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Combination of Telmisartan and Metformin Ameliorate Non-Alcoholic Fatty Liver Experimentally Induced in Rats

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ABSTRACT

Background and aim: Angiotensin II plays a vital role in the development and progression of nonalcoholic fatty liver disease (NAFLD) which is the most common, chronic liver disease worldwide. The present work was designed to evaluate the possible protective effect of telmisartan (AT1 receptor blocker) alone and in combination with metformin on experimentally induced fatty liver in rats and the possible mechanisms underlying this action. **Materials and methods:** Nonalcoholic fatty liver was induced in rats by high-fat diet (HFD). NAFLD rats were divided into 4 groups. HFD untreated group**,** telmisartan treated group 8mg/kg/day, metformin-treated group 150 mg/kg/day and HFD + telmisartan + metformin-treated group. **Results:** The data of the current work revealed that HFD administration significantly deteriorates liver functions, lipid profile, MDA and GSH levels compared to normal control group. Hepatic damage was confirmed with histopathological studies. However, oral monotherapy either with telmisartan or metformin improved the biochemical parameters and histopathological changes with more significant effect of combination therapy over the effect of each drug alone. **In conclusion:** Nonalcoholic fatty liver disease could be ameliorated by coadministration with telmisartan or metformin by improving oxidative stress and liver function with more significant effect of combined therapy over the effect of each drug alone, suggesting new strategy in prevention and treatment of NAFLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world with a prediction to be the most frequent indication for liver transplantation by 2030, as hepatic steatosis may progress to cirrhosis or hepatocellular carcinoma **(Byrne and Targher, 2015)**. There is no much information about the pathogenesis of steatohepatitis, but it may include insulin resistance, impairment of mitochondrial function, increased hepatic content of free fatty acids, chronic inflammation, and fibrosis **(Dumas *et al.*, 2014).** NAFLD usually associated with metabolic syndrome which characterized by central obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low HDL (high-density lipoprotein) **(Demir *et al.*, 2015)**.

Wu *et al.*, (2016) said "There are several therapeutic agents have been used to treat NAFLD patients such as PPARγ activators, antioxidants, hypolipidemic agents and cytoprotective agents. However, no optimal therapeutic strategy has been established, so there is a need for new NAFLD treatment strategies"**.**

Telmisartan is an angiotensin II type 1 receptor (ATI) antagonist which has the longest half-life compared with other angiotensin receptor blockers (ARBs) **(Fujita *et al.*, 2007).**

Telmisartan is effective in mild to moderate hypertension, improves insulin sensitivity in Type 2 DM, and improves cholesterol and triglyceride levels. As most of the patients of NAFLD have features of metabolic syndrome **(Yokohama *et al.*, 2006).**

Metformin, an oral biguanide normoglycemic drug which is widely prescribed to treat individuals with type 2 DM. Metformin produces its action through improves insulin sensitivity and decreases blood glucose level by decreases its intestinal absorption and decreases its production by the liver.**(Rojas and Gomes, 2013).**

This work was designed to evaluate the possible protective effect of telmisartan alone and in combination with metformin on HFD-induced fatty liver in rats and the possible mechanisms underlying this action.

MATERIALS AND METHODS

Animals

Fifty adult male albino rats, obtained from "Experimental animal breeding farm, Helwan – Cairo" were used in the current work. Rats were 150-200 g. They had been acclimatized for two weeks in pharmacology department, Benha faculty of medicine. Rats were allowed to free access to water and standard diet. All experimental protocols were approved by the ethical committee of faculty of medicine, Benha University.

Drugs

Telmisartan (powder), **Metformin** (powder), other chemicals and reagents (Sigma- Aldrich co., Cairo. Egypt.)

