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Original Article

Effects of PPARα, PPARγ, and dual PPARα/γ agonists on liver tissue and biochemical markers in NAFLD-induced Wistar rats

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Abstract

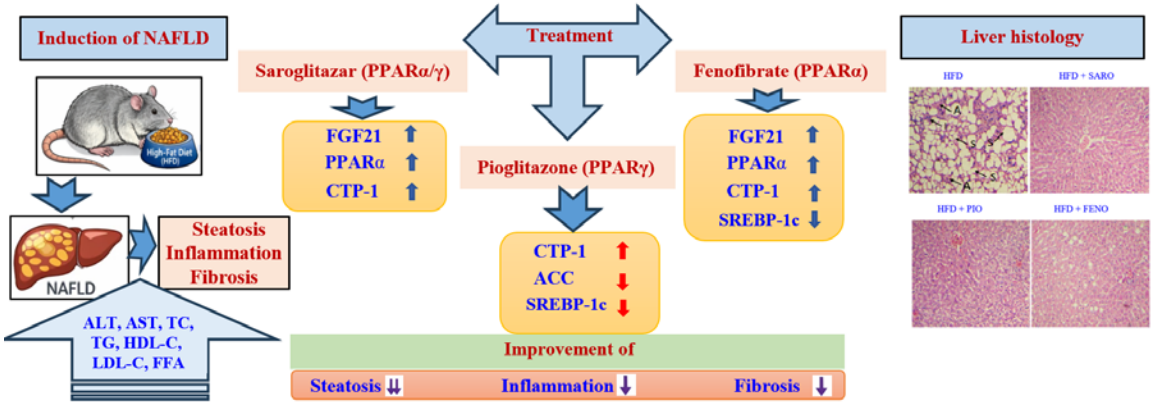
Background and purpose: Non-alcoholic fatty liver disease (NAFLD) is the most common liver-related metabolic disorder worldwide, affecting approximately 25% of the global population. Peroxisome proliferator-activated receptor (PPAR) agonists play an important role in NAFLD management through modulation of lipid metabolism and insulin sensitivity. This study compared the effects of fenofibrate (PPARα agonist), pioglitazone (PPARγ agonist), and saroglitazar (dual PPARα/γ agonist) in a high-fat diet (HFD)-induced NAFLD rat model.

Experimental approach: NAFLD was induced in Wistar rats by feeding a high-fat diet. Animals were assigned to control, HFD, and HFD-treated groups receiving saroglitazar (3 mg/kg), pioglitazone (30 mg/kg), or fenofibrate (100 mg/kg) for six weeks. Anthropometric parameters, serum liver enzymes, lipid profile, fasting blood glucose, and HOMA-IR were assessed. Histopathological changes were evaluated using H&E and Masson's trichrome staining. Hepatic expression of FGF21, CPT-1, PPARα, SREBP-1c, and ACC was analyzed by quantitative RT-PCR.

Findings/Results: All three PPAR agonists significantly improved hepatic steatosis, inflammation, and fibrosis compared with the HFD group, despite variability in biochemical and metabolic parameters. Among the treatments, saroglitazar demonstrated the most pronounced histological improvement, although systemic metabolic improvements were comparable among all three agents.

Conclusion and implications: Fenofibrate, pioglitazone, and saroglitazar exert beneficial effects on NAFLD progression through partially distinct mechanisms. Dual PPARα/γ activation appears to preferentially enhance hepatic histological outcomes, supporting the therapeutic relevance of saroglitazar in NAFLD.

Keywords: Fenofibrate; NAFLD; NASH; Pioglitazone; Saroglitazar.



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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological condition characterized by excessive fat accumulation in hepatocytes, occurring in the absence of significant alcohol consumption or other specific liver-damaging factors. NAFLD has become one of the leading causes of liver disease in developed countries, and its prevalence is increasing in developing nations as well. According to a 2020 epidemiological survey, approximately 1.7 billion people globally are affected by NAFLD, making it a significant health threat worldwide (1,2).

The global prevalence of NAFLD has reached 25% of the adult population, highlighting the widespread impact of this condition. The rising incidence of NAFLD is closely linked to modern lifestyle factors, including high energy intake and sedentary behavior, which contribute to a range of associated diseases such as obesity, type 2 diabetes, and cardiovascular disorders (3,4). NAFLD encompasses a spectrum of liver diseases, ranging from simple fatty liver (steatosis) to more severe forms such as nonalcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and even hepatocellular carcinoma (1).

The initial stage of NAFLD is characterized by simple liver steatosis, where fat content exceeds 5-10% of liver weight. Approximately up to 47% of individuals with NAFLD will progress to NASH, and 25-30% of NASH patients will eventually develop irreversible liver fibrosis (1). Liver fibrosis can progress to cirrhosis, which is generally considered irreversible (5). While simple hepatic steatosis is generally considered benign and not associated with high mortality, the progression to more severe forms of the disease significantly raises the risk of fatal outcomes (1). Therefore, implementing effective anti-fibrotic therapies is essential to prevent further liver damage and preserve liver function (6).

The precise mechanisms underlying NAFLD are still not fully understood, and the condition involves a complex interplay of genetic, environmental, and metabolic factors (7). NAFLD is closely linked to diet-induced dyslipidemia, insulin resistance, and other metabolic comorbidities, which in turn promote obesity, type 2 diabetes, and cardiovascular diseases (3). The bidirectional relationship between NAFLD and these conditions, particularly type 2 diabetes mellitus (T2DM), necessitates a comprehensive and interdisciplinary approach to treatment (8).

Despite the significant burden that NAFLD and NASH place on patients and healthcare systems, no approved pharmacological therapies currently exist to target these conditions, underscoring the urgent need for new therapeutic strategies (3,9).

