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ORIGINAL ARTICLE

Aleglitazar treats non-alcoholic steatohepatitis in mice through PPAR dependent pathway

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a common form of chronic liver disease, a more severe form of NAFLD is non-alcoholic steatohepatitis (NASH). Hepatic injury in NASH is caused by oxidative stress and inflammation brought on by lipotoxicity. Aleglitazar is a dual peroxisome proliferator-activated receptor (PPAR) agonist which has anti-inflammatory, antioxidant, and anti-apoptotic effects **Aim:** To investigate the possible curative effect of Aleglitazar against NASH as well as the possible involvement of PPAR in this effect **Methods:** NASH was induced using high-fat diet. Mice received Aleglitazar (10 mg/kg/day), Aleglitazar (10 mg/kg/day, i.p.) + PPAR- γ blocker (GW-9962) (1mg/kg/day, i.p.) and Aleglitazar (10 mg/kg/day, i.p.) + PPAR-α blocker (MK-886) (10 mg/kg/day, i.p.) for 6 weeks. Blood samples were taken for analysis of alanine and aspartate transaminases (ALT&AST), tumor necrosis factor (TNF-α), matrix metalloproteinase-1 (MMP-1), adiponectin, cholesterol, high-density lipoprotein (HDL), and triglyceride levels. At the same time, the livers of the mice were removed for light microscopic examination and the detection of reduced glutathione (GSH) content **Results:** Increases in triglyceride, cholesterol, MMP-1, TNF- α, AST, ALT, and triglyceride levels were observed in NASH animals, whereas reductions in HDL, adiponectin, and GSH content were observed along with vesicular steatosis, lobular inflammation, and hepatocyte ballooning and degeneration. Aleglitazar treatment improved histopathological alterations and lowered MMP-1, TNF- α, AST, ALT, triglyceride, and cholesterol levels while increasing HDL, adiponectin, and GSH levels. The use of GW-9662 and MK-886 significantly abrogated the hepatic protective effect of Aleglitazar as well as reduced the anti-inflammatory and antioxidant effects **Conclusion:** Aleglitazar cured NASH in mice through activation of PPAR, and its anti-inflammatory and antioxidant properties may have had a positive impact.

Keywords: NAFLD, TNF-α, MMP-1, Adiponectin, PPAR

INTRODUCTION

There is uprising evidence that the commonest worldwide chronic liver disease is non-alcholic fatty liver disease (NAFLD) due to increasing obesity prevalence and control of viral hepatitis (1). NAFLD may be existing only as fatty liver accumulation which does not cause steatohepatitis, and may be presented as non-alcholic steatohepatitis (NASH), the disease which is characterized by liver damage or scarring and inflammation (2). The pathogenesis of NASH is complicated and mainly includes oxidative stress and lipotoxicity. Furthermore, endotoxins, inflammatory cytokines, and chemokines are few inflammatory mediators produced by liver cells such as hepatocytes, hepatic stellate cells (HSCs), portal fibrocytes, and immune cells such as neutrophils, macrophages, natural killer (NK) cells, and lymphocytes and used by the immune system to contribute to NASH (3). Likewise, interactions in the liver between different immune cell types and liver cells were stated (4). Aleglitazar is a dual PPAR-γ and PPAR-α agonist that is non-thiazolidinedione which has been developed for the prospective

management of hyperglycemia and dyslipidemia in individuals suffering from type 2diabetes (T2DM) as it lowers blood sugar and modifies lipid levels (5). It was revealed that Aleglitazar prevented complications of diabetes on the pancreatic tissue, renal tissue, and eyes of male Zucker diabetic fatty rats (6). In the treatment of T2DM patients, studies confirmed that Aleglitazar could reduce PPAR-related side effects and weight gain (7). In the liver, spleen, and intestines of cirrhotic rats with portal hypertension, chronic Aleglitazar therapy dramatically increased PPAR-α/PPAR-γ receptors and decreased tumor necrosis factor- α (TNF- α) and nuclear factor kappa beta (NF- κB) expression (8).

This study aimed to research the probable curative impact of Aleglitazar against NASH in mice and its probable mechanism of action.

MATERIALS AND METHODS

Drugs: Aleglitazar powder, GW-9662 (The PPAR-γ blocker), and MK-886 (The PPAR- α blocker) were purchased from Sigma Aldrich (St.Louis, MO) and the required dosage of each of the three research medications was dissolved in Dimethylsulfoxide (DMSO) just before usage.

Animals and experimental design: Male CD1 albino mice weighing 25–30 g were got from the Faculty of Veterinary Medicine at Zagazig University in Egypt when they were 8–10 weeks old. Mice were kept in rooms that had a constant temperature of 23±2 ^{O}C and a 12-hour light/dark cycle. Before the trial, mice were given a week to adapt and were given unlimited access to filtered water and a regular chow diet.