Experimental design

After acclimatization for 2 weeks, rats divided into 5 experimental groups, 10 rats each. **Group (1): The normal control group** was fed with standard chow diet with no medication. **Group (2): Fatty liver non treated group:** the rats of this group received HFD orally (1% cholesterol and 10% coconut oil) with no medication **(Zou *et al.*, 2006)**. **Group (3): Telmisartan treated fatty liver group (TEL),** rats of this group received HFD with oral administration of telmisartan 8 mg/kg/day for 60 days (**Zhang *et al.*, 2015). Group (4): Metformin treated fatty liver group (MET),** rats of this group received HFD with oral administration of metformin 150 mg/kg/day for 60 days **(El-Lakkany *et al.*, 2016). Group (5): Telmisartan + Metformin treated fatty liver group (TEL+MET)**, rats of this group received HFD with oral administration of telmisartan + metformin for 60 days.

At the end of the experiment, overnight fasted rats were anaesthetized by inhalation of ether and blood samples were collected from rat tail and processed for biochemical investigation. Then rats were sacrificed and Liver of each rat was dissected immediately, washed with ice-cold saline and divided into 2 parts. The first one was immediately frozen at -80°C and used for biochemical determinations, this portion latterly was minced and homogenized. The crude homogenate was centrifuged at 7.700 for 30 minutes at 4^0c and the resultant supernatant was used for assay of tissue malondialdehyde and reduced glutathione **(Halliwell and Chirico, 1993).** The second part preserved in 4% formalin for histopathological examination.

Parameters measured

Blood samples were obtained and allowed to clot then centrifuged at 3000 rounds/ minute for 15 minutes. Sera were separated and kept at - 20ºC till used for biochemical investigation.

Liver function tests: were performed on samples by colorimetric methods (Siedel *et al*., 1991):

a- SGPT (ALT) & SGOT (AST).

b- Albumin- Globulin ratio (A/G ratio).

Determination of serum lipid profile:

a- Determination of triglycerides (TG) (bioMerieux- France):

This was carried out by enzymatic colorimetric test according to (GPO- PAP) method **(Fossati, 1982).**

b- Evaluation of total serum cholesterol (TC): this was carried out by enzymatic colorimetric test **(Siedel *et al*., 1991).**

c- Measurement of serum HDL-cholesterol:

This is carried out by method depends on (Separation of high- density lipoproteins (HDL) and determination of cholesterol bound to these fractions) **(Lopes *et al*., 1977).**

d- Calculation of serum LDL- cholesterol: **(Frirdewald *et al*., 1972).**

Evaluation *of hepatic malondialdehyde (MDA) level:* (Halliwell and Chirico, 1993)

Evaluation *of hepatic reduced glutathione (GSH) level:* (Halliwell and Chirico, 1993)

Histopathological examination: using Hematoxylin and Eosin (H&E) stain **(Dury and Wallington, 1967).**

Statistical analysis: (Goldstone, 1983).

Data were summarized as mean ± S.D. Comparisons between groups were done using the one-way analysis of variance (ANOVA). The difference between groups was compared using Tukey test. P values < or equal 0.05 was considered statistically significant.

RESULTS

Hepatic parameters in different studied groups:

ALT and AST levels were significantly ($p<0.05$) increased in untreated fatty liver group compared to control group while TEL treated and MET treated fatty liver groups showed significant ($p<0.05$) decrease of these parameters compared to untreated group. Co-administration of (TEL+MET) significantly decreased ($p<0.05$) these parameters compared to monotherapy groups and untreated group but still at significant ($p<0.05$) higher level compared to control group.

Meanwhile, A/G ratio significantly ($p<0.05$) decreased in untreated fatty liver group compared to control group. In TEL and MET treated fatty liver groups, there were significant ($p<0.05$) increase of A/G ratio compared to untreated group. Co-administration of (TEL+MET) significantly ($p<0.05$) increase this parameter compared to monotherapy groups and untreated group but still at significant ($p<0.05$) lower level compared to control group.

Table (1): Hepatic parameters in different studied groups (mean ± SD).

