

AMELIORATIVE EFFECT OF PIOGLITAZONE AND ROUSVASTATIN ON HFD/STZ-INDUCED HEPATIC INJURY IN RATS

Short title: Effect of Pioglitazone and Rosuvastatin on Diabetic Liver Injury

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ABSTRACT

Approximately 90% of people with diabetes mellitus (DM) are estimated to have type 2 diabetes (T2DM), which causes hepatic dysfunction. Pioglitazone is an antidiabetic medication that ameliorates diabetic liver dysfunction. Rosuvastatin is an antihyperlipidemic drug that alleviates the hepatic damage caused by T2DM. This study's objective was to look into the therapeutic potential of pioglitazone and rosuvastatin in combination in the management of diabetic liver injury induced by a high-fat diet (HFD) and streptozotocin (STZ). Forty male albino rats weighing 150 ± 15 g were used and split into five groups (8 rats in each). Groups II, III, IV, and V undergo induction of T2DM by feeding rats with HFD for 1 month and injecting a low dose of STZ. Group I, however, remained a negative control. Group II was not given any treatment; Group III was treated with pioglitazone; Group IV was treated with rosuvastatin; and Group V was treated with a combination of pioglitazone and rosuvastatin. After 28 days, serum liver enzyme levels and protein profiles were assessed. Expression of the inflammatory cytokines and Cytochrome P2E1 (CYP2E1) genes in the liver was also evaluated. Furthermore, hepatic tissue underwent histopathological examination. The results demonstrated considerable enhancement of the parameters under investigation in groups that were treated with pioglitazone, rosuvastatin alone, or their combination in comparison to the diabetic group. However, the effects of combined therapy are greater than those of monotherapy. In conclusion, concomitant administration of pioglitazone and rosuvastatin had greater potential for attenuating diabetic liver injury than monotherapy.

Key words: Diabetes mellitus, hepatic dysfunction, antihyperlipidmic, inflammatory cytokines, pioglitazone, rosuvastatin.

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INTRODUCTION

Diabetes mellitus is an illness distinguished by persistent hyperglycemia. The primary cause of T2DM is reduced insulin sensitivity in target tissues and decreased β -cell activity with inability to generate enough insulin. Numerous organs are impacted by T2DM, including the liver, which is essential for controlling the metabolism of fats, carbohydrates, and proteins (Shibabaw *et al.*, 2019). Standard values of blood glucose are achieved through the conversion of glucose to glycogen, facilitated by the liver, which is essential in maintaining glucose homeostasis. The process by which glucose is converted to glycogen is changed in the case of T2DM. Alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are markers of liver integrity. They are increased in liver dysfunction because of their release from the liver into the blood. Furthermore, elevated levels of these enzymes are thought to be an indicator of liver damage in T2DM (Zhang *et al.*, 2023). Numerous investigations have revealed an elevation in inflammatory mediators, like tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), in individuals with DM (Haddadi and Cheraghi-Poor, 2023). In addition, high levels of the CYP2E1 enzyme may be the main source of oxidative stress and stress-mediated liver damage in diabetes (Maksymchuk *et al.*, 2017).

Pioglitazone is a thiazolidinedione derivative that can enhance the metabolism of glucose and, via decreasing insulin resistance in type 2 diabetic patients' adipose, liver, and skeletal muscle tissues, has been utilized as an antidiabetic medication all over the world. It primarily acts by activating peroxisome proliferator-activated receptor- γ , which raises adiponectin levels and lowers TNF- α and free fatty acids released by adipocytes. Pioglitazone, also ameliorates liver dysfunction in T2DM as it decreases liver enzymes (Asakawa *et al.*, 2023).

Rosuvastatin is a fully synthetic statin and functions as a competitive inhibitor of the 3-hydroxy-3-methylglutaryl coenzyme (Guo and Cai, 2021). Its therapeutic application not only involves the treatment of hypercholesterolemia and coronary artery disease but also normalizes serum transaminases in nonalcoholic fatty liver disease (NAFLD) patients with hyperlipidemia (Abdul-Kafy *et al.*, 2019).

This study was aimed to investigate the potential advantages of co-delivery of rosuvastatin and pioglitazone for the treatment of diabetic liver damage induced by HFD and STZ administration.

MATERIALS AND METHODS

Chemicals, reagents and kits:

The streptozotocin was purchased from Sigma-Aldrich, USA. Citric acid and sodium citrate were bought from SD Fine-Chem Ltd., Mumbai, India. Pioglitazone was derived from Unipharma Company, Egypt. Rosuvastatin was obtained from AstraZeneca, Egypt. Isoflurane 1% was acquired from Hospira, Inc., USA. Kits for albumin, total protein, ALT, ALP, and AST were acquired from Spinreact, Spain. RNeasy mini extraction kits were obtained from Qiagen, Germany. Complementary DNA (cDNA) synthesis kits and SYBR Green PCR Master Mix were bought from Thermo Scientific, USA. DNase I was purchased from Fermentas, Lithuania. All the primers were synthesized by Applied biotechnology, Egypt.

Experimental animals:

Fourty adult male albino rats weighing between 150 ± 15 grams were acquired from the Assiut University Faculty of Veterinary Medicine's animal house, Egypt. The study was carried out under the approved ethical regulation of the Faculty of Medicine, Assiut University's Institutional Review Board, No. 04-2023-100075. Rats were kept at cages in standard laboratory conditions with food and water *Ad Libitum*. Before beginning the

research, rats were given two weeks to adapt to regular laboratory surroundings (Sotohy *et al.*, 2019).

