs.

c) Sirius red stained sections (Fig. 3, Table 5)

Sections of control group showed few fine collagen fibers surrounding the central veins and the portal tracts. In NAFLD group, small fibrous tissue septa were observed extending from the portal triads. Administration of metformin or losartan with the HFD (Met-P, Los-P) significantly prevented the increase in the amount of collagen fibers around the central vein and portal tracts compared to NAFLD group confirmed by image analysis. On the other hand when compared to control group, losartan succeeded to return the area occupied by collagen to the normal values (non-significant increase), while metformin failed in this (significant increase). No fibrous septa were observed in both Met-P and Los-P groups.

Table 5: Fat (F.) and Collagen (C.) area percentages by image analysis (%) in different groups sacrificed at 8 weeks

	CONTOL	NAFLD1	Met-P	Los-P
F. area	2.18 ± 0.43	41.75 ± 3.86 *	7.09 ± 0.78*'#	8.02 ± 0.87*'#
C. area	0.78 ± 0.08	5.17 ± 0.87*	2.25 ± 0.61*'#	1.26 ± 0.26#'$

The data is presented as mean ± standard deviation.
P > 0.05 = non-significant, *P < 0.05* = significant
- * = significant as compared to control
- # = significant as compared to NAFLD
- $ = significant as compared to Met-P