Parameters Groups	ALT (U/L)	AST (U/L)	A/G ratio
Control group	43.7± 4.71	46.15± 6.4	3.2± 0.4
Untreated fatty liver group	146. 7± 14.3[a] ↑235.6%*	167±16.4[a] ↑261.8%*	1.3± 0.12[a] ↓59.3%*
TEL treated group	81.6± 9.1[a,b] ↓44,4%**	97.5±8.8[a,b] ↓41.6%**	1.8± 0.2[a,b] ↑38.4%**
MET treated group	78.2± 8.4[a,b.] ↓46.7%**	89.8±9.3[a,b] ↓46.2%**	1.9± 0.3[a,b] ↑46.1%**
TEL+MET treated group	62.2±5.9 [a,b, c,d] ↑57.6%**	65.8±6,2 [a,b, c,d] ↑60.5%**	2.4±0.2[a,b, c,d] ↑84.6%**

a: Significant versus normal control group.

b: Significant versus fatty untreated group.

c: Significant versus TEL treated group.

d: Significant versus MET treated group.

N.B: % change is calculated in relation to control group (*) and untreated group (**).

Serum lipid profile in different studied groups:

Serum cholesterol, TG, LDL-c levels were significantly ($p<0.05$) increased with significant ($p<0.05$) decrease in serum HDL-c level in untreated fatty liver group compared to control group while in TEL treated and MET treated fatty liver groups, there was significant ($p<0.05$) decrease of these parameters with significant increase of HDL-c level ($p<0.05$) compared to untreated group. Co-administration of (TEL+MET) significantly ($p<0.05$) decrease levels of these parameters and significantly ($p<0.05$) increase HDL-C level compared to monotherapy groups and untreated group without significant difference ($p>0.05$) in HDL-C level and still at significant ($p<0.05$) higher level in other parameters compared to control group.

Table (2): Serum lipid profile in different studied groups (mean ± SD).

Parameters / Groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)
Control Group	95.7±10.2	99.2±12.1	45.7±6.1	38.7±4.3
Untreated fatty liver Group	188.4± 16.7[a] ↑96.8%*	172.7± 21.2[a] ↑73.3*	112.4± 12.8[a] ↑154.9%*	21.1±2.6[a] ↓36.1%*
TEL treated Group	156.4 ± 16.5[a,b] ↓17%**	152± 14.3[a,b] ↓12%**	87.2± 9.2[a,b] ↓22.4%**	27.2±4.1[a,b] ↑75.9%**
MET treated Group	151.8± 16.3[a,b] ↓19.4%**	145± 13.5[a,b] ↓16%**	82.9± 8.6[a,b] ↓26.2%**	28.4±3.8[a,b] ↑63.1%**
TEL+ MET treated Group	120±13.6[a,b,c,d] ↓36.3%**	123±11.6[a,b,c,d] ↓28.7%**	62.3±7.2[a,b,c,d] ↓44.5%**	36.5±4.[b,c,d] ↓19.4%**

a: Significant versus normal control group.

b: Significant versus fatty untreated group.

c: Significant versus TEL treated group.

d: Significant versus MET treated group.

N.B: % change is calculated in relation to control group (*) and untreated group (**).

Level of Hepatic (MDA) and (GSH) in different studied groups:

Hepatic (MDA) was significantly ($p<0.05$) increased and hepatic (GSH) was significantly ($p<0.05$) decreased in untreated fatty liver group compared to control group, while in TEL treated and MET treated groups, there was significant ($p<0.05$) decrease of (MDA) and significant ($p<0.05$) increase of (GSH) levels. Both groups showed insignificant difference between them, but still at significant higher level ($p<0.05$) compared to control group. Co-administration of (TEL+MET) significantly ($p<0.05$) increase (GSH) and significantly ($p<0.05$) decrease (MDA) levels compared to monotherapy groups and untreated group but (MDA) still at significant higher level ($p<0.05$) compared to control group.

Table (3): Level of Hepatic (MDA) and (GSH) in different studied groups. (Mean ± SD).