Induction of DM:

The experimental T2DM in rats was induced by HFD and low dose of STZ. HFD contained 58% fat, 25% protein, and 17% carbohydrate as a percentage of total calories. Rats were fed with HFD for 4 weeks. Then, animals, after an overnight fast, received intraperitoneal injections of a low dosage of STZ (35 mg/kg B.W.) dissolved in 0.1 M citrate buffer (pH 4.5). 72 hours after STZ injection, blood samples were obtained from the tail, and the fasting serum glucose levels were calculated using the digital glucometer. Rats that had a fasting blood glucose level of 250 mg/dl or higher were regarded as diabetic and chosen for the experiment (Furman, 2015, Abdel-Mohsen *et al.*, 2023). One day after confirmation of diabetes induction, pioglitazone (10 mg/kg) and rosuvastatin (10 mg/kg) and their combination were used for treatment for 4 weeks.

Experimental design:

Five groups of rats (8 rats per group) were allocated in this study:

- **Group I (-ve control):** Rats in this group received the standard laboratory diet and were injected intraperitoneally with STZ solvent: 0.1 M citrate buffer, pH 4.5.
- **Group II (Non-treated diabetic rats):** HFD/STZ-induced diabetic rats were given no treatment.
- **Group III (Diabetic + pioglitazone):** Diabetic rats were administered pioglitazone orally at a dosage of 10 mg/kg once daily for 4 weeks.
- **Group IV (Diabetic + rosuvastatin):** Diabetic rats received treatment with rosuvastatin orally at a

dosage of 10 mg/kg once daily for 4 weeks.

- **Group V (Diabetic + pioglitazone and rosuvastatin):** Diabetic rats were administered pioglitazone orally at a dosage of 10 mg/kg, orally and after one hour, they were given rosuvastatin orally at a dosage of 10 mg/kg once daily for 4 weeks.

Sample preparation:

Serum sample:

After the end of the experiment, the overnight-fast rats anesthetized with isoflurane. Blood samples were taken by capillary tube from orbital plexus in plain tubes. Serum was separated by centrifugation for 15 minutes at 3000 rpm, and it was then kept at -20°C until the time of the biochemical examination (Abdelrahim *et al.*, 2019).

Liver sample:

Following the collection of blood samples, the animals were sacrificed, and the liver was dissected and divided into two portions. The first portion was stored at -80°C for IL-1 β , TNF- α , and CYP2E1 gene expression, and the second liver portion was kept in 10% neutral buffered formalin to be examined histopathologically.

Liver enzymes activity assays:

Determination of serum AST, ALT, and ALP levels were performed via AST, ALT, and ALP enzymatic commercial kits according to the manufacturer's protocol (Bergmeyer *et al.*, 1986).

Serum protein profile assays:

Serum total protein levels were determined using a total protein colorimetric kit (Biuret Reagent) according to the manufacturer's protocol (Lubran, 1978). Further, serum albumin was estimated using the albumin commercial kit, according to Dumas *et al.* (1971).

Evaluation of gene expression of inflammatory mediators and CYP 2E1 by real time-PCR:

Total RNA was extracted from the tissue samples in accordance with the manufacturer's instructions using the RNeasy mini extraction kit. The spectrophotometry was employed at wavelength 260 to determine the concentration and the 260:280 ratio to select the pure samples that were in the range of 1.8 and 2.0. After then, DNase I was used to clean up the DNA contamination, and cDNA was produced using a RevertAid First Strand cDNA Synthesis Kit in line with the manufacturer's instructions. The primer sets for measuring the mRNA levels of particular genes were generated using the Rattus

norveicus sequences present in Gen Bank (Table 1). The primer3 tool was used to build the primers. SYBR Green PCR Master Mix was used in real-time PCR analysis to assess the relative expression of the selected genes (Thermo Scientific Cat number: 4309155). Applied Biosystems' ABI Prism StepOnePlus Real-Time PCR System as directed by the manufacturer. For each sample, the PCR reactions were conducted twice. The expression levels were normalized for the housekeeping gene beta-actin. The data on gene expression were analyzed using the DDCT method.

Table 1: The primer sets used to determine the amounts of TNF- α , IL-1 β , and CYP2E1.

Gene	Sense	Antisense	Accession No.
IL-1 β	TTGAGTCTGCACAG TTCCCC	GTCCTGGGGAAGGCAT TAGG	NM_031512.2
TNF- α	ACACACGAGACGCT GAAGTA	GGAACAGTCTGGGAAG CTCT	NM_012675.3
Cyp2E1	ATGAGTTTTCTGGA CGGGGG	TTTGATGCGGGCCTCA TTA	NM_031543.2

Histopathological study of hepatic tissue:

Liver specimens were collected and immediately fixed for 24 hours at ambient temperature in 10 % neutral buffered formalin. Subsequently, the samples underwent ethyl alcohol dehydration at increasing rates, followed by a 6-hour xylol clearing period. Subsequently, The samples were then blocked in hard paraffin and cut into sections at a thickness of 5 microns, and stained with hematoxylin and eosin (H&E) for light microscope inspection (M. *et al.*, 2024).

Statistical analysis:

Graph Pad Prism 8 (Graph Pad software Inc., San Diego, USA) was used for data analysis. One-way analysis of variance (ANOVA) is used to determine the statistical significance of differences between the treated groups and the Tukey test was used as a post hoc analysis. P-value of less than 0.05 was considered statistically significant. The means \pm standard errors of means (SEM) were applied to show the data.

RESULTS

Table 2: Effects of single and combined administration of pioglitazone and rosuvastatin on serum AST, ALT, ALP, total protein, and albumin of diabetic rats.