Peroxisome proliferator-activated receptors (PPARs), a group of ligand-activated transcription factors in the nuclear hormone receptor superfamily (8), play a crucial role in regulating lipid and glucose metabolism, as well as energy balance, inflammation, and fibrosis. Different PPAR isoforms, including PPARα and PPARγ, have distinct tissue distributions and regulatory roles in energy metabolism (7). PPARα regulates lipid homeostasis by controlling peroxisomal and mitochondrial β-oxidation and fatty acid transport. In contrast, PPARγ primarily mediates adipocyte differentiation and enhances insulin sensitivity (10). These receptors often form heterodimers with retinoic acid X receptors, which bind to peroxisome proliferator response elements (PPREs) in the promoters of target genes. In addition to their classical function, some PPAR isoforms also exert anti-inflammatory effects through PPRE-independent transcriptional repression (7).

PPAR modulation has long been used in the treatment of metabolic diseases such as T2DM and dyslipidemia, and more recently, has been investigated for its potential therapeutic effects in liver diseases, particularly NAFLD. Given the pathophysiological and epidemiological links between metabolic disorders and NAFLD, PPAR modulators, including PPARα agonists like fenofibrate, PPARγ agonists like pioglitazone, and dual PPARα/γ agonists such as saroglitazar, have been studied for their impact on liver function and disease progression in NAFLD (7).

Saroglitazar has recently been introduced and approved in India for the management of

diabetic dyslipidemia and NASH. Preclinical and clinical studies have shown that saroglitazar not only improves hepatic steatosis and inflammation but also favorably modulates the serum atherogenic lipoprotein profile, thereby potentially lowering cardiovascular risk in patients with NAFLD (11). Despite these promising findings, comparative data directly evaluating the differential metabolic and histopathological effects of selective PPARα agonists, PPARγ agonists, and dual PPARα/γ agonists remain limited. In particular, it is unclear whether improvements in systemic metabolic markers consistently translate into superior hepatic histological outcomes.

Building on our previous work investigating lipid metabolism and PPAR-related pathways in metabolic disorders (12), the present study was designed to compare the effects of saroglitazar, pioglitazone, and fenofibrate on liver histopathology, biochemical parameters, and the expression of key lipid metabolism-related genes (sterol regulatory element-binding protein 1c (SREBP-1c), carnitine palmitoyl transferase 1 (CPT-1), PPARα, acetyl-CoA carboxylase (ACC), and fibroblast growth factor 21 (FGF-21)) in Wistar rats with high-fat diet-induced fatty liver. This comparative approach aims to clarify the distinct metabolic signatures of these PPAR agonists in NAFLD/NASH.

MATERIALS AND METHODS

The high-fat emulsion

A high-fat emulsion diet was prepared following the method outlined by Zou *et al*. (13), designed to induce NAFLD and NASH by providing 4243 kcal/L of energy. The composition of the high-fat diet (HFD) included 77% fat, 9% carbohydrates, and 14% protein, along with added vitamins and minerals. The emulsion was stored at 4 °C and heated to 42 °C in a water bath before use.

In vivo experimental design

Forty-four adult male Wistar rats were obtained from the Experimental Animal Center of Ahvaz Jundishapur University of Medical Sciences. Animals were housed under standard laboratory conditions (12/12-h light/dark cycle, temperature of 25 ± 2 °C, and relative humidity of 55-60%) with free access to standard chow and water during the acclimatization period.

After acclimatization, rats were randomly assigned to a normal control (NC) group and the HFD groups. The NC group initially included 10 rats and received a standard laboratory diet. Rats in the HFD groups were administered a high-fat emulsion (10 mL/kg/day) by oral gavage and had free access to an 18% sucrose solution to induce metabolic disturbances associated with NAFLD/NASH. An equivalent volume of saline was administered to the NC group.

In the middle of week 6, rats in the HFD groups received a single intraperitoneal injection of streptozotocin (STZ; 30-35 mg/kg), freshly prepared in citrate buffer, to exacerbate insulin resistance and promote a NASH-like phenotype. Three days after STZ administration, fasting blood glucose (FBG) levels were measured, and rats with glucose concentrations > 200 mg/dL were considered diabetic.

At the end of week 6, two rats from the NC group and two rats from the HFD group were sacrificed under anesthesia for histopathological evaluation. Liver tissue examination confirmed the presence of hepatic steatosis and inflammatory changes consistent with NAFLD/NASH.

Following histological confirmation of the disease model, pharmacological treatments were initiated from week 7 and continued until the end of the experimental period. All remaining animals completed the study, resulting in a final sample size of n = 8 rats per group for biochemical analyses, gene expression measurements, and histopathological scoring.

All animal procedures were reviewed and approved by the Institutional Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (approval code: HLRC-9807) and were conducted in strict accordance with national and international guidelines for the care and use of laboratory animals, including the ARRIVE 2.0 guidelines and the American Veterinary Medical Association (AVMA) recommendations. Adequate measures were taken to minimize pain, suffering, and distress.

Anesthesia and euthanasia were performed using ketamine (90 mg/kg) and xylazine (9 mg/kg) administered intraperitoneally (14). No unexpected deaths or adverse effects occurred during the study.

Drug preparation and administration

Saroglitazar was obtained from Zydus Cadila (India), while fenofibrate and pioglitazone were purchased from Sobhan Daru Pharmaceutical Company (Iran). Tablets of saroglitazar (4 mg), pioglitazone (30 mg), and fenofibrate (200 mg) were finely powdered separately using a mortar and pestle and suspended in 0.5% carboxymethyl cellulose (Na-CMC; Sigma, USA) solution. Fresh suspensions were prepared daily and administered once daily by oral gavage during the treatment period. The doses were as follows: saroglitazar at 3 mg/kg body weight, pioglitazone at 30 mg/kg body weight, and fenofibrate at 100 mg/kg body weight (15).

Drug treatments were initiated at the start of the eighth week and continued for six weeks. As a result, the experimental design included five groups in total, as shown in Fig. 1.