Groups	Hepatic (MDA) nmol/g	Hepatic (GSH) nmol/g
Control group	32.3± 2. 9	0.252±0.02
Untreated fatty liver group	93.4 ±9.2[a] ↑189.1%*	0.08±0.005 [a] ↓68.2%**
TEL treated group	61.5 ±2. 6[a,b] ↓34.2%**	0.181±0.02 [a,b] ↑126.3%*
MET treated group	58.4 ±4. 5[a,b] ↓37.4%**	0.172±0.018 [a,b] ↑115%*
TEL+MET treated group	40.1±4. 2[a,b,c,d] ↓57%**	0.241 ±0.031 [b,c,d] 201.2%**

a: Significant versus normal control group.

b: Significant versus fatty untreated group.

c: Significant versus TEL treated group.

d: Significant versus MET treated group.

N.B: % change is calculated in relation to control group (*) and untreated group (**).

Histopathological examination:

Control group showed normal liver architecture with (a) normal central vein, (b) normal Hepatocytes (Fig 1). NAFLD non treated group showed (a) marked hydropic changes, (b) marked steatosis and (c) some necro-inflammatory foci in hepatic nodule (Fig 2). While TEL treated group and MET treated group showed preserved liver architecture, (a) hydropic

changes, (b) some inflammatory foci in hepatic nodule of lesser degree than NAFLD non treated rats (Fig 3, 4). Combination (TEL + MET) treated rats showed preserved liver architecture, (a) hepatocytes showed (a) steatosis and (b) some inflammatory foci of lesser degree than monotherapy with TEL, with MET and in NAFLD non treated rats as in (Fig 5).

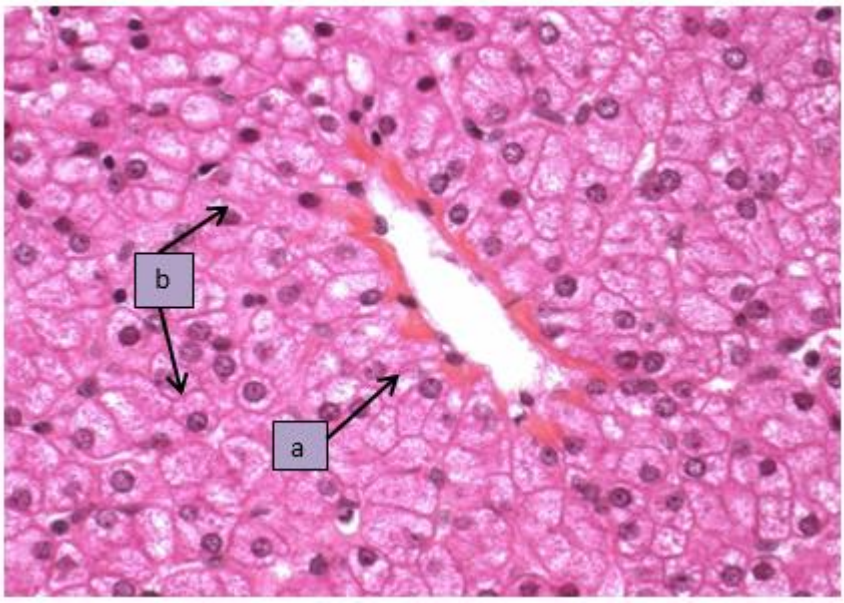

Fig. (1): Photomicrograph of a cut section in the liver of a control rat showing: (a) normal central vein & (b) normal hepatocytes (H&Ex40).