	-ve control rats	Non-treated diabetic rats	Diabetic + pioglitazone	Diabetic + rosuvastatin	Diabetic+ pioglitazone+rosuvastatin
Serum AST	33.04 ± 2.09	220.9 ± 3.67 ****	98.5 ± 1.25 #####	95.7 ± 2.3 #####	53.55 ± 1.59#### @@@&&&&
Serum ALT	34.16±2.05	201.1± 3.4 ****	78.4 ± 2.4#####	78.8 ± 3.16 #####	54.04 ± 1.7 ####@@@@&&&&
Serum ALP	134.2 ± 3.9	485.9 ± 4.7 ****	256.7 ± 2.25 #####	277.1 ± 3.56 #####	235.1 ± 3.27 ####@@@@&&&&
Serum total protein	6.99 ± 0.212	3.74 ± 0.155 ****	5.11 ± 0.1 #####	4.9 ± 0.15 #####	5.36 ± 0.13 #####
Serum albumin	4.01 ± 0.126	1.99 ± 0.09 ****	2.77 ± 0.065 #####	2.86 ± 0.08 #####	3.01 ± 0.064 #####

For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). #####: Significantly different (P < 0.0001) from untreated diabetic rats. @@@@: a significant difference from diabetic + piog. (P < 0.0001). &&&&: a significant difference from diabetic + rosuv. (P < 0.0001). Piog. (pioglitazone). Rosuv.(rosuvastatin).

Effect of single and combined administration of pioglitazone and rosuvastatin on serum liver enzymes levels and serum protein profile of diabetic rats:

Serum levels of AST, ALT, and ALP were found to be significantly higher, while total protein and albumin were significantly lower in diabetic rats that were left untreated compared with -ve control rats. Conversely, as compared to diabetic rats not receiving treatment, the blood levels of AST, ALT, and ALP showed a highly significant

reduction, but total protein and albumin showed a highly significant increase in rats treated with pioglitazone, rosuvastatin, or a combination of these drugs. Diabetic rats that were treated with a combination of pioglitazone and rosuvastatin revealed a significant decrease in AST, ALT, and ALP with no significant change in total protein or albumin in comparison to diabetic rats treated with pioglitazone or rosuvastatin alone. These findings are presented in Table (2) and are shown in figure (1), (2), (3), (4) and (5).

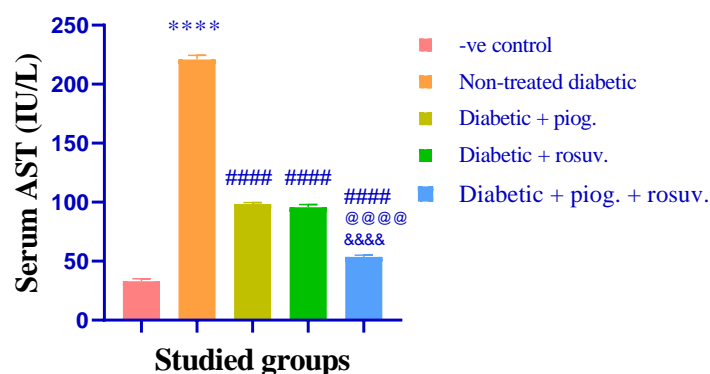


Figure (1): Effects of single and combined administrations of pioglitazone and rosuvastatin on the serum AST of diabetic rats. For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). #####: Significantly different (P < 0.0001) from untreated diabetic rats. @@@@: a significant difference from diabetic + piog. (P < 0.0001). &&&&: a significant difference from diabetic + rosuv. (P < 0.0001). Piog. (pioglitazone). Rosuv.(rosuvastatin).

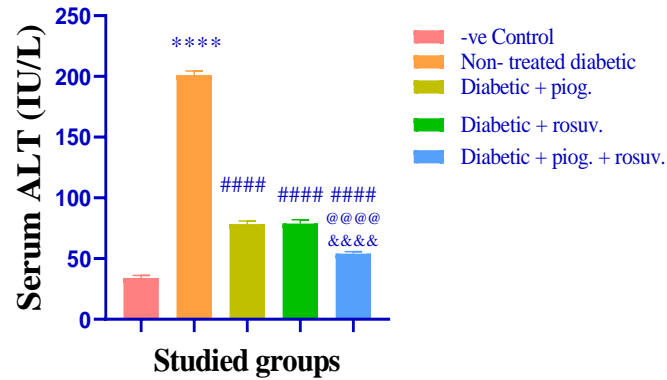


Figure (2): Effect of single and combined administration of pioglitazone and rosuvastatin on the serum ALT of diabetic rats. For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). ####: Significantly different (P < 0.0001) from untreated diabetic rats. @@@@: a significant difference from diabetic + piog. (P < 0.0001). &&&&: a significant difference from diabetic + rosuv. (P < 0.0001). Piog. (pioglitazone). Rosuv.(rosuvastatin).

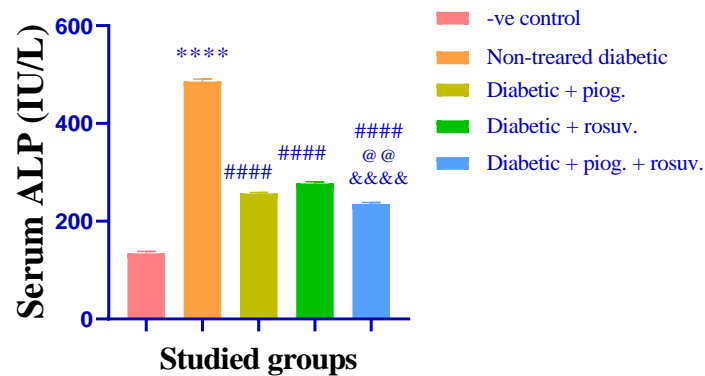


Figure (3): Effects of single and combined administrations of pioglitazone and rosuvastatin on the serum ALP of diabetic rats. For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). ####: Significantly different (P < 0.0001) from untreated diabetic rats. @@: a significant difference from diabetic + piog. (P=0.0015). &&&&: a significant difference from diabetic + rosuv (P < 0.0001). Piog. (pioglitazone). Rosuv.(rosuvastatin).