Mice were divided into six groups (6mice/group): **group 1** (**Control)** fat calories represented only 10% of diet ; **group 2** (**NASH)** mice received a high-fat diet (HFD) (71% from fat, 11% from carbohydrate, and 18% from protein) according to Ching *et al.* (2017) for 7 weeks (9); **group 3 (DMSO)** mice received DMSO (10mg/kg, i.p.) according to Hanslick *et al* (2009) (10) daily for 6 weeks starting from the 8th week after NASH induction; **group 4 (Aleglitazar)** mice received Aleglitazar (10 mg /kg/day, i.p.) (7) for 6 weeks (11) given after NASH induction starting from the 8th week; **group 5 (GW-9962)** mice received GW 9962 (1mg/kg/day, i.p.) (12) before given Aleglitazar (10mg/kg, i.p.) for 6 weeks after NASH induction starting from 8th week and **group 6 (MK-886)** received PPAR-α blocker MK 886 (10mg/kg/day, i.p.) (13) before Aleglitazar (10mg/kg, i.p.) administration for 6weeks after NASH induction starting from 8th week. Mice were assessed daily so that suitable dosages were delivered and followed up.

Dose selection Aleglitazar dose was designated according to Chen et al (2017) (7) who gave mice 10 mg/kg/day of Aleglitazar to test the drug's ability to protect cardiomyocytes from the damaging effects of hyperglycemia.

NASH induction A high-fat diet (71 percent calories from fat, 11 percent calories from carbohydrates, and 18 percent calories from protein) was used for seven weeks to induce NASH (9).

Sampling and tissue dissection The left and middle liver lobes, as well as blood from the heart, were collected after CO2 asphyxiation was used to put down the mice. Each group's liver tissues were divided into two equal groups, one for homogenization and the other for histological research. AST, TNF-α, MMP-1, lipid profile (triglyceride, cholesterol & HDL), and adiponectin levels were assessed using sera. To determine the GSH concentration, the left and median hepatic lobes were washed with ice-cold saline, dried, and then utilized. The liver samples were treated in a Potter-Elvehjem homogenizer after being suspended in phosphate buffer (50 mmol/L, pH 6) at five times the tissue volume. To use the raw homogenate for various tests, it was aliquoted and frozen at -80°C (14).

Detection of liver enzyme levels: serum ALT and AST activity were measured enzymatically using commercial kits obtained from Spinreact (Gerona, Spain).

Measurement of serum TNF-α: a mouse TNF-PicoKineTM ELISA kit was used to test serum TNF. (15)

Estimation of hepatic GSH content Colorimetric analysis was used (16)

Detection of MMP-1: levels were measured using a mouse matrix MMP-1 Elisa kit following product instructions (17)

Measurement of adiponectin: A mouse matrix Adipoq [ELISA kit] was used (18)

Determination of lipid profile: HDL, triglycerides, and total plasma cholesterol were determined quantitatively, enzymatically, and colorimetrically in serum. (19)

Histopathological Examination: Both the left and middle lobes of the liver were used to collect the samples, which were then processed, fixed in 10 percent buffered formalin (pH 7.2), and then embedded in paraffin wax. Light microscopy inspection required the creation of sections with a 5-mm thickness and H&E staining (20).

Statistical analysis: ANOVA was utilized to compare all groups in a single direction. For group comparison, the least significant difference (LSD) was applied. Data were expressed as mean± SEM. P values of 0.05 were recognized as significant. Statistical Package of Social Services version 22 was used to evaluate the data collected (SPSS).

RESULTS

Effect of Aleglitazar on serum levels of ALT &AST: When compared to the control group, NASH dramatically raised the serum levels of ALT and AST. In comparison to the NASH group, the ALT and AST values in the DMSO-treated group were insignificant. In comparison to the NASH and DMSO group, Aleglitazar (10 mg/kg/day) significantly reduced ALT and AST levels. By using GW-9662, the mean increased from 25.66±1.42 to 33.0±0.77 for ALT and from 30.16±0.98 to 55.33± 1.62 for AST compared to the Aleglitazar group. The use of MK-886 increased the mean from 25.66±1.42 to 54.0 ±1.29 for ALT and from 30.16±0.98 to 65.33 for AST in comparison to the Aleglitazar group as shown in Table 1.

Effect of Aleglitazar on TNF-α and MPP-1: As shown in table 2, when compared to the control group, NASH significantly increased the serum levels of TNF- α and MPP-1. TNF-α and MPP-1 were not significant in the DMSO-treated group compared to the NASH group. TNF-α and MPP-1 levels were significantly lowered by Aleglitazar (10 mg/kg/day) in comparison to the NASH and DMSO groups. By using GW-9662, there was an increase in the mean from 68.46 ± 1.35 to 93.37±1.41 for TNF-α and from 81.61±0.91 to 103.45±0.89 for MMP-1 compared to Aleglitazar group. MK-886 increased the mean from 68.46±1.35 to 106.62±1.40 for TNF-α and from 81.61±0.91 to 146.05± 1.55 for MMP-1 compared to the Aleglitazar group.