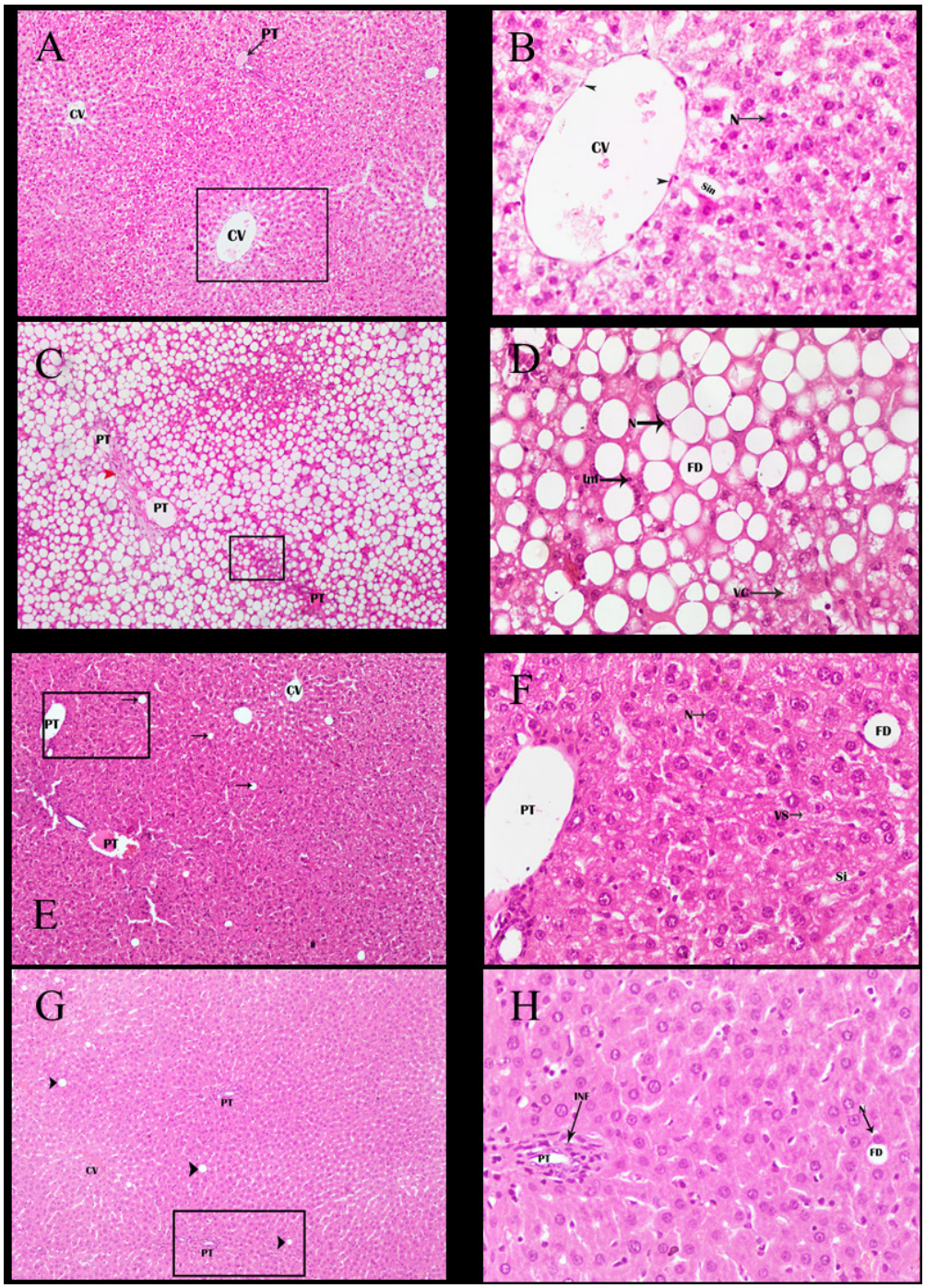

Fig. 1: A, B: photomicrographs of liver section of the control group, showing polyhedral hepatocytes arranged in anastomosing plates radiating from central vein (CV), lined by flat endothelial cells (arrowheads) and polyhedral hepatocytes arranged in flat anastomosing plates with vesicular nuclei (N) separated by hepatic sinusoids (Sin), C, D: photomicrographs of liver section from NAFLD group showing fibrous septa (red arrowhead) extending between the portal tracts (PT), hepatocytes distended with large fat droplets (FD) pushing the nucleus (N) to one side. The cytoplasm of some hepatocytes appears vacuolated (VC) and inflammatory cell infiltrate (Inf) could be seen between the hepatocytes. E, F: photomicrographs of liver section from Met-P group showing plates of hepatocytes with vesicular nuclei (N) arranged in cords radiating from the central veins (CV) separated by wide sinusoidal spaces (Si), few hepatocytes contains small and large sized lipid droplets (arrows, FD). The cytoplasm of some hepatocytes appeared vacuolated (VS). G, H: photomicrographs of liver section from Los-P group showing normal hepatocytes with vesicular nuclei (N), few hepatocytes loaded with fat droplets (arrowhead, FD) with moderate inflammatory cell infiltrate (INF) around the portal tracts (PT). A, C, E, and G: hematoxylin-eosin stain, original magnification: ×100. B, D, F and H: hematoxylin-eosin stain, original magnification: ×400