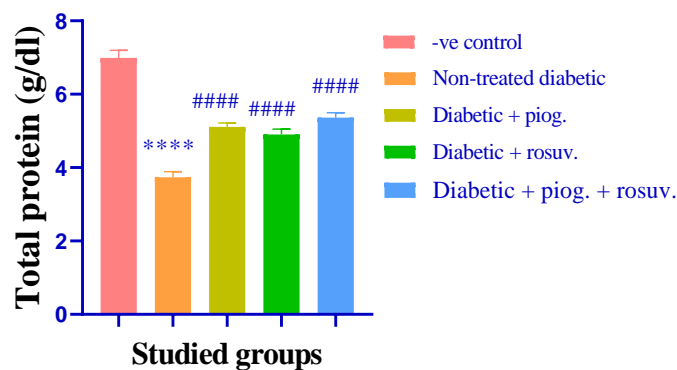


Figure (4): Effect of single and combined administration of pioglitazone and rosuvastatin on serum total protein of diabetic rats. For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). ####: Significantly different (P < 0.0001) from untreated diabetic rats. Piog. (pioglitazone). Rosuv.(rosuvastatin).

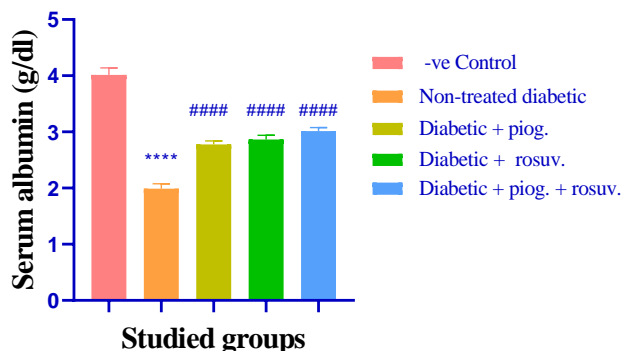


Figure (5): Effect of single and combined administration of pioglitazone and rosuvastatin on the serum albumin of diabetic rats. For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). #####: Significantly different (P < 0.0001) from untreated diabetic rats. Piog. (pioglitazone). Rosuv.(rosuvastatin).

Effect of single and combined administration of pioglitazone and rosuvastatin on gene expression of hepatic inflammatory markers:

Induction of diabetes in rats has led to a significant increase in TNF-α and IL-1β levels in their livers. while treatment with pioglitazone, rosuvastatin, or combination therapy of them for 28 consecutive days was found to produce a significant reduction in raised hepatic

TNF-α and IL-1β levels in the diabetic rats. Diabetic rats that received pioglitazone and rosuvastatin together revealed a significant reduction in TNF-α and IL-1β in comparison to diabetic rats treated with pioglitazone alone and showed a significant reduction in IL-1β with no change in TNF-α levels in comparison to diabetic rats treated with rosuvastatin alone. These results are given in Table (3) and figure (6) and (7).

Table 3: Effect of single and combined administration of pioglitazone and rosuvastatin on the transcript levels of hepatic TNF-α, IL-1β, and CYP 2E1 in diabetic rats.

	-ve control	Diabetic non-treated rats	Diabetic + pioglitazone	Diabetic + rosuvastatin	Diabetic+ pioglitazone+rosuvastatin
Hepatic TNF-α	1 ± 00	6.8 ± 0.21 ****	5.6 ± 0.06 ##	5.1 ± 0.1 #####	4.8 ± 0.2 ##### @
Hepatic IL-1β	1 ± 00	7.7 ± 0.07 ****	5.8 ± 0.08 #####	5.6 ± 0.1 #####	4.3 ± 0.04 ##### @@@@ &&&&
Hepatic CYP2E1	1 ± 00	9.7 ± 0.03 ****	7.5 ± 0.07 #####	7.7 ± 0.1 #####	6.1 ± 0.3 ##### @@@@ &&&&

For every group (n = 8), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). #####: Significantly different (P < 0.0001) from untreated diabetic rats. @@@@: a significant difference from diabetic + piog. (P < 0.0001). &&&&: a significant difference from diabetic + rosuv. (P < 0.0001). Piog. (pioglitazone). Rosuv.(rosuvastatin).

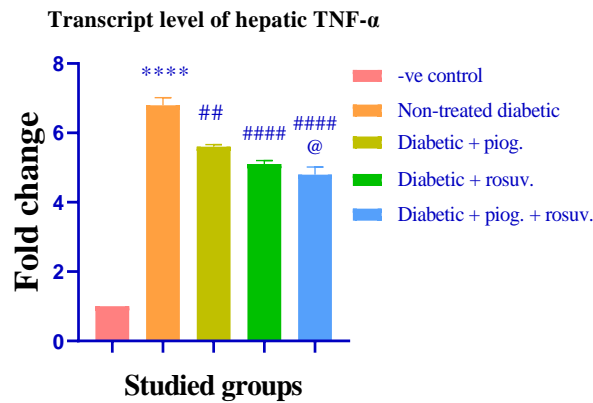


Figure (6): Effect of single and combined administration of pioglitazone and rosuvastatin on hepatic TNF-α in diabetic rats. For every group (n = 3), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). #####: Significantly different (P < 0.0001) from untreated diabetic rats. @: a significant difference from diabetic + piog. (P = 0.0206). Piog. (pioglitazone). Rosuv.(rosuvastatin).