Effect of Aleglitazar on adiponectin and GSH: As displayed in table 2, both the hepatic GSH content and the serum level of adiponectin were considerably lower in NASH compared to the control group. Adiponectin levels and GSH content in the DMSO-treated group did not differ substantially from those in the NASH group. When compared to the NASH group, Aleglitazar (10 mg/kg/day) significantly enhanced the levels of adiponectin and GSH. GW-9662 decreased the mean from 6.34±0.15 to 4.43±0.156 for adiponectin and from 52.86±1.85 to 32.3±0.79 for GSH compared to the Aleglitazar group. MK-886 decreased the mean from 6.34±0.15 to 1.84± 0.12 for adiponectin and from 52.86± 1.85 to 20.61±0.53 for GSH in comparison to the Aleglitazar group.

Effect of Aleglitazar on lipid profile: When compared to the control group, NASH significantly reduced HDL levels with a significant increase in triglyceride and cholesterol levels in the serum. In comparison to the NASH group, the DMSO treatment did not result in any appreciable alterations to the examined parameters. In comparison to the NASH group, Aleglitazar (10 mg/kg/day) significantly reduced triglyceride and cholesterol levels while significantly raising HDL levels. Using GW-9662 increased the mean from 76.69±0.8 to 99.63±0.47 for TG and from 182.36± 0.58 to 199±0.89 for cholesterol and decreased the mean from 42.49±1.01 to 31.29±1.82 for HDL compared to Aleglitazar group. MK-886 increased the mean from 76.69±0.8 to 4.43±0.156 for TG and from 182.36±0.58 to 218.2± 0.73 for cholesterol and decreased the mean from 42.49±1.01 to

23.28±1.06 for HDL in comparison to Aleglitazar group as presented in table 3.

Effect of Aleglitazar on total body weight, liver mass, and ratio: When compared to the control group, NASH significantly increased total body weight, liver mass and decreased the ratio between them. In comparison to the NASH group, the DMSO treatment did not result in any considerable variations to the examined parameters. In comparison to the NASH group, Aleglitazar (10 mg/kg/day) significantly reduced total body weight, liver mass and increased the ratio between them. treatment with GW-9662 increased the mean total body weight to 48.33±0.31, liver mass to 2.35±0.32, and ratio to 4.81±0.03 compared to the Aleglitazar group. Using MK-886 increased the mean of total body weight to 47.66±0.61, liver mass to 2.26±0.04, and ratio to 4.80±0.11 compared to the Aleglitazar group as presented in table 4.

HISTOPATHOLOGICAL FINDINGS

The histological findings revealed normal liver structure, hepatocytes with a central nucleus and eosinophilic cytoplasm, normal central veins, and no steatosis, ballooning, or hepatocyte degeneration [Fig. 1a]. However, the liver in the NASH group exhibited lobular inflammation, macro and microvesicular steatosis, hepatocyte ballooning, and hepatocyte degeneration [Fig.1b]. On examination of DMSO group, it showed the same picture as the NASH group in addition to that some hepatic cells showed a signet ring appearance [Fig. 1c] .Aleglitazar (10 mg/kg) treated groups showed decrease in the number of inflammatory cells and the degree of steatosis with less obvious hepatocyte ballooning and degeneration [Fig. 1d, e& f]. As PPAR-γ antagonist [GW 9662] was administered, improvement of the NASH picture of inflammation, steatosis, and degeneration of hepatocytes was seen but not as better as before its administration, compared with the picture seen with Aleglitazar treated group [10mg/kg] as the number of inflammatory cells, steatosis and hepatocyte degeneration was more [Fig. 1g] and when PPAR-α antagonist [MK 866] was administered, improvement of the NASH picture of inflammation, steatosis, and hepatocytes was observed but not as before its administration, compared with the picture seen with Aleglitazar treated group [10mg/kg] as the number of inflammatory cells, steatosis and hepatocyte degeneration was more, and it was noticed that the picture seen with MK-887 was less good than that seen with PPAR-γ antagonist [GW 9662] [Fig. 1h]. Histopathological scoring was done according to Klenier et al (2005) (21) as described in Table 5, and revealed that, the NASH group showed high pathological scoring (7) as well as the DMSO group (8) compared to the control group (0). Treatment with Aleglitazar reduced the score to (5) compared with the NASH group. Using GW-9662 showed a pathological score of (5) while the MK-886 group showed a pathological score of (6) as shown in table 6.