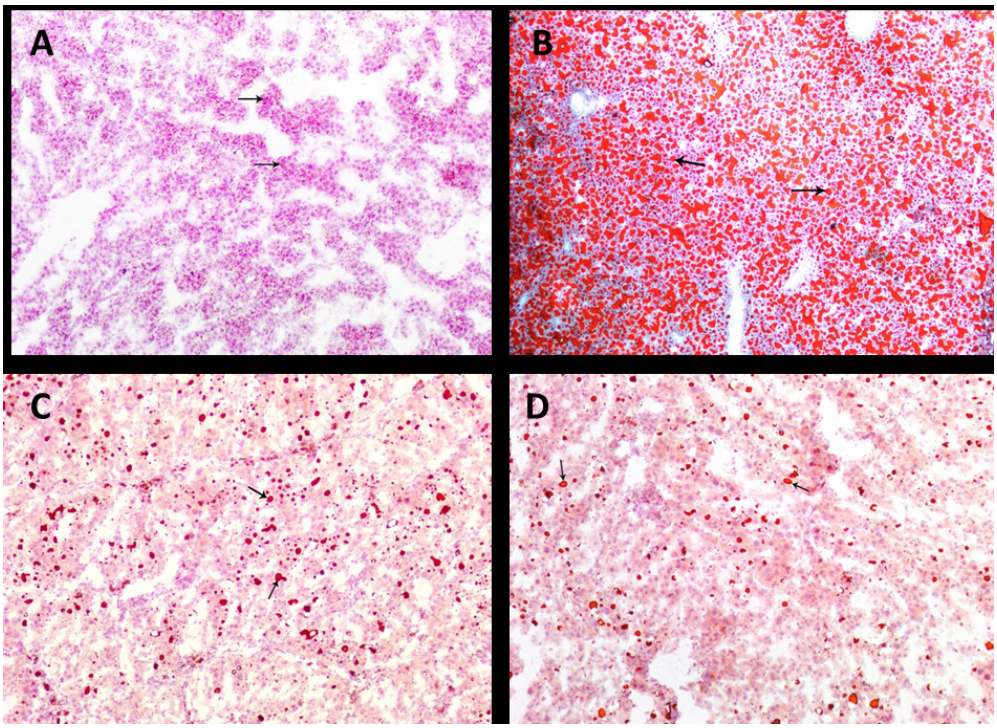

Fig. 2: A: photomicrograph of a frozen section of liver of control group showing small sized red lipid droplets (arrow) scattered in the hepatocytes, B: photomicrograph of a frozen section of liver of NAFLD group showing large amount of different size fat droplets (arrow) in the hepatocytes, C: photomicrograph of a frozen section of liver of Met-P group showing little amount of variable sized fat droplets (arrow), D: photomicrograph of a frozen section of liver of Los-P group showing little amount of variable sized fat droplets (arrow). (Oil Red O; original magnification: ×100).

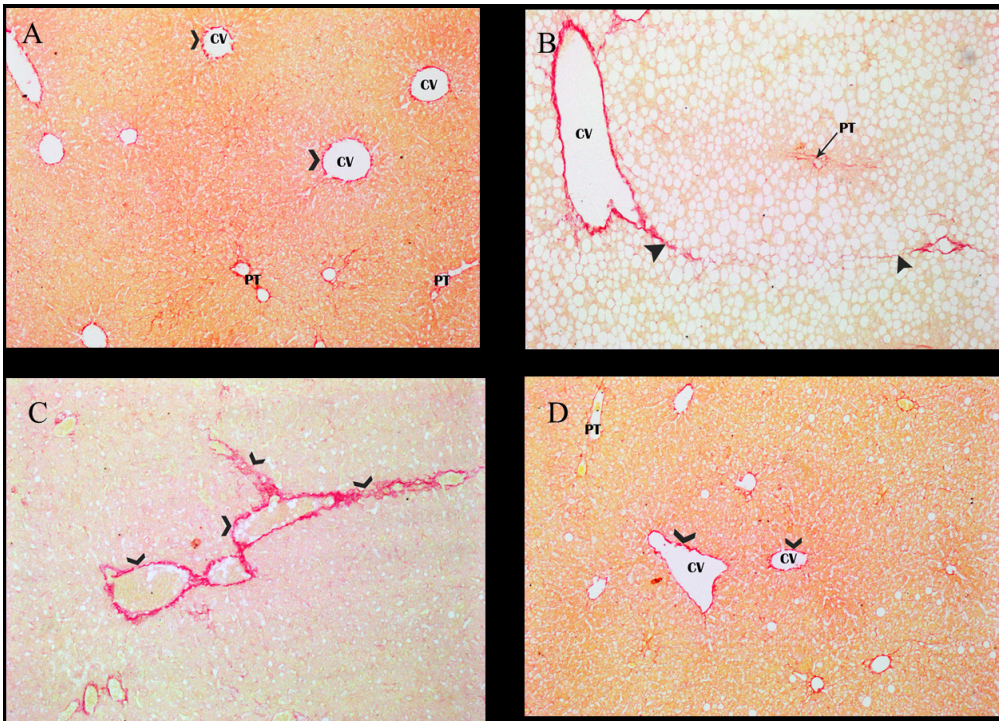

Fig. 3: A: photomicrograph of a section of liver of the control group showing little amount of collagen fibers (arrow head) around central vein (CV) and portal tract (PT), B: photomicrograph of a section of liver of NAFLD group showing increased amount of collagen fibers around central vein (CV) and portal tract (PT) with small septa (arrowheads), C: photomicrograph of a section of liver of Met-P group showing slight increase in the amount of collagen fibers in the portal tract (arrowheads), D: photomicrograph of a section of liver of Los-P group showing little amount of collagen around central vein (CV) and portal tract (PT) (arrowheads) (Sirus red; original magnification: ×100).