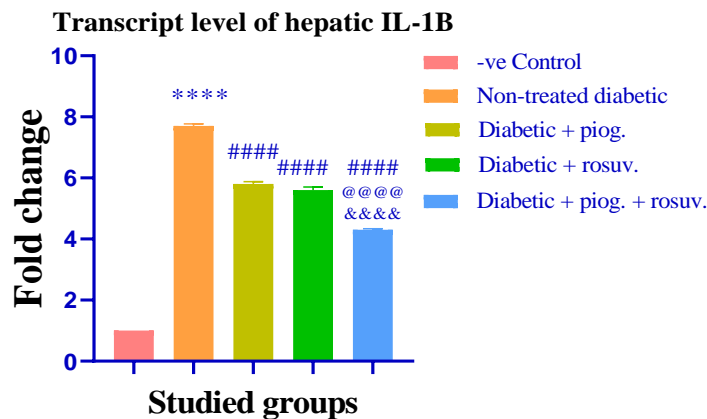


Figure (7): Effect of single and combined administration of pioglitazone and rosuvastatin on hepatic IL-1β of diabetic rats. For every group (n = 3), the data displays the mean ± SEM. ****: a significant difference from the -ve control (P < 0.0001). #####: Significantly different (P < 0.0001) from untreated diabetic rats. @@@@: a significant difference from diabetic + piog. (P < 0.0001). &&&&: a significant difference from diabetic + rosuv. (P < 0.0001). Piog. (pioglitazone). Rosuv.(rosuvastatin)

Effect of single and combined administration of pioglitazone and rosuvastatin on the transcript level of CYP2E1 in the liver of diabetic rats:

CYP2E1 was significantly increased in the diabetic rats' livers, which were not treated in contrast to -ve control rats. However, diabetic rats that received pioglitazone, rosuvastatin, or their combination for 28

days showed a reduction that was significant in comparison with the diabetic group that was not receiving treatment. In addition, pioglitazone and rosuvastatin coadministration elicited a significant decrease in hepatic CYP2E1 in comparison to a single administration of pioglitazone or rosuvastatin.

Transcript level of hepatic CYP2E1

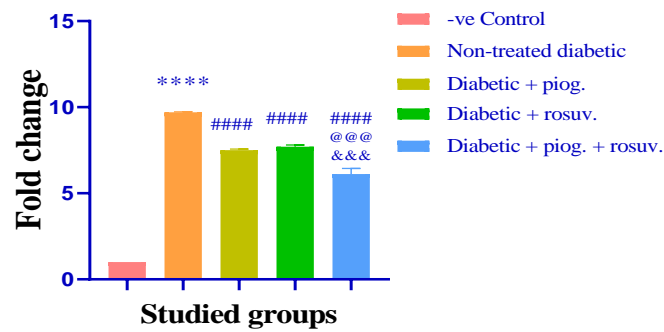


Figure (8): Effect of pioglitazone, rosuvastatin and their combination on transcript level of hepatic CYP2E1 of diabetic rats. For every group (n = 3), the data displays the mean \pm SEM. ****: a significant difference from the -ve control ($P < 0.0001$). ####: Significantly different ($P < 0.0001$) from untreated diabetic rats. @@@: a significant difference from diabetic + piog. ($P = 0.0006$). &&&: a significant difference from diabetic + rosuv. ($P = 0.0002$). Piog. (pioglitazone). Rosuv.(rosuvastatin).

Histopathological investigation of hepatic tissue in different experimental groups:

The hepatic tissues of the control group showed normal hepatic parenchyma divided into lobes. Every lobe is separated into many typical lobules. These are composed of sinusoids and plates of parenchymal cells, hepatocytes, radially arranged around a central vein. Lobules are not clearly distinguished from one another (Figure. 9). While severely congested portal blood vessels and sinusoids were detected in the diabetic group, also bile ductal hyperplasia with different degrees of vacuolar degeneration of the hepatic parenchyma, especially around the central veins. Some cases revealed focal aggregation of lymphocytic cells in between hepatocytes,

injury of the intima of blood vessels causing hemorrhage, and activation of von Kuffer cells (Figure 10). Regarding to the diabetic group treated with pioglitazone, alteration of hepatic tissues was presented by minimal centrilobular necrosis with pycnotic nuclei of some hepatocytes. In addition to dilated blood sinusoids (Figure. 11). The diabetic rats that received rosuvastatin showed normal hepatic parenchyma with mild lymphocytic cell infiltrates. Also, mild vacuolar degeneration and dilated sinusoids were noticed (Figure. 12). By combining the two drugs (pioglitazone and rosuvastatin) in diabetic rats, there was an observable improvement in the microscopy of the liver. Only mild congestion of portal blood vessels was seen (Figure. 13).