Blood and tissue samples

At the end of the six-week drug treatment period, rats were euthanized *via* intraperitoneal (IP) injection of a ketamine and xylazine mixture (90/9 mg/kg) after a 12-h fasting period. Blood samples were collected from the aorta, and serum was separated by centrifugation at 4500 rpm for 15 min. Liver tissues were excised, washed with ice-cold saline, and then weighed after drying. The liver index was calculated based on these measurements. The liver tissue was subsequently divided into two portions: one was fixed in 10% formalin for histological examination, and the other was used for RNA extraction and gene expression analysis.

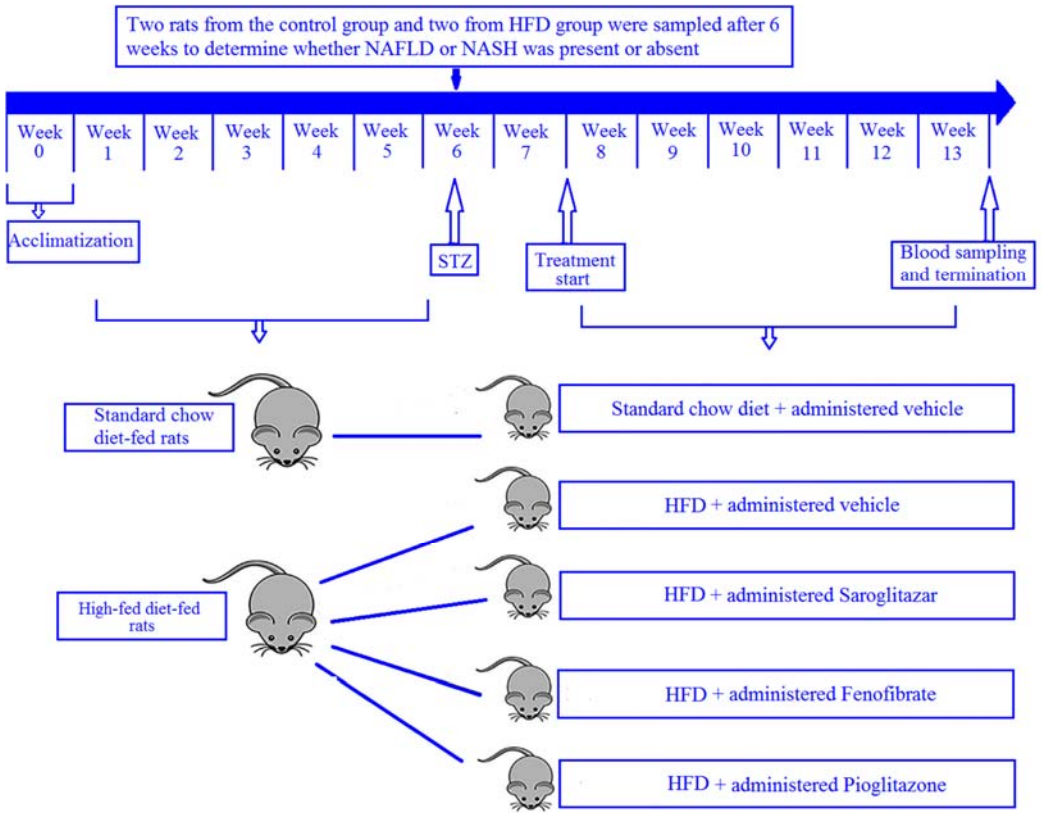

Fig. 1. Experimental group design and drug treatment protocol. NAFLD, Non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; STZ, streptozotocin; HFD, high-fat diet.

Table 1. The primer sequences of the used genes.

Gene	Forward primer	Reversed primer
SREBP-1c	TCTTGACCGACATCGAGACAT	CCTGTGTCTCCTGTCTCACC
FGF21	ACACAATTCCAGCTGCCTTG	TAGAGGCTTTGACACCCAGG
ACC	TTAAGGGGTGAAGAGGGTGC	CACTTCCAAAGACCTAGCC
PPARα	TGGTGCATTTGGGCGTATCT	CACGAGCGCTAAGCTGTGA
CPT-1α	AGCCCTGAGACAGACTCACA	ATCACGAGGGTCCGTTTTCC
β-actin	CCCATCTATGAGGGTTACGC	TTTAATGTCACGCACGATTTC

Biochemical measurements

Serum biochemical parameters were assessed to evaluate liver injury and lipid metabolism. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using a Roche 6000 automated analyzer with the corresponding assay kits, as markers of hepatocellular damage. Lipid profile parameters, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were determined by standard enzymatic colorimetric methods (Pars Azmoon, Tehran, Iran). Free fatty acids (FFA) were measured using a commercially available kit (Randox Laboratories Ltd., Crumlin, UK) as an indicator of lipid metabolism dysregulation in NASH.

Gene expression analysis

Total RNA was extracted from frozen liver tissue using the BioFACT™ Total RNA Prep kit (Ver. 2.0, BioFACT, following the manufacturer's protocol. cDNA synthesis was then carried out *via* reverse transcription using the BioFACT™ RT-kit (BioFACT, Daejeon, Korea). The resulting cDNA was analyzed through quantitative real-time polymerase chain reaction (qRT-PCR) using the SYBR Green PCR reagent (Takara Bio, Otsu, Japan) and the ABI StepOnePlus qPCR system. Beta-actin (ACTB) served as the internal reference gene for normalizing target mRNA expression. The primer sequences used are listed in Table 1.

The ΔCt method was employed to compare the expression of each gene to the internal control gene, β-actin (ΔCt = Ct of target gene - Ct of internal control). The fold change in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ formula, which quantifies the relative expression difference between the target and control groups. Results are presented as the mean ± SD.