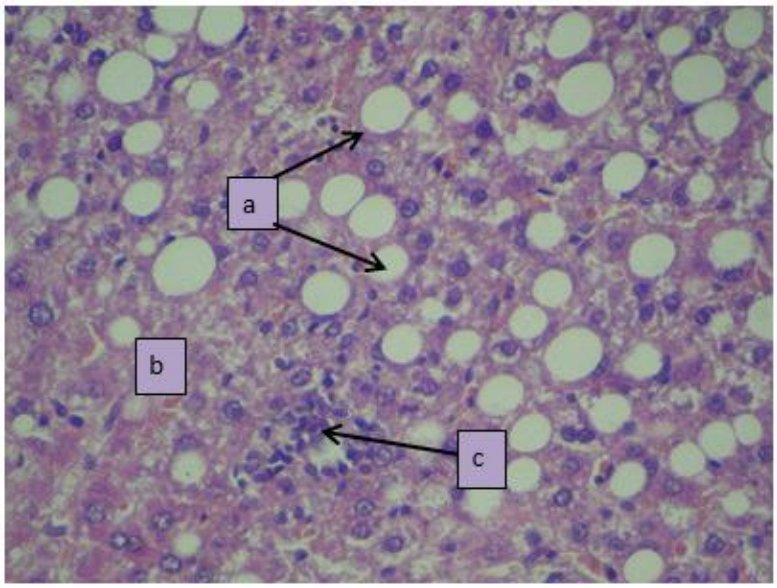

Fig. (2): Photomicrograph of a cut section in the liver of fatty untreated rat showing: severe (a) hydropic changes, (b) steatosis and (c) inflammatory cell infiltration (H&Ex40).

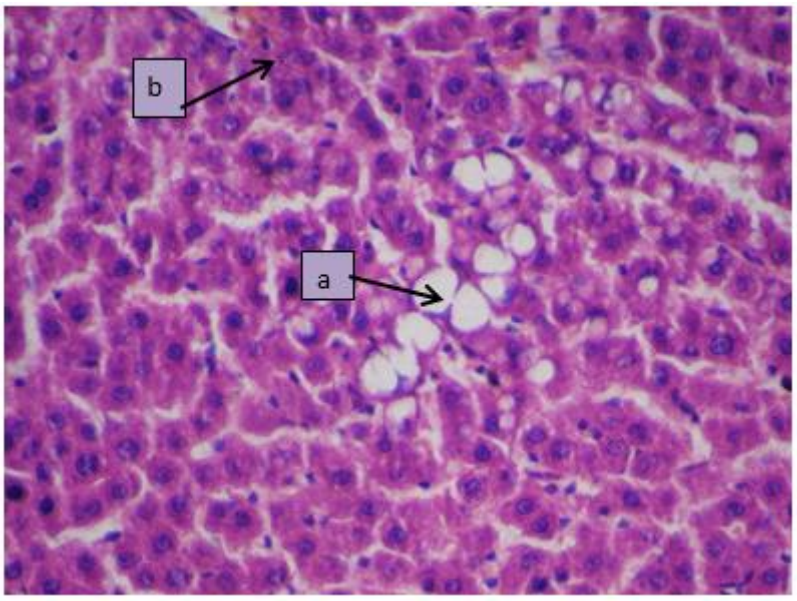

Fig. (3): Photomicrograph of a cut section in the liver of telmisartan treated rat showing: moderate (a) hydropic changes and (b) inflammatory cell infiltration (H&Ex40).