d) Anti TNF-α immune-stained sections (Fig. 4, Table 6)

Sections of control group showed few TNF-α positive reaction in the lining of the hepatic sinusoids. NAFLD sections showed significant increase of TNF-α positive reaction in the hepatocytes and in the hepatic sinusoids lining compared to the control group. This finding was confirmed by measuring the area percentage of positive TNF-α reaction which revealed significant increase compared to control group. In liver sections of Met-P, Los-P groups, TNF-α positive reaction was observed in the hepatocytes and in the hepatic sinusoids lining. By image analysis, administration of metformin or losartan (Met-P, Los-P groups) significantly prevented the increase in the TNF-α positive reaction compared with NAFLD group. On the other hand, losartan succeeded to normalize the area occupied by TNF-α positivity, whereas metformin failed to normalize it. However, the decline in the area stained by TNF-α in Los-P group when compared with Met-P group was non-significant.

Table 6: TNF-α positive cells area percentage by image analysis in different groups sacrificed at 8 weeks

	CONTOL	NAFLD1	Met-P	Los-P
Area	1.24 ± 0.28	14.35 ± 2.37*	5.11 ± 1.48*'#	3.68 ± 1.02#'$

The data is presented as mean ± standard deviation.
P > 0.05 = non-significant, *P < 0.05* = significant
- * = significant as compared to control
- # = significant as compared to NAFLD
- $ = significant as compared to Met-P

