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RESEARCH ARTICLE OPEN ACCESS

Neuregulin-4 as a novel target of dapagliflozin in high-fat diet-induced fatty liver disease in rats

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Abstract
Objective: The present study aimed to evaluate the hepatoprotective effects of dapagliflozin in a rat model of high-fat/high-fructose diet-induced fatty liver disease and to investigate its association with Neuregulin-4 (NRG4) expression.
Methods: Forty adult male Wistar albino rats were randomly allocated into five groups (n = 8 each): control, high-fat/high-fructose diet (HFD), pioglitazone-treated (10 mg/kg/day + HFD), dapagliflozin-treated (1 mg/kg/day + HFD), and vehicle-treated (1% DMSO + HFD). Pioglitazone and dapagliflozin were administered orally for 14 days before induction and continued throughout the 8-week induction period. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured, while hepatic malondialdehyde (MDA), reduced glutathione (GSH), and NRG4 levels were determined using ELISA-based methods. Histopathological and fibrosis assessments were performed using hematoxylin and eosin and Masson's trichrome staining.
Results: The HFD group demonstrated significant hepatic injury, characterised by increased serum ALT, AST, and hepatic MDA levels, together with reduced GSH and NRG4 levels compared with the control group. Treatment with dapagliflozin significantly improved liver enzyme profiles, attenuated oxidative stress, restored antioxidant status, and increased hepatic NRG4 levels. Histopathological examination revealed a marked reduction in hepatic steatosis and fibrosis in dapagliflozin-treated rats, with effects comparable to those observed with pioglitazone treatment.
Conclusion: Dapagliflozin administration was associated with improvement of biochemical, oxidative stress, and histopathological alterations induced by a high-fat/high-fructose diet in rats. The findings are consistent with a potential role of NRG4 in the hepatoprotective effects of dapagliflozin; however, further mechanistic studies are required to clarify the underlying molecular pathways.

Keywords: Dapagliflozin, Neuregulin-4, NRG4, Oxidative stress, Hepatic steatosis, MASLD

Plain English Summary

Fatty liver disease is a common health problem that is closely linked to obesity, diabetes, and unhealthy dietary habits. Over time, excessive fat accumulation in the liver can lead to inflammation, tissue damage, and liver fibrosis. Although several treatment options are available, there is still a need for new therapies that can effectively reduce liver injury and improve metabolic health.

Dapagliflozin is a medication commonly used for the treatment of type 2 diabetes. Recent studies suggest that it may also have beneficial effects on fatty liver disease beyond its blood glucose-lowering action. In the present study, we investigated whether dapagliflozin could protect the liver in rats fed a high-fat/high-fructose diet, which is widely used to mimic fatty liver disease in humans.

Our results showed that dapagliflozin treatment improved liver function, reduced oxidative stress, restored antioxidant defence mechanisms, and increased the level of Neuregulin-4 (NRG4), a protein that may play a protective role in liver metabolism. Histopathological examination also demonstrated

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reduced liver damage and fibrosis in dapagliflozin-treated animals compared with untreated animals receiving the high-fat diet.
These findings suggest that dapagliflozin may have beneficial effects in metabolic liver disease and support the need for future studies to better understand the underlying molecular mechanisms and their potential clinical applications.