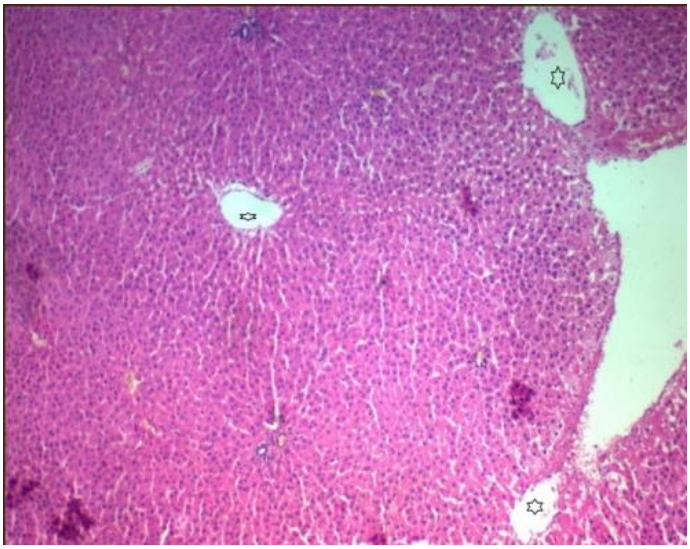

Fig. 1a: Photomicrograph of normal liver tissue showing three central veins surrounded by rows of hepatocytes without hepatocyte ballooning or degeneration (H & E x 400) **[Control group]**

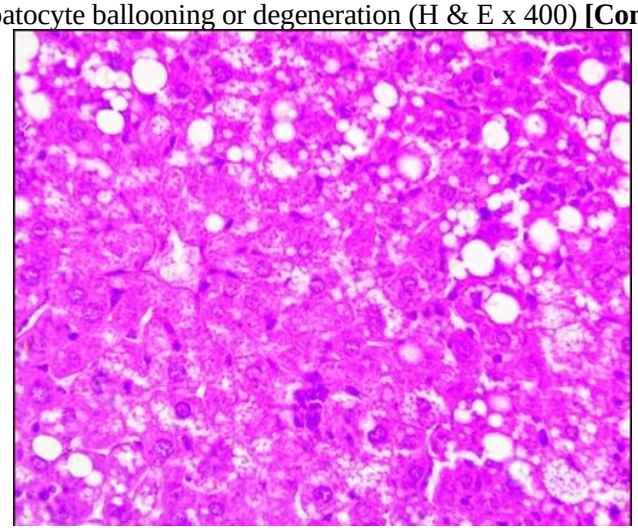

Fig.1b: Photomicrograph of liver tissue taken under a microscope reveals a dilated, congested central vein that is encircled by large clusters of inflammatory cells (H & E x 400) **[NASH group]**

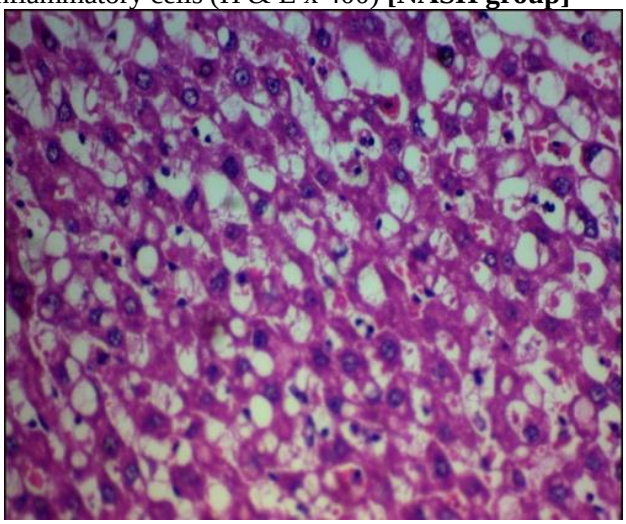

Fig. 1c: Photomicrograph of liver tissue showing severe fatty changes of hepatocytes with vacuolated cytoplasm and signet-ring nuclei (H & E x 400) **[DMSO group]**

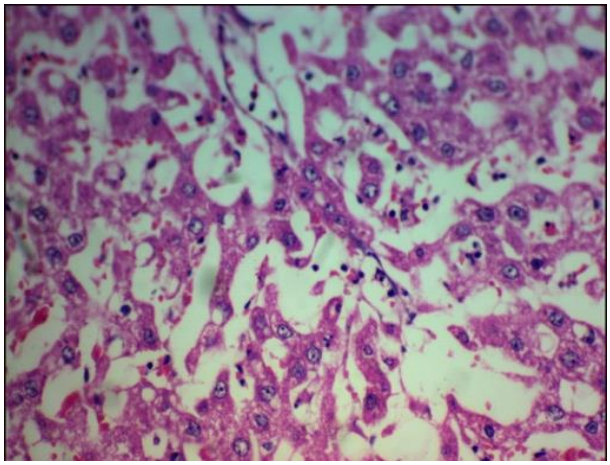

Fig. 1d: Photomicrograph of liver tissue showing fatty changes of hepatocytes and scattered inflammatory cells (H & E x 400) **[Aleglitazar 10 mg].**