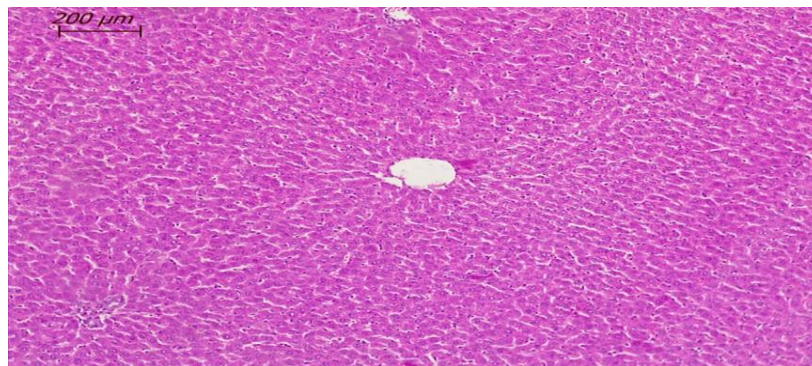


Figure (9): liver from rat (3 months) (-ve control group) showing normal hepatic parenchyma divided into lobes Every lobe is separated into many typical lobules. These are composed of sinusoids and of plates of parenchymal cells, hepatocytes, radially arranged around a central vein. Lobules are not clearly distinguished from one another. (H&E, scale bar: 200 μ m).

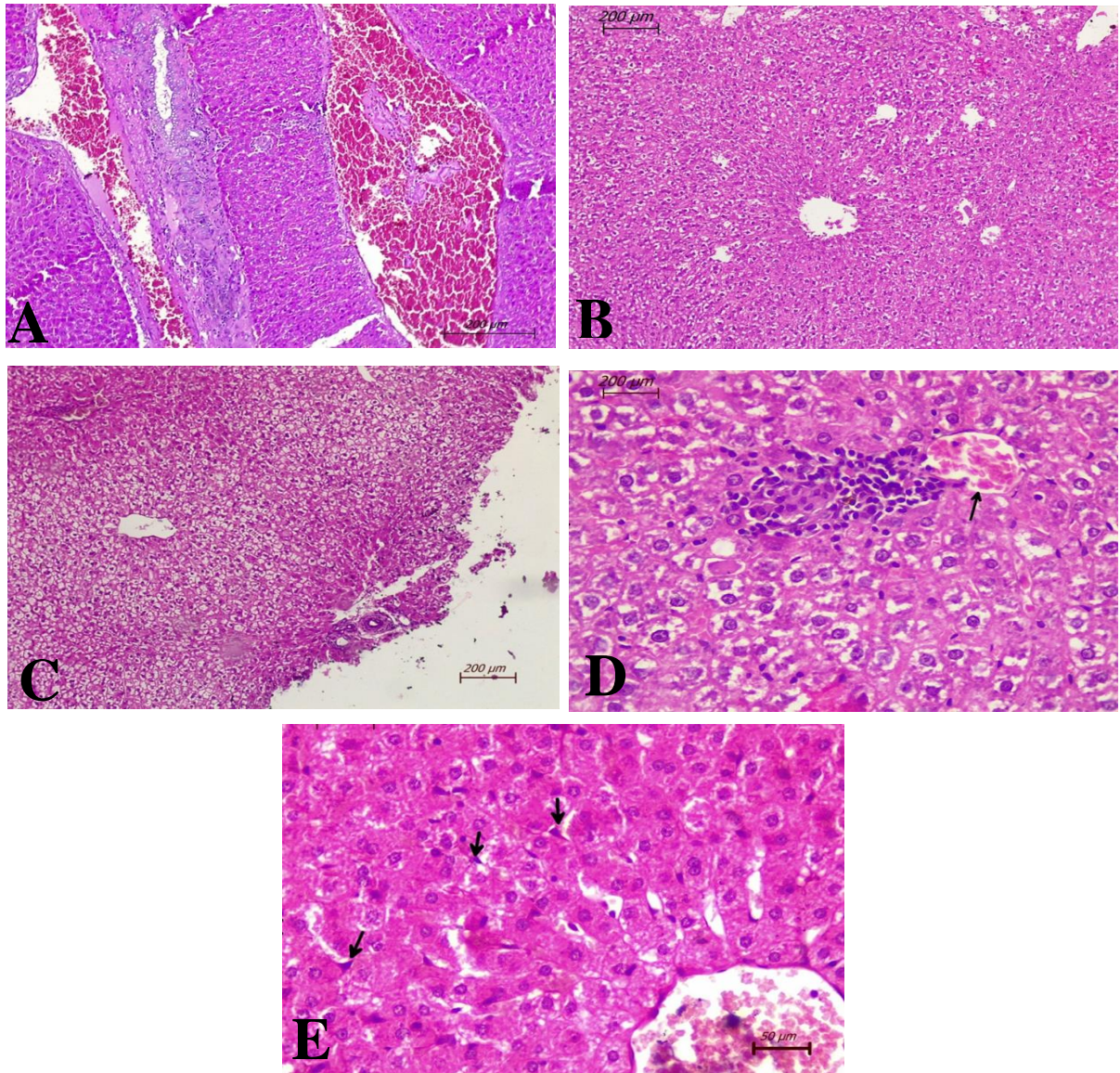


Figure (10): liver from rat (3 months) (Non-treated diabetic group) showing: (A) normal hepatic parenchyma with severely congested blood vessels and bile ductal hyperplasia, (B) mild vacuolar degeneration of hepatic parenchyma especially around central veins, (C) severe vacuolar degeneration of hepatic parenchyma especially around central veins, (D) vacuolar degeneration of hepatic parenchyma, focal aggregation of lymphocytic cells and injury of intima of blood vessels (arrow). A, B, C and D: H&E, scale bar: 200 μm, (E) activation of von kuffer cells (arrows). (H&E, scale bar: 50 μm).