Histopathological evaluations

For histopathological analysis, liver tissue samples were fixed in 10% buffered formalin for 24 h, dehydrated in a graded alcohol series, and embedded in paraffin. Sections of 6-7-µm thickness were prepared using a microtome and mounted on glass slides. After deparaffinization and rehydration, the sections were stained with hematoxylin and eosin (H&E) for evaluation of steatosis, lobular inflammation, and hepatocyte ballooning, and with Masson's trichrome for fibrosis assessment. Steatosis was graded based on the percentage of hepatocytes containing macrovesicular fat using a 4-point scale (grade 1: 0-25%; grade 2: 26-50%; grade 3: 51-75%; grade 4: 76-100%) (2). The degrees of inflammation, necrosis, and fibrosis were evaluated as the mean score obtained from 10 randomly selected microscopic fields per slide, using a validated 0-3 semi-quantitative scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe), as described by Avni *et al*. (16). This scoring system was adapted from previously published experimental studies and allows comparative evaluation of histopathological changes across groups. Although it is not identical to the NAFLD activity score (NAS), it provides a reproducible semi-quantitative assessment suitable for experimental NASH models.

Statistical analysis

Data are presented as the mean ± SD. Comparisons between the NC and HFD groups were performed using independent-sample t-tests. For comparisons among all five experimental groups, one-way analysis of variance (ANOVA) was used, followed by the Tukey-Kramer post hoc test to identify specific group differences. All statistical analyses were carried out using GraphPad Prism version 8.0 (GraphPad Software, San Diego, USA). *P*-values < 0.05 were considered statistically significant.

RESULTS

Changes in body weight, liver weight, liver index, and liver TG in rats

After six weeks of high-fat emulsion administration, hepatic steatosis was confirmed. NASH model induction led to significant increases in body weight, liver weight, liver index (liver weight/body weight), and liver TG compared to the control group. Following drug treatment with fenofibrate, pioglitazone, and saroglitazar, significant changes in these variables were observed compared to the HFD group, as shown in Fig. 2.

H&E staining revealed marked hepatocyte ballooning, steatosis, and inflammatory infiltration in the HFD group, which were significantly improved in all treatment groups, with the saroglitazar group showing the lowest histopathological scores among the treated groups. Masson's trichrome staining showed extensive fibrotic deposition in HFD rats, while fibrotic changes were visibly attenuated following treatment, with saroglitazar again demonstrating superior histological improvement. Slides were analyzed under magnification of ×100.

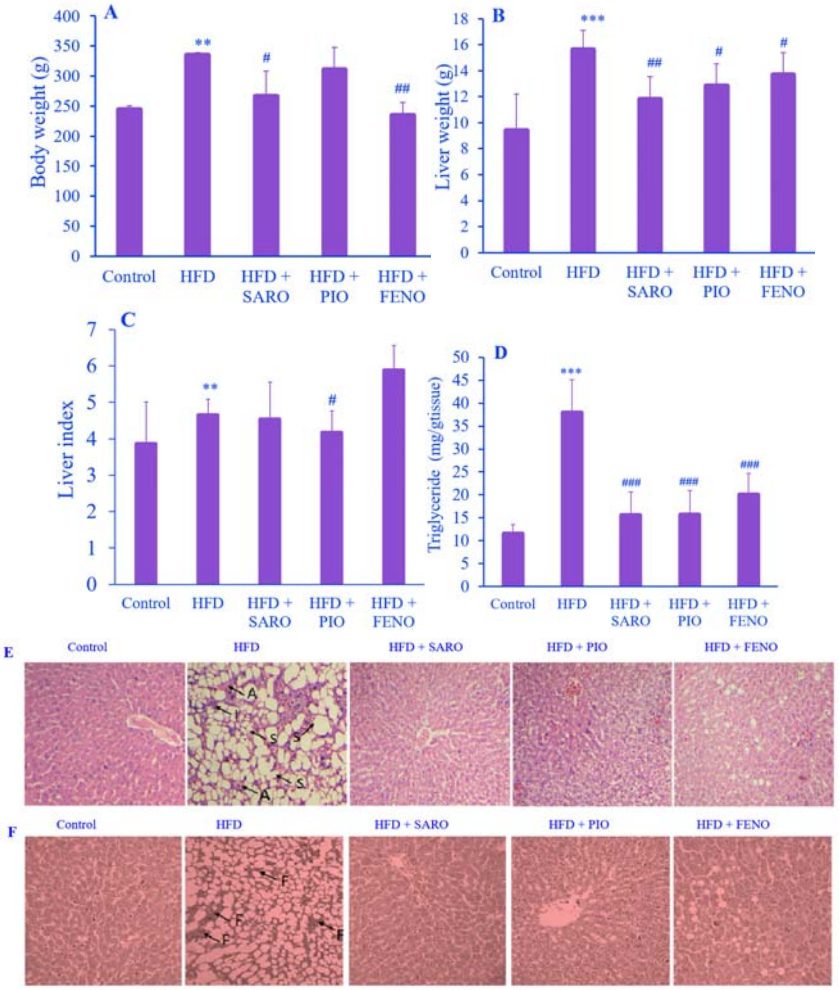

Fig. 2. Comparison of (A) body weight, (B) liver weight, (C) liver index, and (D) hepatic triglyceride content after 12 weeks of HFD-induced NAFLD and subsequent treatment with SARO (3 mg/kg), PIO (30 mg/kg), and FENO (100 mg/kg). (E and F) H&E and Masson's trichrome staining (×100) revealed improved hepatic architecture and reduced fibrosis in treated groups, particularly in the saroglitazar group. Data are presented as mean ± SD, n = 8. $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$ indicate significant differences in comparison with the control group; $^{\#}P \leq 0.5$, $^{\#\#}P \leq 0.01$, $^{\#\#\#}P \leq 0.001$ versus HFD group. In pathology images (E and F): A, accumulation of RBCs; I, inflammation; S, steatosis; F, fibrosis. NAFLD, Non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; STZ, streptozotocin; HFD, high-fat diet; SARO, saroglitazar group; PIO, pioglitazone group; FENO, fenofibrate.

Table 2. Effects of daily administration of SARO, PIO, and FENO on serum biochemical parameters in Wistar rats with HFD-induced fatty liver. Values are presented as mean ± SD (n = 8). ***$P \leq 0.001$ indicate significant differences in comparison with the control group; $^{\#}P \leq 0.5$, $^{\#\#}P \leq 0.01$, $^{\#\#\#}P \leq 0.001$ versus HFD group.