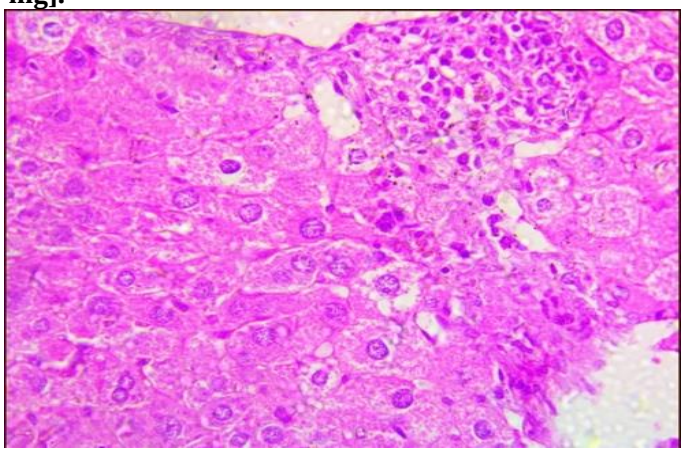

Fig. 1e: Photomicrograph of liver tissue showing fatty changes of hepatocytes and scattered inflammatory cells, mild lobular inflammation, mild ballooning degeneration of some hepatocytes and minimal steatosis (H & E x 400) **[Aleglitazar 10 mg].**

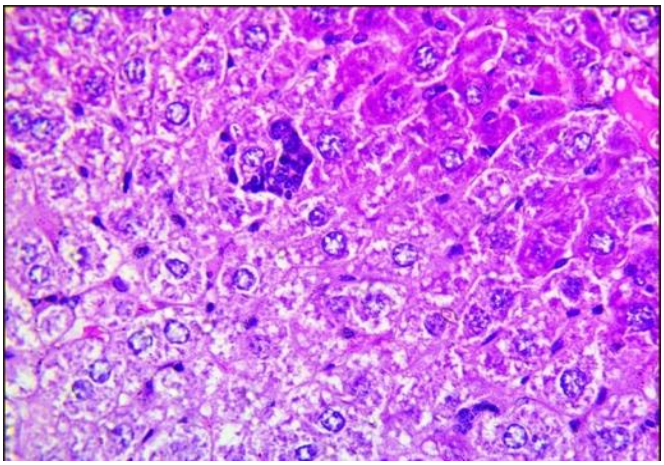

Fig. 1f: Photomicrograph of liver tissue showing scattered inflammatory cells, mild lobular inflammation, and minimal steatosis (H & E x 400) **[Aleglitazar 10 mg].**

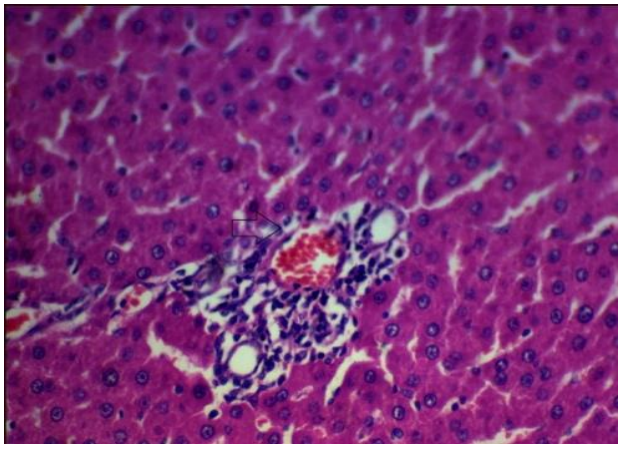

Fig. 1g: Photomicrograph of Liver tissue showing a small portal tract infiltrated with aggregates of inflammatory cells and surrounded by normal hepatocytes (H & E x 400) **[GW 9662 group]**

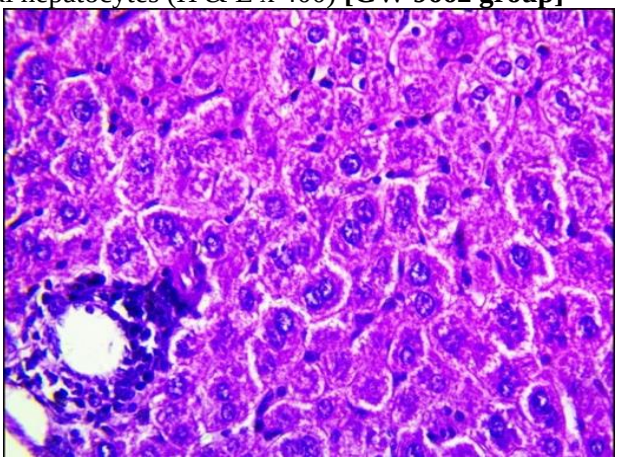

Fig.1h: Photomicrograph of liver tissue showing steatosis, lobular inflammation, ballooning degeneration of hepatocytes (H & E x 400) **[MK 886 group]**