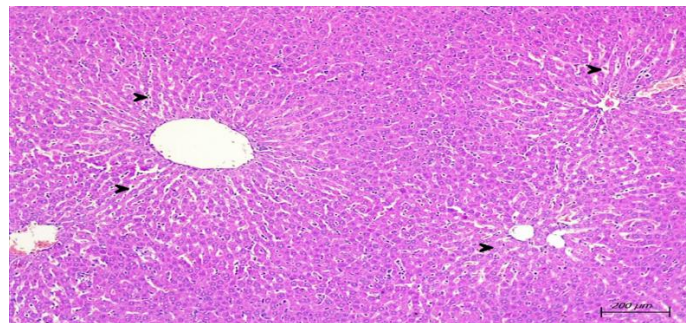


Figure (11): liver from rat (3 months) (Diabetic + pioglitazone) showing minimal centrilobular necrosis (arrow head), dilated blood sinusoids with pycnotic nuclei of some hepatocytes (H&E, scale bar:200 μm).

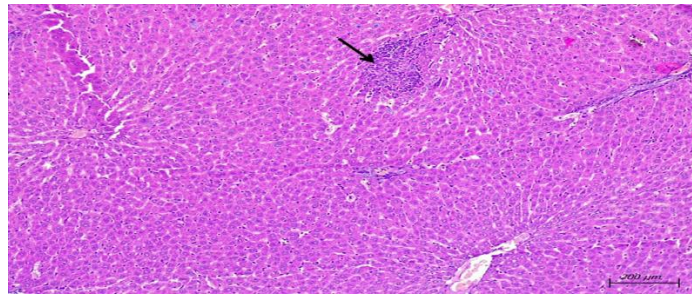


Figure (12): liver from rat (3 months) (Diabetic + rosuvastatin) showing normal hepatic parenchyma with mild lymphocytic cell infiltrates (arrow). Lobules are indistinctly separated from one another. (H&E, scale bar: 200 μ m).

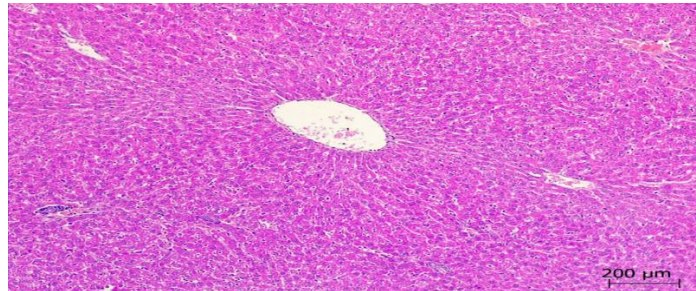


Figure (13): liver from rat (3 months) (Diabetic + pioglitazone + rosuvastatin) showing normal hepatic parenchyma with mild congestion of sinusoids and blood vessels (H&E, scale bar: 200 μ m).

DISCUSSION

Diabetes impacts many endogenous organs, but the liver is one of the most vital ones (Ciardullo and Perseghin, 2022). So, this experiment was aimed to determine the therapeutic impact of pioglitazone and rosuvastatin, either alone or combined, on liver damage caused by the HFD and STZ.

Transaminases, including ALT and AST, are very sensitive biomarkers linked to hepatic dysfunction (Yazdi *et al.*, 2019). Significant increases in AST and ALT activity in the serum of diabetic rats may be mainly due to their leaking into the bloodstream from the hepatocyte's cytoplasm as a result of STZ-related liver damage (Abdel-Mohsen *et al.*, 2023). Conversely, elevated serum ALP levels suggested biliary obstruction, most likely as a result of edematous compression (Giribabu *et al.*, 2015). The DM model's observed rise in all liver enzyme activities is consistent with the findings of Atta *et al.* (2023), who found that HFD-fed/STZ rats had elevated AST, ALT, and ALP activities. Untreated diabetic rats showed altered protein metabolism as seen by a decrease in

blood levels of albumin and total protein. Similar results were reported in another investigation (Gad-Elkareem *et al.*, 2019). This decrease may be linked to decreased availability of mRNA for protein synthesis, decreased total RNA content, increased gluconeogenesis using glucogenic amino acids, increased muscle protein breakdown, and impaired liver function (Gad-Elkareem *et al.*, 2019).

The significant decline in liver biomarkers that were seen in diabetic rats receiving treatment with pioglitazone is similar to results obtained by Alshabi *et al.* (2021), who found that levels of liver biomarkers were substantially reduced in the pioglitazone-treated diabetic group in contrast to rats with diabetes. The current study's findings further demonstrated that rosuvastatin therapy at 10 mg/day in a diabetic model for 4 weeks resulted in a notable decline in ALP, AST, and ALT activity, while reduced albumin and total protein significantly increased. These results are comparable to the findings obtained from a study by Ahmed and colleagues (Ahmed *et al.*, 2012), who indicated that rosuvastatin

improved NAFLD patients' liver abnormalities. In contrast, findings from the study of Dizaye and Mohammed (2019) differed from our study's findings since they showed no discernible decline in serum AST and ALT but a notable rise in ALP in hyperlipidemic rats treated with rosuvastatin.

The hepatic tissue section's histological analysis verified this biochemical findings. The histopathological changes of liver in HFD/STZ rat model showed hepatocellular necrosis and deformed morphology, vacuolar degeneration, bile duct hyperplasia, mononuclear cellular infiltrations and activation of kupffer cells which confirm elevation of liver biomarkers. Those histological finding were previously described by (Rodríguez *et al.*, 2018, Faddladdeen and Ojaimi, 2019).