Introduction

Metabolically-dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), is a leading cause of chronic liver disease worldwide. It encompasses a spectrum of conditions ranging from simple steatosis, with or without mild inflammation, to non-alcoholic steatohepatitis (NASH), which is characterised by persistent inflammation and accelerated progression to fibrosis. Type 2 diabetes raises the risk of cirrhosis and its consequences, and NAFLD has a strong association with components of metabolic syndrome. Advanced liver fibrosis is a crucial prognostic indicator for liver-related outcomes and total mortality, and it may be evaluated using a variety of non-invasive techniques, even though cardiovascular disease and extrahepatic cancer are the main causes of death in individuals with NAFLD (1). Increased liver fat levels greater than 5.5% are indicative of non-alcoholic fatty liver disease (NAFLD), which is linked to obesity, type 2 diabetes, chronic renal disease, and cardiovascular disease. About 75% of those with type 2 diabetes have non-alcoholic fatty liver disease.
NAFLD can be classified as either non-alcoholic steatohepatitis (NASH), the more aggressive variant, or non-alcoholic fatty liver (NAFL) based on histological grading from liver biopsies. NASH is more likely to occur in people with type 2 diabetes, and the condition has a worse prognosis (2).
Globally, NAFLD is thought to affect 30.2% of the population. The incidence is 30.9% in Asia, 16.1% in Australia, 30.2% in Europe, 29% in North America, and 34% in South America, according to regional reports (3).
Non-alcoholic fatty liver disease (NAFLD) is influenced by nutrition, metabolic factors, and insulin resistance (IR), although the underlying pathogenic pathways are still unclear (4).
The liver and adipose tissue communicate closely. Adipose tissue dysfunction in an insulin-resistant (IR) state and dysregulation in lipid metabolism are the causes of steatosis in the liver (5). Obesity-related metabolic illnesses are partly caused by dysregulated release of adipokines, a range of physiologically active chemicals secreted by adipose tissue (6).
Neuregulin 4 (NRG-4) belongs to the epidermal growth factor (EGF) family and is thought to be an adipokine that functions as an endocrine, paracrine, or autocrine signal by releasing the EGF-like domain upon proteolytic cleavage (7, 8, 9, 10). Angiogenesis, thermogenesis, glucose metabolism, lipid metabolism, and neurogenesis are among the physiological and pathological processes in which it is involved. In addition, it reduces inflammatory factors and inhibits apoptosis in inflammatory disorders (11).
NRG-4 improves NAFLD by increasing autophagy, decreasing hepatocyte death, and improving lipid metabolism. Recent studies have shown that NRG-4 suppresses hepatic lipogenesis by inhibiting LXR signalling to downregulate genes such as Srebf1, Fasn, Scd1, and Acaca. Furthermore, it promotes autophagy by activating AMPK and blocking mTOR signalling, thereby aiding in hepatic lipid clearance. Additionally, via modifying STAT5 and JNK1/2 signalling, NRG-4 decreases hepatocyte apoptosis and inflammation, which helps prevent NASH from developing from basic steatosis. These pathways show how NRG-4 can both regulate lipid metabolism and act as a preventative factor against the progression of NAFLD (11).
By preventing glucose reabsorption in the proximal renal tubule and boosting its excretion in urine, sodium-glucose co-transporter 2 (SGLT2) inhibitors, a novel class of oral antidiabetic drugs, improve insulin resistance and lower blood glucose levels (12), and by downregulating SREBP-1c and preventing the synthesis of new liver fat, they improve IR enhances lipid metabolism (13).
Several existing preclinical studies have demonstrated the potential of SGLT2 inhibitors to alter energy metabolism toward increased fat oxidation. This may lead to an overall decrease in body weight and fat content as well as decreased hepatic ectopic fat storage (14).
Additionally, by reducing fat content, SGLT2 inhibitors can prevent adipose cells from releasing pro-inflammatory cytokines, which reduces the inflammatory effects, one of the main factors contributing to the evolution of NASH and liver fibrosis (15, 16).
Dapagliflozin is a very selective SGLT-2 inhibitor. Clinical trials have shown that dapagliflozin lowers visceral and liver fat in people with T2D and NAFLD, making it a potential therapy option for NAFLD, On the other hand the use of pioglitazone, an agonist of the peroxisome proliferator-activated receptor gamma (PPAR-γ), in patients with non-alcoholic fatty liver disease (NAFLD) has shown to increase insulin sensitivity

and reduce hepatic steatosis, inflammation, and disease progression (17). Thus, the present study aimed to evaluate the effects of dapagliflozin and pioglitazone on diet-induced fatty liver disease, with particular emphasis on hepatic NRG4 expression, lipid metabolism, and liver function.

Materials and Methods

Experimental Animals

The present experimental study was conducted on forty healthy adult male Wistar albino rats weighing 250–300 g. The animals were obtained from the Animal House, College of Medicine, University of Babylon, Iraq. Before the beginning of the experiment, all rats were allowed to acclimatise to the laboratory environment for two weeks.

The animals were housed in standard polypropylene cages under controlled environmental conditions (22–25°C, relative humidity 50–60%, and a 12-hour light/dark cycle). Standard laboratory chow and tap water were provided ad libitum throughout the acclimatisation period. Animals were observed daily to monitor their general health and well-being.

After the acclimatisation period, the rats were randomly allocated into five experimental groups (n = 8 per group). All experimental procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of the College of Medicine, University of Babylon, Iraq (Ref. No. 4329, Date: 6/7/2025)

Study Design and Experimental Groups

Following acclimatisation, the animals were randomly assigned into five experimental groups, with eight rats in each group:

Group I (Control): Rats received a standard laboratory diet throughout the experimental period.

Group II (HFD Group): Rats received a high-fat/high-fructose dietary regimen for eight consecutive weeks to induce hepatic steatosis.

Group III (Pioglitazone + HFD): Rats were administered pioglitazone orally at a dose of 10 mg/kg/day for 14 days before induction and continued to receive treatment during the subsequent 8-week high-fat/high-fructose feeding period (18).

Group IV (Dapagliflozin + HFD): Rats received dapagliflozin orally at a dose of 1 mg/kg/day for 14 days before induction and throughout the 8-week induction period (19).

Group V (Vehicle + HFD): Rats received 1% dimethyl sulfoxide (DMSO) orally during the experimental period in addition to the high-fat/high-fructose diet.

The total duration of the experimental protocol was ten weeks, including a two-week pretreatment phase followed by an eight-week induction and treatment phase. The pretreatment regimen was designed to evaluate the potential preventive effects of dapagliflozin and pioglitazone against diet-induced hepatic injury

Induction of Hepatic Steatosis

Experimental hepatic steatosis was induced using a combined high-fat/high-fructose dietary model. The diet consisted of standard laboratory chow supplemented with rendered sheep fat together with a 20% (w/v) fructose solution supplied as drinking water throughout the induction period.