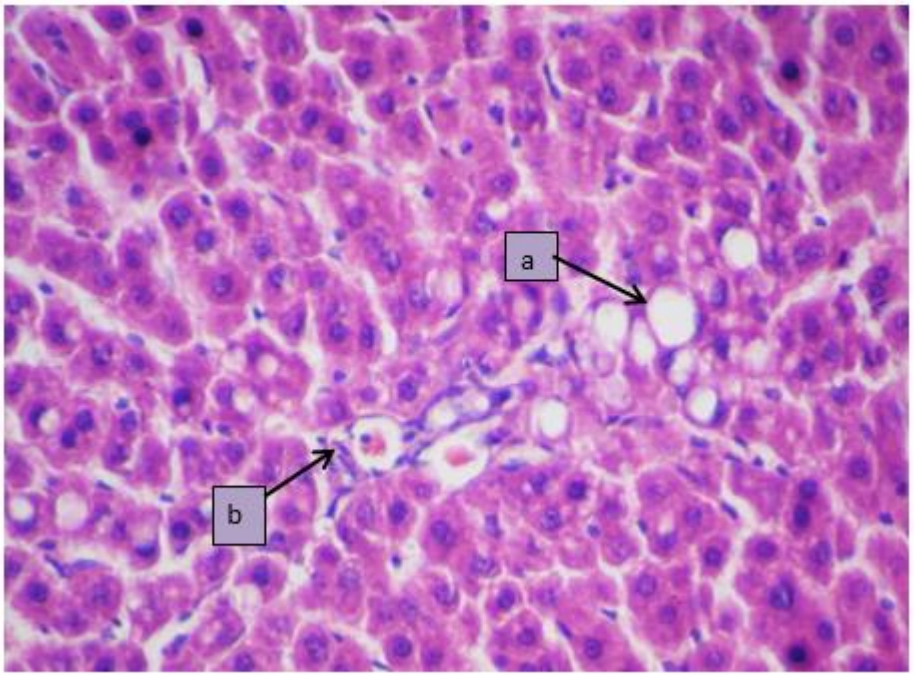

Fig. (4): Photomicrograph of a cut section in the liver of metformin-treated rat showing: moderate (a) hydropic changes and (b) inflammatory cell infiltration (H&Ex40).

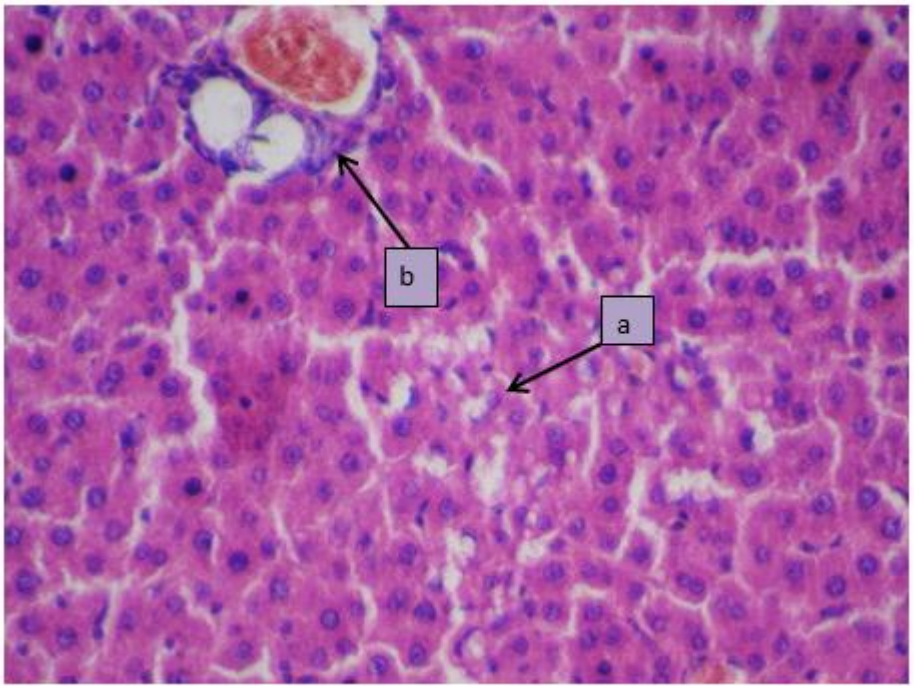

Fig. (5): Photomicrograph of a cut section in the liver of telmisartan +metformin treated rat showing: mild (a) hydropic changes and (b) inflammatory cell infiltration (H&Ex40).

DISCUSSION

"Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. It is present in 30% of the general adult population and found predominantly in obese people with high-fat diets and inactive lifestyles"**, (Mavrogiannaki and Migdalis, 2013).**

In the present study, NAFLD was induced in rats by HFD which considered similar to human NAFLD in relation to pathophysiology, including obesity, IR and hepatic steatosis **(Carabelli *et al.*, 2011).**

The data in the current work revealed that 60 days after high-fat diet administration, there was significant elevation in liver enzymes with significant reduction of A/G ratio, HDL-c and hepatic GSH, also there was significant increase in triglycerides, total cholesterol, LDL-c and hepatic MDA. Histopathological examination supported the biochemical analysis; these results were coincided with **(Olorunnisola *et al.*, 2012, Rath *et al.*, 2016 and Koo *et al.*, 2017)**.