Variables	Normal control	HFD	HFD + SARO	HFD + PIO	HFD + FENO
ALT (IU/I)	31.57 ± 3.30	70.57 ± 5.82***	38.5 ± 5.83###	39.18 ±5.62###	41.68 ± 4.43###
AST (IU/I)	44.71 ± 3.25	106.0 ± 20.07***	55.88 ± 5.89###	58.0 ± 4.12###	61.40 ± 4.47###
ALP (IU/I)	313.2 ± 29.81	553.0 ± 45.71**	515.1 ± 44.98	345.5 ± 42.31##	382.7 ± 32.39#
TG (mg/dL)	44.57 ± 9.14	115.10 ± 16.32***	42.13 ±13.70###	51.34 ±16.70###	54.57 ± 15.20###
TC (mg/dL)	71.57 ± 4.63	124.1 ± 7.49***	72.13 ± 9.64###	76.63 ± 9.22###	85.45 ± 8.30###
HDL (mg/dL)	44.00 ± 3.82	27.71 ± 5.03***	31.07 ± 6.22	34.15 ± 2.65###	34.65 ± 4.38###
LDL (mg/dL)	18.66 ± 3.33	71.40 ± 7.25***	33.45 ± 15.48###	33.55 ± 4.71###	33.55 ± 6.41###
FFA (mg/dL)	9.99 ± 3.04	29.52 ± 4.37***	23.40 ± 5.68	21.26 ± 5.02##	18.56 ± 4.11##
FBG (mg/dL)	105.70 ± 7.54	254.30 ± 8.95***	148.50 ± 10.0###	134.10 ± 6.78###	127.40 ± 8.22###
Insulin (IU/I)	3.68 ± 1.07	3.079 ± 1.088	4.86 ± 0.94#	3.627 ± 1.22	3.677 ± 1.63
HOMA-IR (unitless)	0.96 ± 0.26	1.92 ± 0.68**	1.78 ± 0.29	1.14 ± 0.37##	1.03 ± 0.62##

SARO, Saroglitazar group; PIO, pioglitazone group; FENO, fenofibrate; HFD, high-fat diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FFA, free fatty acids; FBG, fasting blood glucose; HOMA-IR is a dimensionless index calculated as follows: (fasting insulin [μU/mL] × fasting glucose [mg/dL]) / 405.

Table 3. Quantitative histopathological evaluation in control and experimental groups. Values are presented as mean ± SD (n = 8 mice/group). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ indicate significant differences in comparison with the control group; $^{\#}P \leq 0.5$, $^{\#\#}P \leq 0.01$, $^{\#\#\#}P \leq 0.001$ versus HFD group.

Groups	Accumulation of RBCs	Inflammation	Steatosis	Fibrosis
Control	0.13 ± 0.04	0.21 ± 0.09	0.00 ± 0.00	0.00 ± 0.00
HFD	1.53 ±0.24**	1.42 ± 0.31**	3.84 ± 0.43***	2.12 ± 0.35***
HFD + SARO	0.15 ± 0.5#	0.11 ± 0.04##	0.00 ± 0.00###	0.00 ± 0.00###
HFD + FENO	0.55 ± 0.22*#	0.61 ± 0.18*##	1.2 ± 0.4***##	0.00 ± 0.00###
HFD + PIO	0.89 ± 0.28**#	0.47 ± 0.16*#	0.52 ± 0.17***##	0.00 ± 0.00###

HFD, High-fat diet group; SARO, saroglitazar group; PIO, pioglitazone group; FENO, fenofibrate.

The effects of HFD and drug treatments on serum biochemical parameters

As shown in Table 2, HFD significantly increased serum ALT, AST, alkaline phosphatase (ALP), TG, TC, LDL-C, and FFA levels compared with the controls. Treatment with saroglitazar, pioglitazone, and fenofibrate significantly reduced ALT and AST levels compared with the HFD group. ALP levels were significantly reduced by pioglitazone and fenofibrate, whereas saroglitazar did not produce a statistically significant change in ALP.

All three drugs significantly reduced TG, TC, and LDL-C compared to the HFD group. HDL-C increased significantly in the fenofibrate and pioglitazone groups but not in the saroglitazar group. FFA levels were significantly reduced in the pioglitazone and fenofibrate groups, but not with saroglitazar. Regarding glycemic indices, HFD rats showed a marked increase in FBG in comparison with the control group. All three treatments significantly reduced FBG versus the HFD-treated group. Insulin levels remained unchanged in the pioglitazone and fenofibrate groups but increased significantly in the saroglitazar group.

HOMA-IR decreased significantly following fenofibrate and pioglitazone treatment, whereas saroglitazar produced a numerical but non-significant reduction, consistent with Table 2.

The effects of the treatments on histopathological parameters

Based on the results presented in Table 3, the group receiving the HFD showed a significant increase in steatosis, hepatic fibrosis, inflammation, and RBC accumulation compared to the control group after 6 weeks. The mean steatosis score in the HFD group was 3.84 ± 0.43, indicating severe fat accumulation. Treatment with saroglitazar reduced this score to 0.00 ± 0.00, indicating a marked normalization of histological steatosis scores. Pioglitazone and fenofibrate also reduced the steatosis score, though less prominently than saroglitazar.

All three treatments significantly reduced inflammation compared to the HFD group, but the improvement was more pronounced with saroglitazar.

Similarly, RBC accumulation decreased from 1.53 ± 0.24 in the HFD group to 0.15 ± 0.05 after saroglitazar treatment. Pioglitazone and fenofibrate also showed improvement; however remained higher compared with saroglitazar.

Fibrosis scoring showed no detectable fibrosis in the control group. However, HFD treatment led to a considerable increase in fibrosis score compared to the control group, which returned to 0.00 ± 0.00 in the groups treated with saroglitazar, pioglitazone, and fenofibrate, indicating normalization of fibrosis scores following therapy.