Table 1: Effect of Aleglitazar on serum levels of ALT and AST

n=6	Control	NASH	DMSO 10mg/kg/day	Aleglitazar 10mg/kg/day	GW-9962 1mg/kg/day	MK-886 10mg/kg/day
ALT (U/L)	13.5± 1.23	59.0± 4.12 #	57.0± 4.10 #	25.66± 1.42 #*	33.0± 0.77 $#*	54.0± 1.29 $#
AST (U/L)	17.83± 0.70	68.5± 1.7 #	66.3± 1.23 #	30.16± 0.98 *#	55.33±1.62 $#*	65.33± 1.41 $#

Data represent mean± SEM ALT: alanine aminotransferase, AST: aspartate aminotransferase
NASH: non-alcoholic steatohepatitis, DMSO: dimethyl sulfoxide.
Significant to control group*Significant to NASH and DMSO groups **$** Significant to Aleglitazar 10mg group.

Table 2: Effect of Aleglitazar on serum levels of MPP-1, TNF-α & adiponectin and hepatic GSH

n=6	Control	NASH	DMSO 10mg/kg/day	Aleglitazar 10mg/kg/day	GW-9962 1mg/kg/day	MK-886 10mg/kg/day
MMP-1 (ng/ml)	78.13± 1.51	155.3± 5.33 #	152.4± 5.33 #	81.61± 0.91 #*	103.45±0.89 $#*	146.05±1.55 $#

TNF_α (pg/ml)	17.05± 0.78	107.04± 2.57 #	104.6± 2.57 #	68.46± 1.35 #*	93.37± 1.41 $#*	106.62±1.40 $#
Adiponectin (pmol/ml)	6.92± 0.16	1.9± 0.11 #	2.04± 0.11 #	6.34±0.15 #*	4.43± 0.16 $#*	1.84±0.12 #$
GSH (nmol/mg)	63.86± 0.99	24.34± 0.76 #	23.03± 0.76#	52.86± 1.85#*	32.3± 0.79 $*#	20.61±0.53 #$

Data represent mean± SEM

TNF: tumor necrosis factor, MPP-1: matrix metalloproteinase, GSH: reduced glutathione NASH: non-alcoholic steatohepatitis, DMSO: dimethyl sulfoxide.

Significant to control group * Significant to NASH and DMSO groups.

$ Significant to Aleglitazar 10mg group

Table 3: Effect of Aleglitazar on serum levels of triglyceride, cholestrol and HDL

n=6	Control	NASH	DMSO 10mg/kg/day	Aleglitazar 10mg/kg/day	GW-9962 1mg/kg/day	MK-886 10mg/kg/day
Triglyceride (mg/dl)	55.66± 1.04	125.23± 0.93 #	123.02± 0.99 #	76.69±0.8 #*	99.63± 0.47 #*$	124.8±1.18 #$
Cholestrol (mg/dl)	150.28 ± 2.4	222.30± 1.63 #	224.32± 1.54 #	182.36±0.58 #*	199.0±0.89 #$*	218.2±0.73 #$
HDL (mg/dl)	58.41± 0.73	24.43± 1.05#	25.45± 1.2 #	42.49± 1.01 #*	31.29±1.82 #*$	23.28±1.06 #$

Data represent mean± SEM
HDL: high-density lipoprotein, NASH: non-alcoholic steatohepatitis, DMSO: dimethyl sulfoxide.
#Significant to the control group. *Significant to NASH and DMSO groups.
$Significant to Aleglitazar 10mg group.

Table 4: Effect of Aleglitazar on Total body weight, liver mass and ratio" at sacrifaction time"

n=6	Control	NASH	DMSO 10mg/kg/day	Aleglitazar 10mg/kg/day	GW-9962 1mg/kg/day	MK-886 10mg/kg/day
Total body weight (gm)	44.50±0.92	51.50±0.36 #	52.37±2.003 #	47.17±0.74 #*	47.66±0.61 #$*	48.33±0.31 #$
Liver mass (gm)	1.99±0.38	2.57±0.46 #	2.25±0.05 #	2.25± 0.05 #*	2.26±0.04 #$*	2.35±0.32 #$
Ratio	4.47± 0.01	4.41± 0.03 #	4.59±0.18 #	4.78± 0.07 #*	4.80±0.11 #*$	4.81±0.03 #$

Data represent mean± SEM NASH: non-alcoholic steatohepatitis, DMSO: dimethyl sulfoxide.
#Significant to the control group.*Significant to NASH and DMSO groups.
$Significant to Aleglitazar 10mg group.