The data of the present work revealed lower level of albumin/globulin ratio in model group, this coincide with **(Lu *et al.*, 2007).**

The reduction in A/G ratio level explained by **(Norazmir *et al.*, 2010)** who decided that reduced A/G ratio may be due to decreased protein intake or increased protein requirement for free radical neutralization.

The present study revealed higher levels of serum ALT and AST markers in untreated fatty liver group, this agrees with **(Wang *et al.*, 2015, Lonardo *et al.*, 2017 and Tsai and Lee, 2018)** who found higher activities of these markers in response to oxidative stress induced by high-fat diets.

Wu *et al.*, (2016) suggested that" the Renin-Angiotensin system (RAS) may play a potent role in the development and progression of NAFLD, due to accumulation of triglycerides, decreases hepatic fatty acid oxidation, alters very low-density lipoprotein secretion, and increases lipogenesis in the liver".

Telmisartan a non-peptide antagonist selectively blocks AT1R, which does not increase the levels of bradykinin or decrease plasma levels of angiotensin-II or aldosterone **(Jin *et al.*, 2007).**

In the present study, administration of telmisartan daily orally (8 mg /kg) for 60 days to NAFLD model significantly decreased serum ALT activity, serum AST activity, total serum cholesterol level, serum TG level , serum LDL-C and hepatic MDA with significant elevation of A/G ratio, serum HDL-C and GSH compared with NAFLD untreated rats. The results of this study were coincided with **(Fujita *et al.*, 2007)**.

The results of the present study run in parallel with that of ***(*Sirag *et al., 2011)***, who reported that increase albumin/globulin ratio after treatment with Candesartan cilexetil is indicative of the increase in the immunity activity of the hepatic tissue.

Also**, (Wu *et al.*, 2016)** reported that, there was downregulation of low-density lipoprotein receptor in livers of mice treated with telmisartan.

The present study found that, there was significant decrease in hepatic MDA and significant increase in hepatic GSH levels in telmisartan treated group compared to untreated fatty liver

group, these agree with **(Fathy *et al*., 2019)** who revealed that improve MDA and GSH levels in CCl_4-induced liver fibrosis in rats.

Result of current study showed histopathological improvement in liver architecture in telmisartan-treated rats, livers showed moderate ballooning degeneration and inflammatory cell infiltration without steatosis; this is in line with **(Kuwashiro *et al*., 2011 and Yki, 2016).**

Alam *et al*., (2016) used randomized controlled clinical trials reported that significant improvements were observed in histology and fibrosis in NASH patients 1 year after TEL treatment.

Hepatoprotective effect of telmisartan may be explained by its modulatory effect on PPARγ, as telmisartan is PPARγ agonist and so it can decrease insulin resistance and improve hepatic steatosis through modulation of this receptor. **(Benson *et al*., 2004, Fuentes *et al*., 2015 and Borém *et al*., 2018)**

Jin *et al*., (2007) also reported that telmisartan could inhibit the activation of HSCs. Following treatment with telmisartan, TGF-β1, TIMP-1, TIMP-2 and matrix metalloproteinase-13 were significantly downregulated, and the pathological results demonstrated that the liver fibrosis score of the treatment group was significantly improved, suggesting that TEL may have the roles against hepatic fibrosis.

Nakagami *et al*., (2010) reported that TEL may upregulate PPARγ and increase the production of hepatocyte growth factor, thus improving liver fibrosis in rats with NASH.

Also **(Park *et al*., 2019)** revealed that "telmisartan efficiently improve and prevent the progression of NASH in diabetic nonalcoholic steatohepatitis (NASH) mice, the amelioration of NASH likely occurred via regulation of inflammatory and fibrosis-related responses, and an integrated analysis of transcriptional and non-transcriptional genes regulated by telmisartan identified cross-talk between angiotensin-PPAR-NFκB pathways that could contribute to the effects of telmisartan on NASH".