The effects of the treatments on hepatic mRNA expression and serum FGF-21 levels

As shown in Fig. 3, after six weeks of HFD, hepatic expression of FGF-21 and serum levels of FGF-21 were significantly reduced in the HFD group compared to the control group. Treatment with fenofibrate and saroglitazar significantly normalized both hepatic expression and serum levels of FGF-21. No significant difference was observed between fenofibrate and saroglitazar in hepatic expression; however, fenofibrate produced a slightly greater increase in serum levels. On the other hand, treatment with pioglitazone did not show a significant change in hepatic expression or serum levels of FGF-21 compared to the HFD group.

The effects of the treatments on hepatic mRNA expression of PPARα and CPT-1α

As shown in Fig. 4, the hepatic mRNA expression of PPARα did not show a significant change after 6 weeks of HFD feeding in the HFD group compared to the control group. Treatment with fenofibrate and saroglitazar significantly increased hepatic PPARα expression compared to the HFD group, with a greater increase observed in the fenofibrate group. However, pioglitazone treatment did not result in a significant change in hepatic PPARα expression compared to the HFD group.

Hepatic CPT-1α mRNA expression significantly decreased after 6 weeks of HFD feeding compared to the control group. Treatment with fenofibrate, saroglitazar, and pioglitazone significantly normalized CPT-1α expression. The increase was greater in the fenofibrate group compared to the saroglitazar and pioglitazone groups.

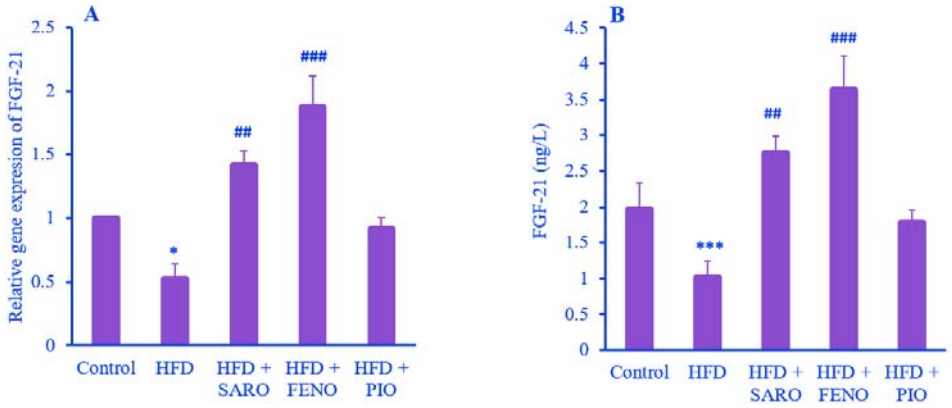

Fig. 3. Effect of administration of SARO (3 mg/kg), PIO (30 mg/kg), and FENO (100 mg/kg) on hepatic mRNA expression of FGF-21 and serum FGF-21 levels in Wistar rats with HFD-induced fatty liver. (A) Expression levels were measured using RT-PCR. mRNA expression data were normalized to β-actin (ACTB) and presented as $2^{-\Delta\Delta Ct}$ values. The relative fold change ($2^{-\Delta\Delta Ct}$) of FGF-21expression is shown as bar graphs, with values compared to the control group. (B) Results from four independent experiments are presented as mean ± SD of changes in serum FGF-21 levels relative to the control group. *$P \leq 0.05$ and ***$P \leq 0.001$ indicate significant differences in comparison with the control group; $^{\#\#}P \leq 0.01$and $^{\#\#\#}P \leq 0.001$ versus HFD group. SARO, Saroglitazar group; PIO, pioglitazone group; FENO, fenofibrate; HFD, high-fat diet; FGF-21, fibroblast growth factor 21.

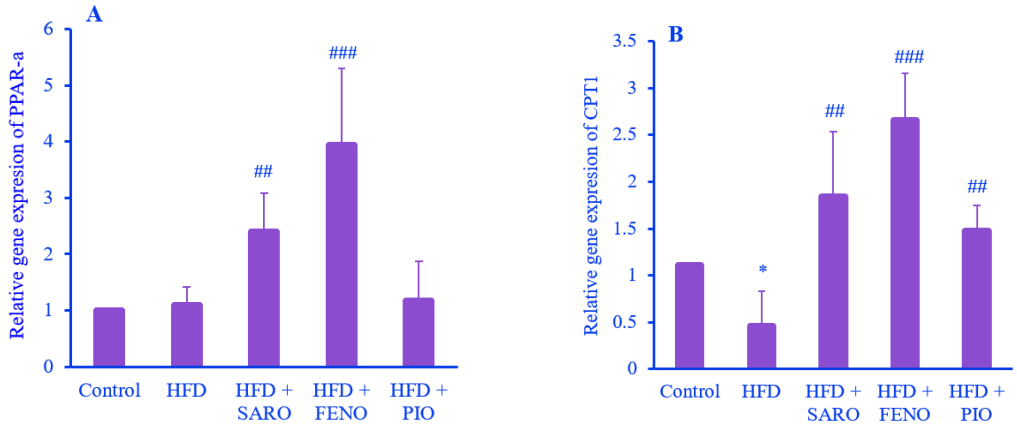

Fig. 4. Effect of SARO (3 mg/kg), PIO (30 mg/kg), and FENO (100 mg/kg) administration on hepatic mRNA expression of PPARα and CPT1α in HFD-induced fatty liver in Wistar rats. Expression levels were measured using RT-PCR. mRNA expression data were normalized to β-actin (ACTB) and presented as $2^{-\Delta\Delta Ct}$ values. The relative fold change ($2^{-\Delta\Delta Ct}$) of PPARα and CPT1α expression is shown as bar graphs, with values compared to the control group. Data are presented as mean + SD (n = 8 mice/group). $^{*}P \leq 0.05$ indicates significant differences in comparison with the control group; $^{\#\#}P \leq 0.01$ and $^{\#\#\#}P \leq 0.001$ versus HFD group. SARO, Saroglitazar group; PIO, pioglitazone group; FENO, fenofibrate; HFD, high-fat diet; FGF-21, fibroblast growth factor 21.