Table (5): Constituents of nonalcoholic fatty liver disease activity score [21]

Element	Definition	Score
Steatosis	< 5%	0
	5%-33%	1
	> 33%-66%	2
	> 66%	3
Lobular inflammation	No foci	0
	< 2 foci per 200 × field	1
	2-4 foci per 200 × field	2
	> 4 foci per 200 × field	3
Ballooning	None	0
	Few balloon cells	1
	Many cells/prominent ballooning	2

Table (6): Histopathological scoring

n=6	Control	NASH	DMSO 10mg/kg/day	Aleglitazar 10mg/kg/day	GW-9962 1mg/kg/day	MK-886 10mg/kg/day
Steatosis [0-3]	0	3	2	2	3	2
Lobular inflammation [0-3]	0	3	4	2	1	2
Ballooning [0-2]	0	1	2	1	1	2
Score [0-8]	0	7	8	5	5	6

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is swiftly rising to the top of the list of causes of liver injury around the world (22). When fat accumulation is the magnitude of impairment, it is well-tolerated, however, in a subgroup of individuals, disease advancement occurs leading to liver fibrosis and failure due to hepatic inflammation (23). The liver parenchyma experiences significant collagen deposition because of injury and repair cycles, which can lead to cirrhosis and other observable fibrotic alterations and when steatosis occurs after tissue damage, the condition is referred to as NASH (23). Another frequent and extremely harmful late-stage consequence is the development of liver cancers, particularly hepatocellular carcinoma (24). As a result, there is a growing need for novel medicines for NASH with excellent safety profiles.

Aleglitazar is a brand-new, well-balanced dual PPAR agonist which has a strong and high affinity for both PPAR-α and PPAR-γ (IC50 = 0.0028 M and 0.0046 M, respectively) according to in vitro binding and transactivation experiments (25). Because of its balanced affinity for both receptors, Aleglitazar produces a dual PPAR agonist effect with clinically anticipated properties and a higher level of safety compared to unbalanced dual PPAR agonists that have been linked to adverse consequences such as edema, weight gain, and renal problems (5). Herein, the current work was designed to study the possible curative effect of Aleglitazar against NASH and to determine whether PPAR may have played a role in this outcome.The results of the current study showed a significant increase in ALT and AST serum levels in the NASH group compared to the control group. In addition, NASH was linked to a disrupted histopathological image of the liver, which was manifested by hepatocyte inflammation, fatty infiltration, degeneration, and ballooning. Similarly, (26) reported increased ALT and AST levels in nonalcoholic steatohepatitis rats with the destruction of hepatic lobules and inflammatory cellular infiltration. Moreover, (27) showed that there was an increase in the level of hepatic transaminases in the NASH, obese, ovariectomized mice model. In the same context, (28) detected the existence of steatosis, hepatocellular ballooning, and lobular inflammation in NAFLD.Furthermore, Song et al showed that hepatic steatosis induced by a high-fat diet increased the level of AST, ALT, and alkaline phosphates, and increased the level of total cholesterol, triglyceride, and LDL (29).Our work demonstrated that Aleglitazar reduced the levels of AST, ALT and improved hepatic histopathological alterations, and following the previous findings, (8) showed that Aleglitazar (0.3mg/kg) reduced ALT levels in a rat model of increasing hepatic and splanchnic problems in cirrhotic rats with portal hypertension through reducing TNF-overproduction and systemic/local inflammation. Furthermore, (30) confirmed that Saroglitazar, a dual PPAR agonist, given at a dose of (3mg/kg) decreased the serum level of AST and ALT and improved NASH histopathological alterations. Additionally, (31) demonstrated that Saroglitazar (3 mg/kg) improved liver histopathology in experimental NASH models and avoided liver fibrosis through the modulation of inflammatory cytokines and adiponectin.It's well-known that HFD causes free fatty acids to undergo mitochondrial β-oxidation with excess electron flow consuming cytochrome c

oxidase resulting in accumulation of reactive oxygen species which elicit proinflammatory signaling of NF-κB transcriptional factor, and so inducing NF-κB-dependent proinflammatory molecules, like TNF-α (32). Additionally, through an NF-κB/p65 dependent pathway, TNF-α stimulates the production of MMPs, primarily, MMP-1 and MMP-3. (33). MMPs are proteases that can remodel the extracellular matrix and has been shown that they also have significant physiologic impacts on immunological modulation, cell signaling, and transcriptional control (34).