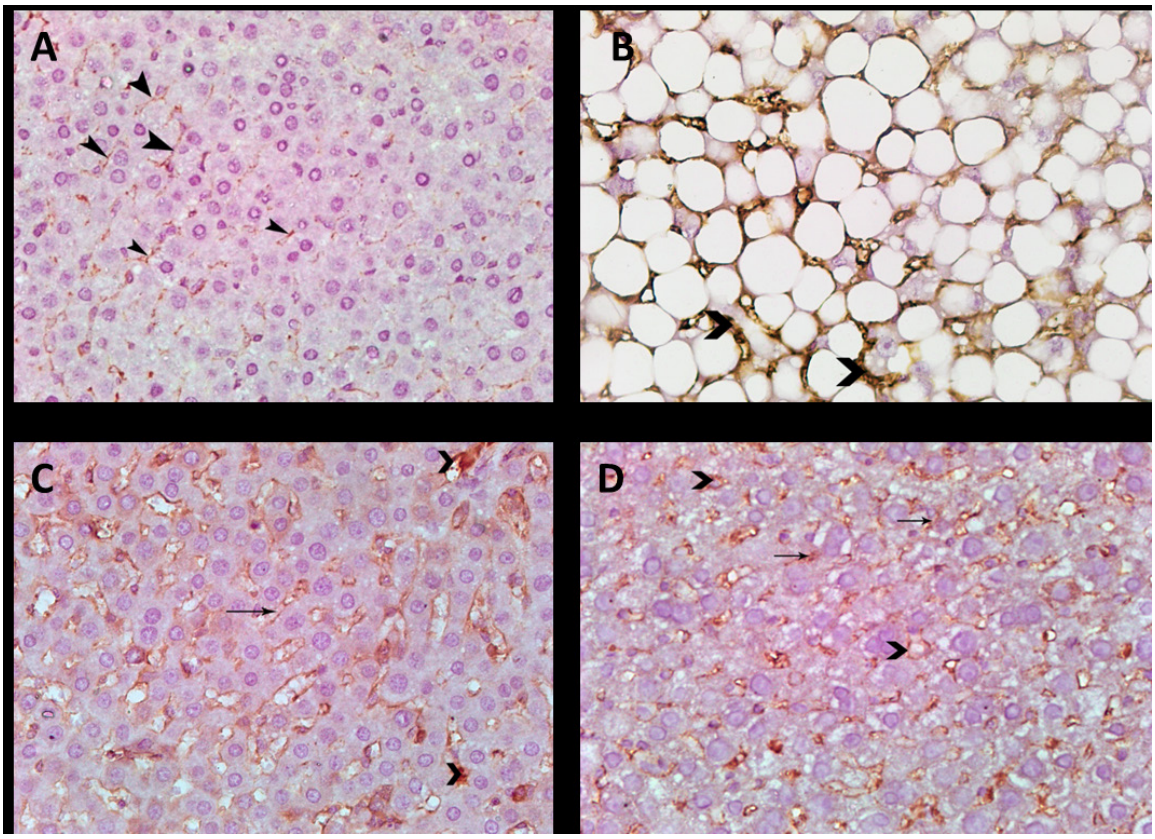

Fig. 4: A: photomicrograph of liver sections of the control group, showing TNF-α positive reaction in the hepatic sinusoids (arrowhead), B: photomicrograph of liver sections of the NAFLD group showing increased amount of TNF-α positive reaction in between the hepatocytes (arrowhead), C: photomicrograph of liver sections of the Met-p group showing TNF-α positive reaction in the hepatic sinusoids (arrows) and some hepatocytes (arrowhead), D: photomicrograph of liver sections of the Los-p group showing TNF-α positive cells (arrowhead) and TNF-α deposition in the hepatic sinusoids (arrows) (TNF-α; original magnification: x 400).

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by excessive lipid accumulation in the liver in the absence of alcohol consumption[22].

Many animal models were tried to mimic the natural history of human NAFLD. The first type of models are the genetic models by knockout of certain genes involved in lipid metabolism such as SREBP-1c transgenic mice, Ob/ob mice, Db/db mice, Zucker rat and others. The second type of models are the dietary types including Methionine and choline deficient diet, fat rich diet, cholesterol and cholate diet and fructose rich diet[23].

Fructose controls the activity of glucokinase, which is the principle enzyme involved in hepatic glucose metabolism. Fructose potently enhances the liver glucose uptake and glycogen synthesis. Therefore, excessive fructose intake leads to postprandial hypertriglyceridemia, which increases visceral adipose deposition, insulin resistance and hepatic steatosis[24].

Many animal studies found that the high fat diet represent the detrimental eating habits of the Western diet and mimic the etiology of NAFLD. Animal study on mice showed that the administration of high-fat foods and high-fructose/sucrose liquids leads to a synergistic effect that may induce liver inflammation and fibrogenesis[11, 24, 25].

Therefore, in our current study, we used a dietary model including combination of high fat diet (20 gm butter/100 gm diet) and fructose added in significant amount to drinking water. The duration sufficient to produce NAFLD was 8 weeks. This is in agreement with[26].

In this study, the histopathological picture of a NAFLD rat model (HFD) showed distorted hepatic architecture with micro-vesicular and macro-vesicular steatosis and inflammatory cell infiltration by Hx & E examination and significant increase in the area occupied by fat by oil red o staining when compared with the control group. These findings were in agreement with Ganz *et al*.[27] in their study on mice. Kawasaki *et al*.[28] examined livers of Wistar rats were fed fructose rich diet for 5 weeks and found significantly higher grades of steatosis of macrovesicular type and intralobular inflammation than those in control rats.

Additionally, in our study we found a significant increase in the area occupied by collagen fibers when compared with the control group. This finding agrees with[29] Kohli *et al*. who found that non-genetically modified mice maintained on a high fat diet present a NASH-like phenotype including hepatic steatosis, inflammation with significant fibrosis.

In our study, there was significant increase in the body weight and liver weight when compared with the control group. These findings agreed with Buettner *et al*.[30]. In addition, the level of TC and TG in HFD animals was significantly higher. Yao *et al*.[31], reported the same result. They attributed this elevation to lipid oxidation impairment and mitochondrial dysfunction. Also, the serum levels of hepatic enzymes ALT and AST were significantly increased. These results agreed with Yin *et al*., Ni and Wang and Zhu *et al*.[32-34]

Administration of HFD for 8 weeks progressively increased the serum levels of FBS significantly. This finding agreed with Frantz *et al*.[35] who explained it by gluconeogenesis enhancement and increased GLUT2 level in the hepatocytes under the effect of HFD.