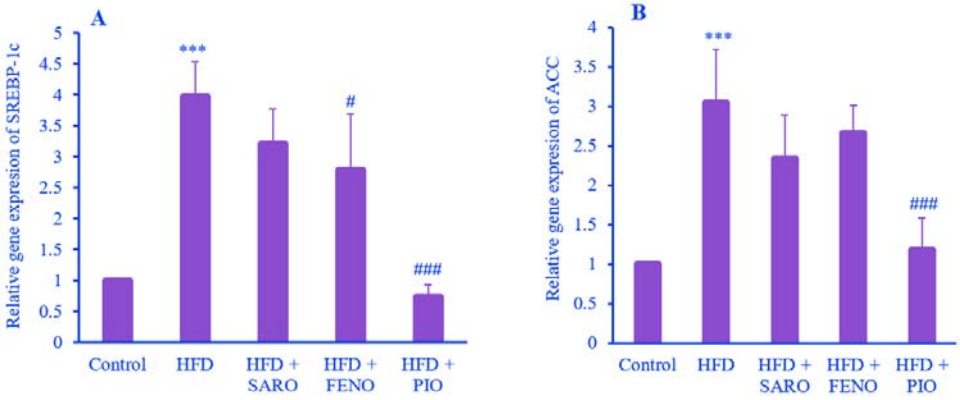

Fig. 5. Effect of SARO (3 mg/kg), PIO (30 mg/kg), and FENO (100 mg/kg) administration on hepatic mRNA expression of (A) SREBP-1c and (B) ACC in HFD-induced fatty liver in Wistar rats. Expression levels were measured using RT-PCR. mRNA expression data were normalized to β-actin (ACTB) and presented as $2^{-\Delta\Delta Ct}$ values. The relative fold change ($2^{-\Delta\Delta Ct}$) of SREBP-1c and ACC expression is shown as bar graphs, with values compared to the control group. Data are presented as mean + SD (n = 8 mice/group). $^{***}P \leq 0.001$ indicates significant differences in comparison with the control group; $^{\#}P \leq 0.05$ and $^{\#\#\#}P \leq 0.001$ versus HFD group. SARO, Saroglitazar group; PIO, pioglitazone group; FENO, fenofibrate; HFD, high-fat diet; SREBP-1c, sterol regulatory element-binding protein 1c; ACC, acetyl-CoA carboxylase.

The effects of the treatments on hepatic mRNA expression of SREBP-1c and ACC

As shown in Fig. 5, after 6 weeks of HFD feeding, hepatic SREBP-1c gene expression significantly increased in the HF group compared to the control group. Treatment with pioglitazone and fenofibrate significantly reduced SREBP-1c expression compared to the HFD group. This reduction was more significant in the pioglitazone group compared with the fenofibrate group, while saroglitazar had no significant effect.

Hepatic ACC gene expression significantly increased in the HFD group compared to the control group. After pioglitazone treatment, hepatic ACC expression significantly decreased compared with the HFD group, whereas fenofibrate and saroglitazar did not significantly alter ACC expression compared with the HFD group.

DISCUSSION

Since NAFLD and NASH are multifactorial conditions involving insulin resistance, steatosis, inflammation, oxidative stress, mitochondrial dysfunction, and fibrosis, and given that both PPARα and PPARγ receptors play key roles in regulating these pathways, agonists targeting these receptors are expected to exert beneficial effects in managing these disorders and may contribute to the amelioration of NASH.

PPARα is mainly expressed in hepatocytes, and its activation helps prevent steatosis and steatohepatitis by reducing liver fat accumulation and oxidative damage. In contrast, PPARγ is highly expressed in fat cells, where it improves insulin sensitivity and lowers fatty acid delivery to the liver. Accordingly, this study aimed to compare the effects of PPARα (fenofibrate), PPARγ (pioglitazone), and dual PPARα/γ (saroglitazar) agonists on histopathological and biochemical markers in high-fat diet-induced NAFLD in Wistar rats (15).

The results of this study demonstrated that, despite some inconsistent effects on certain biochemical parameters, treatment with saroglitazar, pioglitazone, and fenofibrate significantly improved liver fibrosis and significantly reduced hepatic steatosis and inflammation compared with the HFD group. Saroglitazar produced the most pronounced histological improvement among the treatment groups despite not significantly reducing SREBP-1c or ACC expression. This finding indicates that its beneficial effects are unlikely to be primarily mediated through direct transcriptional suppression of *de novo* lipogenesis. Instead, these effects may be associated with alternative mechanisms, including improved hepatic fatty acid handling, modulation of inflammatory pathways, or tissue-specific effects of combined PPARα and PPARγ activation, although these mechanisms were not directly assessed in the present study.

In addition to gene expression changes, the effects of PPAR agonists on serum lipid profile and liver enzymes warrant consideration. In the present study, fenofibrate and pioglitazone were associated with more pronounced improvements in circulating FFAs and insulin resistance indices, consistent with their established systemic metabolic effects. In contrast, although saroglitazar showed a numerical reduction in FFA levels, this change did not reach statistical significance. Similarly, saroglitazar did not significantly improve ALP or HOMA-IR levels compared with other treatments, suggesting limited effects on certain biochemical markers of liver injury. Notably, despite these modest biochemical responses, saroglitazar achieved superior histological improvement, highlighting a dissociation between circulating metabolic markers and intrahepatic tissue remodeling in this model. Collectively, these findings indicate that combined activation of PPARα and PPARγ may preferentially confer intrahepatic benefits rather than uniformly improving all systemic metabolic parameters.