In the same context, reactive oxygen species buildup results in an oxidative stress state with an imbalance between defense components like GSH and ROS, leading to depletion of antioxidants like glutathione (35). Fatty acids oxidation is known to be controlled by adiponectin, which has a detrimental relationship to obesity and its comorbidities (36). Adiponectin binds to two different receptors named adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) at the cellular level, activating the PPAR- α and mitogen-activated protein kinase (MAPK) receptor inside the cell (37). AdipoR1 and AdipoR2 expression is significantly decreased in an obesity state and so adiponectin expression, leading to insulin resistance (38). According to the current findings, NASH was linked to a drop in HDL levels with increased triglyceride, LDL, and cholesterol levels. Similarly, [39] reported that HDL levels fell in a rat model of NAFLD with elevated triglyceride, LDL, and cholesterol levels which led to the accelerated and more severe hepatic effect. Our results showed that Aleglitazar decreased LDL, triglyceride, and cholesterol levels with the restoration of HDL levels. Similarly, Aleglitazar (0.03 mg/kg) increased HDL by 125% as described by Hansen et al (40). Moreover, (40) reported that Aleglitazar (0.03 mg/kg) abridged triglyceride levels by an average of 89%, furthermore, it reduced LDL levels (41%) and amplified levels of apolipoprotein A-I (17%). In the same context, (41) detected that Aleglitazar, given at a dose of (0.3mg/kg) lowered cholesterol and triglyceride levels in a fatty diabetic rat model through prevention of pancreatic beta cell apoptosis and preservation of insulin.The current findings revealed a decrease in the serum levels of adiponectin and the amount of GSH in the liver. Similarly, (42) supported this finding as he observed that NAFLD was linked to lower levels of GSH in the liver and adiponectin in the blood and explained this as oxidative stress, which accompanied NAFLD, led to lower GSH level, the antioxidant which is censoriously involved in the protein disulfide bond formation and oligomerization of adiponectin. In this context, Aleglitazar increased adiponectin levels in our present study and this result is in line with Jain et al who confirmed that saroglitazar, the dual PPARα/γ agonist, given at a dose of (0.01-3 mg/kg) increased the level of adiponectin and improved the lipid profile in a rat pre-clinical model (43). Similarly, (40) confirmed that Aleglitazar increased the mean levels of adiponectin by 158% from 12.8 µg/mL at baseline to 33.0 µg/mL in a primate model of metabolic syndrome. Moreover, Chen et al showed that Aleglitazar decreased reactive oxygen species and increased total antioxidant capacity and so protected the rat cardiomyocytes from hyperglycemia (7). TNF- α, the inflammatory cytokine, showed significant increase with high fat diet use and this agree with Zou et al who studied the effect of high fat emulsion induced NASH and recorded increase in TNF- α level with PPAR-α down regulation (44). In this aspect, the current investigation showed that Aleglitazar lowered TNF-α serum level and as revealed by previous work done, Tsai et al stated that Aleglitazar decreased TNF-α level in a rat model of hepatic cirrhosis with portal hypertension through PPAR- γ activation (8). In our current study, the MPP-1 level in the NASH group was elevated and similar findings, which were made by Okazaki et al, confirmed that there was elevated MMP levels in NASH and stated the contribution of tissue inhibitors of metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs) to hepatic fibrosis and carcinogenesis (45). Additionally, Ando et al confirmed that serum MMP-1 strongly represented NASH activity, but it lacks any relation to hepatic fibrosis (46). In this context, Aleglitazar decreased the MMP-1 level in our study and this was supported by the

findings of Di Paola et al. who investigated the effectiveness of adelmidrol, a dual PPAR agonist against osteoarthritis, and reported a decrease in MMP-1 level through decrease in TNF-α and interleukin-1 signaling (47). It is important to note that PPARs are nuclear receptors that control a wide range of downstream signals, including metabolic, inflammatory, and oxidative stress pathways. ROS and inflammatory cytokines control the expression of PPAR. (48)

Our results showed that the protective effect of Aleglitazar was attenuated as we used PPAR-γ and PPAR- α blockers suggesting a potential role of both PPAR in our model. However, MK-886, the PPAR-α blocker, showed more attenuation of Aleglitazar's effect. In this context, Tsai et al stated that the PPAR-γ blocker (GW 9662) but not PPAR- α blocker (GW-1929), reduced the protective effect of Aleglitazar against hepatic cirrhosis with portal hypertension and this result is against ours as it showed that aleglitazar's protective effect was mainly via PPAR-γ activation (8). Additionally, Werner et al showed that the effects of Aleglitazar in increasing the number and function of endothelial progenitor cells were only partially reduced by GW9662, which meant that Aleglitazar's protective effect was mediated by both PPAR α and γ (49).

CONCLUSION

Considering all previous findings, we showed for the first time, according to our knowledge, that Aleglitazar stopped the advancement of NASH brought on by a high-fat diet. This impact was partly attributed to its PPAR-mediated, mainly PPAR- α, antioxidant and anti-inflammatory properties.

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Compliance with ethical standards: protocols of the experiment were authorized by the Zagazig University's institutional animal care and usage committee (ZU-IACUC/3/F/82/2022) and followed the ethical principles for laboratory animal research.

Conflict of interest: There are no conflicts of interest, according to the author.

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