Regarding the oxidative stress markers, in the present study the values of MDA was significantly elevated and GSH was significantly reduced when compared with control group. These findings agreed with the finding of Aghazadeh *et al*., Yao *et al*.and Zhu *et al*.[31, 34, 36]

Cytokines play an essential role in changing of NAFLD to steatohepatitis and hepatic fibrosis. One of the important cytokines is TNF-α. In the present model, sections from the rat livers showed marked increase in TNF-α deposition in the hepatocytes and in the kupffer cells inside the hepatic sinusoids and the area occupied by TNF-α staining showed significant increase when compared to the control group. This finding was in agreement with Tobelli *et al*.[21]

Currently, the main treatment for NAFLD/NASH is a modified life style by exercise and diet and there is a deficient data regarding the most

beneficial and suitable pharmacological therapy. However, pharmacological therapeutic agents are used by obese patients with NAFLD often lacks the ability to maintain the healthy lifestyles. The drugs investigated in the management of NAFLD are insulin sensitizers, antioxidants, vitamin E, lipid-lowering drugs, pentoxifylline, ursodeoxycholic acid, angiotensin receptor blockers, n-3 polyunsaturated fatty acids, probiotics, synbiotics, and herbal medicines[37].

In our current study we tested two drugs (metformin and losartan) for their preventive efficacy in the management of NAFLD.

Metformin is an insulin sensitizer, which belongs to biguanides family; and is used to treat diabetes mellitus type 2. On the molecular level, it acts by activation of AMPK, which increases glucose uptake by the muscles, inhibits hepatic gluconeogenesis, inhibits lipogenesis; and stimulate lipolysis in the liver, muscles and adipose tissues.

Literatures focusing on the preventive effect of metformin on NAFLD were deficient. Therefore, in the current study, the preventive effect of metformin on NAFLD was assessed by administration of the drug from the start of NAFLD induction; and it was found that metformin significantly prevented the increase in the body weight and the liver weight and it succeeded to normalize the body weight but not the liver weight. This finding was in agreement with Chen *et al*.[38] Moreover, in the current study, metformin prevented the elevation of ALT, AST and FBS and normalized their values when administered from the start. Not only this, but it ameliorated hepatic oxidative stress. This finding was in agreement with De la Rosa *et al*.[39] who proved the metformin protective role against oxidative stress in primary hepatocytes. This could be explained by metformin role in the induction of heme oxygenase-1 (HO-1), bcl-xl gene expression, and the reduction of c-Jun N-terminal kinase (JNK) activation. JNK is a key player in the pathogenesis of oxidative stress-mediated diseases.

Metformin prevented the elevation of the serum levels of lipid profile (TG & TC) and succeeded to normalize TC, but failed to normalize TG value. Khan and Jena[40] found that metformin treatment significantly decreased the TC and TG. Histopathologically, metformin preserved the hepatic architecture, decreased hepatic steatosis and inflammation. These histopathological findings were confirmed by image analysis and were in agreement with Gujjalaa *et al*.[41] The previous findings were explained by AMPK activation that leads to downregulation of SREBP-1c and fatty acid synthase, thus hepatic lipogenesis and gluconeogenesis decreases, while lipolysis increases. On the other hand, hepatic fibrosis is prevented by metformin administration by TGF-b suppression resulting from AMPK stimulation[6].

In the current study, metformin prevented the increase in the positive TNF-α reaction compared with NAFLD group, but failed to normalize it compared with control group. This finding could be explained as metformin induced activation of AMPK leading to macrophage polarization to the M2 phenotype[40].

Losartan is a selective Angiotensin II receptor blockers (ARBs) type 1 that suppresses the renin-angiotensin system (RAS) which has a role in the mechanisms of insulin resistance and is involved in the pathways of liver damage. It is widely used as an antihypertensive agent that was expected to be effective for treatment of NAFLD. Losartan induce vasodilation in the pancreatic vasculature and enhancing insulin secretion, vasodilation promotes glucose delivery to insulin-sensitive tissues. Other mechanisms are improvement of insulin signaling, decreased sympathetic activity, adjustment of muscle fiber composition and improvement of ionic balance and selective stimulation of PPAR[42].

In the current study, the preventive effect of losartan on the development of NAFLD was assessed by its administration from the start in parallel with NAFLD induction.