Taken together, the present findings suggest that the three PPAR agonists exerted distinct but complementary effects on hepatic metabolism. Fenofibrate and saroglitazar, both possessing PPARα activity, were associated with increased expression of PPARα-related pathways and CPT-1, consistent with enhanced fatty acid β-oxidation. In contrast, pioglitazone primarily influenced lipid metabolism through modulation of lipogenic regulators such as SREBP-1c. Notably, saroglitazar, as a dual PPARα/γ agonist, appeared to integrate elements of both mechanisms, which may partly explain its superior histological improvement despite inconsistent effects on some systemic metabolic parameters. These interpretations are based on observed expression patterns and should be viewed as associative rather than mechanistically definitive.

Notably, saroglitazar and fenofibrate (PPARα-activating agents) significantly increased both hepatic FGF21 mRNA expression and serum FGF21 levels, an effect not observed with the PPARγ-selective agonist pioglitazone when compared to HFD controls. This aligns with FGF21's established role as a metabolic hormone that regulates glucose homeostasis and exerts hepatoprotective effects. Experimental studies in murine systems confirm that PPARα activation upregulates both hepatic and circulating FGF21 levels (7).

In contrast, the PPARγ-selective agonist pioglitazone exerted its therapeutic effects primarily through significant downregulation of SREBP-1c expression, leading to decreased ACC activity and upregulated CPT-1. Given that elevated hepatic SREBP-1c promotes steatosis (17) and ACC accelerates NAFLD progression (18), these data reveal distinct mechanistic pathways. Notably, pioglitazone treatment did not alter PPARα expression, whereas both saroglitazar and fenofibrate (PPARα-activating agents) upregulated PPARα expression, which was accompanied by increased CPT-1 mRNA expression without modulating SREBP-1c or ACC, in parallel with their induction of FGF21 mRNA. This coordinated regulation is consistent with previous reports suggesting that FGF21 is associated with enhanced fatty acid oxidation through pathways involving CPT-1 regulation. However, direct assessment of AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC) phosphorylation, extracellular signal-regulated kinase 1/2 (ERK1/2), or peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) signaling was beyond the scope of the present study (19). These findings align with established literature demonstrating that PPARα and PPARγ coactivators independently regulate CPT-1 through distinct genomic elements. CPT-1 mediates the rate-limiting conversion of long-chain acyl-CoA to acylcarnitine, enabling fatty acid transport across mitochondrial membranes and initiating β-oxidation of long-chain fatty acids (20).

Contrary to our findings, Jain *et al*. reported that neither pioglitazone nor fenofibrate improved hepatic steatosis, showing only partial amelioration of inflammation and liver function. Notably, in their experimental model, saroglitazar treatment significantly reduced the NAFLD activity score (15). Consistent with their findings, our study also demonstrated that saroglitazar had a more pronounced effect on improving hepatic steatosis compared to the other two agonists. Furthermore, multiple studies, including work by Dan Zhang *et al*., support our results by confirming the efficacy of fenofibrate in improving both insulin resistance and hepatic steatosis, albeit through distinct mechanisms (21). Notably, our rodent findings are in line with the clinical observations obtained by Sayadishahraki *et al*. (22), who reported that pioglitazone improved steatosis grade and metabolic parameters in NAFLD patients. This translational consistency highlights the importance of PPARγ-mediated SREBP-1c inhibition in human disease, despite model-specific differences in FGF21 response. Similarly, Zeyu Wang *et al*. reported that pioglitazone exerted beneficial effects on NAFLD in both non-diabetic and diabetic patients, including improvements in liver histopathology, liver enzyme levels, HOMA-IR, and lipid profiles (23). Additionally, Yaghoubi *et al*. demonstrated the therapeutic benefits of both fenofibrate and pioglitazone in patients with fatty liver disease (24).

The divergent results across studies likely reflect inherent methodological differences in (i) animal model genetics, (ii) hepatic steatosis quantification methods, (iii) HFD formulations (varying in duration and lipid composition), (iv) therapeutic intervention periods, and (v) STZ-mediated diabetes induction protocols.

Several limitations of this study should be acknowledged. First, the HFD rodent model may not fully recapitulate the heterogeneity of human NAFLD. Second, tissue-specific PPAR expression patterns and their metabolic effects may vary across species. Third, the long-term effects of PPAR agonist treatment were not assessed in the present study. In addition, the use of low-dose STZ, while effective in accelerating insulin resistance and NASH development, may partially affect pancreatic β-cell function and insulin secretion; therefore, this potential impact should be considered when interpreting the metabolic outcomes. Collectively, given the observed anti-steatotic and anti-fibrotic effects of individual PPARα and PPARγ agonists in the present study, concurrent administration of dual PPARα/γ agonists may offer potential advantages by synergistically targeting multiple disease pathways. We propose that future studies investigate: (1) combinatorial effects of PPAR agonists, (2) optimization of dosing regimens to maximize efficacy while minimizing potential adverse effects, and (3) long-term outcomes of multi-agonist therapies.

CONCLUSIONS

This study demonstrates that all tested PPAR agonists (saroglitazar, pioglitazone, and fenofibrate) significantly improved hepatic inflammation, steatosis, and fibrosis, each through distinct biochemical pathways. While PPARα activation promoted FGF21 and CPT-1 expression, and PPARγ activation suppressed SREBP-1c, indicating different but complementary mechanisms, saroglitazar, acting as a dual PPARα/γ agonist, showed superior efficacy in ameliorating these hepatic abnormalities. These findings highlight the therapeutic advantage of simultaneous activation of both PPAR pathways and suggest that dual agonists like saroglitazar may exert synergistic and more potent effects, offering enhanced potential for the treatment of NAFLD and NASH.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

N. Mohammadtaghvaei contributed to the study conception and design. E. Khoshbakht performed all experiments. H. Yaghooti and B. Taheri contributed to interpreting the results. N. Dinarvand wrote the first draft of the article. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

AI declaration

To improve readability and language, ChatGPT was used. After using this tool, the author(s) reviewed and edited the content and take full responsibility for the content of the publication.

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