Fathy *et al*., (2019) suggested another hepatoprotective mechanism of telmisartan which is attributed to suppression of NO production and iNOS protein expression.

Metformin is an oral normoglycemic drug, it suppresses endogenous glucose production and improves lipid metabolism, also it reduces insulin resistance and increases insulin sensitivity

and this could be explained by its modulatory effects on insulin receptor expression and tyrosine kinase activity **(Viollet *et al*., 2012).**

In the current study, administration of metformin daily orally (150 mg /kg) for 60 days to NAFLD model in significant decrease in serum ALT activity, serum AST activity, total serum cholesterol level, serum TG level , serum LDL-C and hepatic MDA with significant elevation of A/G ratio, serum HDL-C and GSH compared with NAFLD non treated rats, these results coincided with **(Ahmed and Mahmoud 2012)** who reported that metformin administration single orally daily for 5 weeks in experimental HFD induced NASH in rats resulted in significant decrease in serum TG level.

Also, Data from clinical study done by **(Ebeid *et al*., 2012)** noticed that metformin administration daily orally for 8 weeks resulted significant decrease in liver function tests and in total lipid profile with significant elevation of serum HDL-C compared with before treatment in patients with T2DM.

In the present work, as regard histopathological data, it was found that metformin-treated NAFLD rats demonstrated moderate changes of macro-vesicular steatosis, hydropic degeneration (hepatocyte ballooning) and steatohepatitis started to appear if compared to NAFLD untreated rats ,these results coincide with **(Lin *et al*., 2000 and Raso *et al*., 2009).**

Hepatoprotective effect of metformin explained by **(Viollet *et al*., 2012)** who documented that "metformin decreased plasma FFA through its suppression of acetyl-CoA carboxylase (ACC) activity. As ACC is an important enzyme which control the rate of malonyl-CoA synthesis, which is a critical precursor of fatty acid synthesis, and a potent inhibitor of mitochondrial fatty acid oxidation".

Another explanation produced by **(Miller and Birnbaum, 2010)** who revealed that the action of metformin may be mediated by adenosine monophosphate activated protein kinase (AMPK) activation, as AMPK regulates the changes in the hepatic lipid metabolism.

Also, it has antioxidant and anti-inflammatory properties which modulate the progression of steatohepatitis to fibrosis **(Fruci *et al*., 2013).**

In the present study, co-administration of (TEL + MET) daily orally for 60 days to NAFLD model resulted in significant decrease in serum ALT level, serum AST level, total serum

cholesterol level, serum TG level, serum LDL-C and hepatic MDA with significant elevation of serum A/G ratio, HDL-C level and hepatic GSH compared to monotherapy either with TEL alone or MET alone and NAFLD untreated rats.

The results of the present work are in same line with **(Rania *et al.*, 2013)** who documented that" addition of telmisartan to metformin successfully improved serum glucose levels, serum total cholesterol (TC), triglycerides (TG) and adiponectin, suggesting the potential role of telmisartan and metformin combination in treating experimentally induced type II diabetes mellitus in rats".

Also these results in agreement with **(Goyal *et al.*, 2011)** who documented that combined therapy of telmisartan and metformin resulted significant decrease in liver function tests and in total lipid profile with significant elevation of serum HDL-C in HFD induced obesity in Westar rats.

In conclusion, the added benefit from that combination come from that telmisartan and metformin can potentiate each other as each of them act at different mechanism in atrial to prevent and treat NAFLD. The present study suggests that telmisartan in combination with metformin produced a better result than in either of them alone. These results evidenced by better improvement of liver function, lipid profile, antioxidant effect in hepatic tissue and better improvement in hepatic histopathological changes. These findings suggest that administration of telmisartan in combination with metformin in patients prone to develop fatty liver (e.g. hepatitis C, obesity, hyperlipidemia and Diabetes), particularly if these patients have any cardiovascular disease, may be a promising therapy for NAFLD treatment.

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