Regarding FBS, body weight and the liver weight, losartan significantly prevented their increase compared with NAFLD group. However, it failed to normalize them. This finding was in agreement with Shad *et al*.[43] The possible mechanism includes prevention of insulin resistance under the effect of vasodilation and increased glucose uptake by skeletal muscle and activation of PPAR-γ and subsequently

translocation of GLUT 4 to the plasma membrane in the skeletal muscle[42].

Moreover, administration of losartan significantly prevented the increase in the serum levels of liver enzymes (AST & ALT) along with the lipid profile (TG & TC) compared with NAFLD group and succeeded to normalize their levels. Fujita *et al*.[44] observed decreased ALT level by telmisartan in choline deficient diet model of NAFLD, Mohamed *et al*.[45] observed similar findings in a study on another ARB (irbesartan); and Okura *et al*.[46] found that losartan significantly decreased the level of liver enzymes in a steatohepatitis model in non-diabetic rat. The explanation of the decrease in liver enzymes is the reduced release of the inflammatory cytokine TNF-α thus protecting the hepatocytes from HFD-induced injury. The hypolipidemic effect of losartan is explained by prevention of insulin resistance resulting from its vasodilator effect and by down regulation of PPAR.

In the current study, losartan significantly decreased the hepatic level of MDA and significantly increased the hepatic level of GSH compared to NAFLD group. But, it failed to normalize their levels compared to control group. Similar finding was observed by Okura *et al*.[46] who proved that losartan significantly prevent the increase of MDA content in choline deficient diet mouse model of NASH who attributed this to AT-II blocking which inhibit ROS production[47].

Concerning the histopathology, losartan preserved the hepatic architecture, markedly prevented hepatic steatosis confirmed by image analysis. Significant decrease in the area occupied by fat compared with NAFLD group was observed, despite it failed to eliminate the fat as there was significant increase in the area occupied by fat compared with control. This finding was in agreement with Kato *et al*.[48] who studied the effect of irbesartan, on non-alcoholic steatohepatitis using FLS-ob/ob male mice and found that irbesartan decreased the area of hepatic steatosis compared to the model group. They attributed the improvement of hepatic steatosis to the effect of ARBs on the genes regulating lipid metabolism in the form of upregulated PPAR gene and down regulation of SREBP gene with partial agonistic activity of PPAR-γ could improve adipocyte dysfunction, consequently reducing TNF-α and free fatty acids.

Moreover, the amount of fibrous tissue around the portal tracts was reduced after administration of losartan which was confirmed by image analysis and succeeded to return the area occupied by collagen to the normal values (non-significant increase). This finding was in agreement with Yoshiji *et al*.[49]; who attributed the antifibrotic effect of losartan to inhibition of hepatic stellate cells (HSC) activation. Other factors might be included in the antifibrotic effect of losartan such as attenuation of oxidative stress, reduction of macrophage, inflammatory cytokines down-regulation, suppression of TIMP-1 and increased circulating adiponectin levels[42].

In the present study, the administration of losartan significantly prevented the increase in the TNF-α positive reaction in the hepatocytes and in the hepatic sinusoids compared with NAFLD group. Also, it succeeded to normalize the area occupied by TNF-α positive reaction. Similar result was observed by Fujita *et al*.[44]; and Kato *et al*.[48] who found that the number of Kupffer cells infiltrating the liver was reduced and TNF-α gene expression was decreased under the effect of Telmisartan. A-II is known to increase inflammatory cytokines release thus ATR1 blockers reduce the hepatic TNF-α production[42, 50].

In the current study, a detailed comparison on the preventive effects of both metformin and losartan in NAFLD were carried on to assess 2 different pathways in the pathogenesis of NAFLD.

Addressing the preventive effects, each drug was administered at the start of NAFLD induction, metformin produced significant reduction in the body weight, and FBS compared to Los-P group, while no significant difference in the remaining biochemical parameters was observed. This effect could be attributed to AMPK phospholylation by metformin resulting in enhanced insulin signaling leading to decreased gluconeogenesis, increased lipolysis, decreased lipogenesis, and enhanced glucose entry to skeletal muscles[6, 51, 52].