The hepatotherapeutic effects of pioglitazone were subsequently validated by histology, which showed the liver to be significantly improved and to have normal hepatocytes and microvasculature, indicating significant hepatic mitigation. These histopathological results resemble those observed by Alshabi *et al.* (2021). Similarly, Hamouda *et al.* (2022) found that pioglitazone-treated rats showed a reduced degree of inflammatory alterations and enhanced hepatic architecture as compared to the diabetic group. Moreover, rosuvastatin-treated diabetic rat models revealed a substantial improvement in the cellular structure of the liver. These data complement liver histopathology data in the study of Abdul-Kafy *et al.* (2019), in which rats with HFD-induced NAFLD treated with rosuvastatin showed great histopathological improvements in the liver. Also, the coadministration of pioglitazone and rosuvastatin provided a greater hepatotherapeutic effect than monotherapy.

This study demonstrated that the STZ/HFD-induced diabetic group had significantly higher quantities of hepatic inflammatory markers (IL-1 β and TNF- α) than the -ve control group, which supported previous

findings (AlAmri *et al.*, 2020, Abdel-Mohsen *et al.*, 2023). These mediators induce Kupffer cells to promote the inflammation (Omidkhoda *et al.*, 2023).

In this investigation, pioglitazone decreased hepatic pro-inflammatory cytokines. These outcomes resemble those of Hamouda *et al.* (2022), who revealed that pioglitazone mitigates T2DM-induced hepatic injury through a reduction of hepatic TNF- α , IL-6, and IL-1 β contents. Additionally, rosuvastatin improved the alteration of TNF- α and IL-1 β , and that was in accordance with the meta-analysis carried out by Tabrizi *et al.* (2019), which showed that statin use appears to dramatically reduce indicators of inflammation, such as IL-6, IL-1 and TNF- α in individuals with metabolic syndrome and associated illnesses. Further, combination therapy of pioglitazone and rosuvastatin was more effective than monotherapy at reducing TNF- α and IL-1 β .

Oxidative stress and stress-mediated liver damage in diabetes may be primarily caused by elevated CYP2E1 enzyme levels (Maksymchuk *et al.*, 2017). Our results explored a significant elevation in concentrations of CYP2E1 in the hepatic tissue of STZ-induced diabetic rats fed HFD, and these findings matched the previous work of Maksymchuk *et al.* (2017). Also, our investigation revealed that the use of pioglitazone led to a considerable reduction in CYP2E1 levels compared to diabetic rats. So, it prevents oxidative damage to hepatic cells caused by diabetes via suppression of CYP2E1 expression. This observation of our present work was supported by the other reports (Surapaneni *et al.*, 2014). Moreover, rosuvastatin-treated diabetic rats showed lower CYP2E1 levels than diabetic model. The concomitant administration of pioglitazone with rosuvastatin decreased CYP2E1 levels more than pioglitazone or rosuvastatin alone suggesting that interactions between two drugs might have synergistic effect on prevention of HFD/STZ-induced hepatic injury.

CONCLUSION

Overall, the current study found that pioglitazone, rosuvastatin, or both reversed hepatic impairment induced by HFD and STZ via decreasing liver enzymes, inflammatory cytokines, and CYP2E1, increasing serum total protein and albumin, and improving histopathological changes. Moreover, the combination of pioglitazone and rosuvastatin has a synergistic effect on improving most previous parameters. These results present proof of the beneficial effects of the pioglitazone and rosuvastatin mixture in the treatment of hepatic dysfunction caused by diabetes.

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التأثير التحسيني للبيوجليتازون والروسوفاستاتين على الإصابة الكبدية الناجمة عن نظام غذائي غني بالدهون والعلاج بالستربتوزوتوسين في الجرذان

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تشير التقديرات إلى أن ما يقرب من ٩٠% من المصابين بداء السكري في جميع أنحاء العالم يعانون من مرض السكري من النوع ٢ الذي يسبب خللاً في وظائف الكبد. يستخدم بيوجليتازون كدواء مضاد لمرض السكر ويخفف من خلل وظائف الكبد الناتج عن مرض السكر. يستخدم روسوفاستاتين كدواء خافض للدهون وأيضاً للتخفيف من الأضرار الكبدية الناجمة عن داء السكري. كان الهدف من هذه الدراسة هو النظر في الإمكانيات العلاجية للبيوجليتازون والروسوفاستاتين معاً في تخفيف إصابة الكبد الناتجة عن مرض السكري عن طريق الجمع بين نظام غذائي غني بالدهون والعلاج بالستربتوزوتوسين. تم استخدام أربعين ذكراً من الجرذان البيضاء في هذا البحث وتم تقسيمهم إلى خمس مجموعات. (٨ فئران في كل مجموعة). تخضع المجموعة الثانية والثالثة والرابعة والخامسة للإصابة بمرض السكري عن طريق تغذية الجرذان بنظام غذائي غني بالدهون لمدة شهر واحد وحقق جرعة منخفضة من الستربتوزوتوسين. بينما ظلت المجموعة الأولى كمجموعة ضابطة. المجموعة الثانية لم تحصل على أي دواء؛ عولجت المجموعة الثالثة يومياً بالبيوجليتازون. عولجت المجموعة الرابعة يومياً بالروسوفاستاتين. وتم علاج المجموعة الخامسة يومياً بمزيج من البيوجليتازون والروسوفاستاتين. بعد ٢٨ يوماً، تم تقييم نشاط إنزيمات الكبد ومستوى البروتينات في الدم و تم أيضاً تقييم الجينات الخاصة بالالتهاب في الكبد. وعلاوة على ذلك، خضعت أنسجة الكبد للفحص النسيجي. أظهرت النتائج تحسناً كبيراً في المجموعات التي عولجت بالبيوجليتازون أو الروسوفاستاتين أو مزيجهما. لكن كان للتناول المتزامن للبيوجليتازون والروسوفاستاتين قدرة أكبر على تخفيف إصابة الكبد الناتجة عن مرض السكري مقارنة بالعلاج بواحد منهما.