On the other hand, losartan administration at the start significantly improved the histopathological features of NAFLD including significant decrease in the fat area, collagen area and area immune-stained cells with TNF-α compared with Met-P groups. These effects could be explained by more than one mechanism

as ARBs decrease the insulin resistance, rather than this they decrease macrophage infiltration, decrease proinflammatory cytokines production, decrease TIMP4 expression and prevent HSCs activation[42, 49].

CONCLUSIONS

The single administration of metformin or losartan in prevention of NAFLD improves the body and liver weights, decreases the serum levels of liver enzyme, lipid profile and fasting blood glucose. Moreover, they relieve the oxidative stress and decrease the release of tumor necrosis factor alpha. Losartan improves the liver histopathology more than metformin and prevent progression of NASH to hepatic fibrosis, so it carries a great hope as a therapy for NAFLD. Combination of both drugs should be tested for better biochemical and histopathological results.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الدور التحسيني المحتمل للزنجبيل في اعتلال الصائم المعوي الناجم عن اشعاع غاما في ذكور الجرذان البالغة. دراسة بالميكروسكوب الضوئي والميكروسكوب الاليكترونى الماسح

ملخص البحث

الخلفية: تعتبر الأمعاء واحدة من أكثر الأجهزة تضررا من سمية الإشعاع. لذلك، لا تزال معدلات الأمراض والوفيات الناجمة عن التهاب الأمعاء والقولون الإشعاعي مرتفعة. و ذكرت الدراسات الحديثة أن الزنجبيل قد يمتلك خصائص مضادة للالتهابات والأكسدة. لذلك كان الهدف من هذه الدراسة هو استكشاف الدور المحتمل للزنجبيل كعامل وقائي وكعامل علاجي محتمل في اعتلال الأمعاء الناجم عن اشعاع غاما.

المواد والطرق: تم استخدام ست وثلاثون جرذا أمهقا من الذكور البالغين في هذه الدراسة. تم تقسيم الحيوانات بشكل عشوائي إلى ثلاث مجموعات: المجموعة الأولى (I) استخدمت كمجموعة ضابطة، المجموعة الثانية(II) (أشعة جاما): حيث تعرض كل جرذ لجرعة واحدة إشعاعية و قدرها ٠,٧٣٩(١١جراي/ دقيقة) من إشعاع جاما ، المجموعة الثالثة (III) (أشعة جاما و زنجبيل): تم تقسيمها إلى مجموعتين فرعيتين تم تعرض كلاهما للإشعاع كما هو الحال في المجموعة الثانية وتم إعطاؤهم مستخلص الزنجبيل عن طريق الفم مرة واحدة يوميًا ١٢٠ ملغم / كغم. المجموعة الفرعية (-IIIأ): تلقت مستخلص الزنجبيل سبعة أيام قبل الإشعاع بينما المجموعة الفرعية (-IIIب): تلقت مستخلص الزنجبيل سبعة أيام قبل وبعد الإشعاع. في نهاية التجربة تم ذبح جميع الحيوانات، وتم فحص عينات الصائم المعوي بواسطة الميكروسكوب الضوئي والميكروسكوب الإلكتروني الماسح. كما تم إجراء دراسة مورفومترية وتحليل إحصائي.

النتائج: أظهر الفحص الهستولوجى للمجموعة الثانية (II) انفتال واندماج لبعض الخملات. بينما أظهرت الخبايا تكاثر بؤري لخلاياها الطلائية وغزوها بالخلايا الالتهابية. علاوة على ذلك، أظهرت المجموعة الفرعية (-IIIأ) اختلال بؤري لجدار الخبايا. كما لوحظ انخفاض معتد في متوسط عدد خلايا الكأسية وزيادة معتدة في النسبة المئوية للمساحة المتوسطة لألياف الكولاجين، في المجموعات السابقة مقارنة بالمجموعة الضابطة. ومع ذلك، أظهرت المجموعة الفرعية (-IIIب) استعادة معظم هيكل الغشاء المخاطي للصائم ليصبح تقريبا مشابه للمجموعة الضابطة.

الخلاصة: يبدو أن الزنجبيل له تأثير واعد محتمل لتحسين إصابة الأنسجة المعوية الناجمة عن الإشعاع.