For each rat (approximately 250 g body weight), the daily dietary regimen consisted of 14 g of standard chow mixed with 6 g of sheep fat. Fresh sheep fat was liquefied at approximately 50°C and filtered before mixing with the chow to ensure a homogeneous preparation. In addition, animals received freshly prepared 20% fructose solution (20 g fructose dissolved in 100 mL drinking water) ad libitum. Fresh food and fructose solution were prepared daily, and any remaining portions were discarded after 24 hours.

This dietary model was selected because it reproduces the metabolic and histopathological alterations associated with diet-induced hepatic steatosis, including lipid accumulation, oxidative stress, inflammation, and early fibrotic changes

Preparation and Administration of Drugs

Dapagliflozin and pioglitazone powders were obtained from MedChemExpress (MCE, USA). Fresh drug solutions were prepared daily, immediately before administration. Dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) at a concentration of 1% was used as the vehicle to dissolve or suspend the tested compounds.

Pioglitazone was administered orally by gastric gavage at a dose of 10 mg/kg/day, whereas dapagliflozin was administered orally at a dose of 1 mg/kg/day. Both drugs were given for 14 days before induction of hepatic steatosis and were continued throughout the subsequent 8-week induction period. The selected doses were based on previously published experimental studies that demonstrated their beneficial metabolic and hepatoprotective effects.

Animals in the vehicle group received an equivalent volume of freshly prepared 1% DMSO solution by oral gavage according to the same dosing schedule.

Sample Collection

At the end of the experimental period, the animals were fasted overnight (12–14 hours) with free access to water. Rats were anaesthetised

using a combination of ketamine and xylazine before sample collection. The absence of the pedal withdrawal reflex confirmed adequate anaesthesia.

Blood samples were collected by cardiac puncture into plain tubes and allowed to clot at room temperature for approximately 20 minutes. Samples were then centrifuged at 3000 rpm for 15 minutes to separate the serum, which was aliquoted into sterile Eppendorf tubes and stored at −80°C until biochemical analysis.

Following blood collection, the animals were euthanised, and the liver was carefully excised through a midline abdominal incision. Liver tissues were washed with ice-cold normal saline to remove residual blood and then blotted dry. The liver from each animal was weighed, and representative tissue specimens were divided into two portions. One portion was stored at −80°C for biochemical assays, while the other was fixed in 10% neutral buffered formalin for histopathological examination.

Biochemical Analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using commercially available diagnostic kits according to the manufacturers' instructions.

Liver tissue homogenates were prepared under cold conditions using phosphate-buffered saline (PBS, pH 7.4). Hepatic oxidative stress markers, including malondialdehyde (MDA) and reduced glutathione (GSH), were measured using commercially available ELISA kits. Hepatic Neuregulin-4 (NRG4) concentrations were determined using a Rat Neuregulin-4 (NRG4) ELISA Kit (MyBioSource, USA) according to the manufacturer's protocol.

All biochemical measurements were performed in accordance with the instructions provided by the kit manufacturers, and the obtained absorbance values were recorded using a microplate reader.

Histopathological Examination

Liver tissue specimens were fixed in 10% neutral buffered formalin for 48 hours and routinely processed for histological examination. Following dehydration through ascending grades of ethanol and clearing with xylene, the tissues were embedded in paraffin wax. Serial sections of 4 µm thickness were prepared using a semi-automatic microtome and mounted on glass slides.

For the assessment of general hepatic architecture and steatotic changes, tissue sections were stained with haematoxylin and eosin (H&E). Additional sections were stained with Masson's trichrome stain to evaluate collagen deposition and hepatic fibrosis. Histopathological examination and image acquisition were performed using a Zeiss Primo Star 3 light microscope equipped with a Zeiss Axiocam 208 colour digital camera (Zeiss, Germany).

Liver fibrosis was evaluated according to the NASH Clinical Research Network (NASH-CRN/Kleiner) fibrosis staging system. Representative photomicrographs from each experimental group were obtained under appropriate magnifications and used for comparative histopathological assessment.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (IBM SPSS Statistics, version 26). Quantitative data are presented as mean ± standard deviation (SD).

Comparisons among the five experimental groups were carried out using one-way analysis of variance (One-way ANOVA). When a statistically significant overall difference was detected, intergroup comparisons were performed using the Least Significant Difference (LSD) post hoc test. A p-value of less than 0.05 was considered statistically significant.

The symbols used in the tables and figures were defined as follows: $p < 0.05$ versus the control group (*), and $p < 0.05$ versus the induction group (ɣ).

Results

Effect of Treatments on Hepatic Oxidative Stress Markers

Malondialdehyde (MDA)

The induction group (Group B) exhibited a significant increase in hepatic malondialdehyde (MDA) levels compared with the control group (Group A), indicating enhanced lipid peroxidation and oxidative stress. In contrast, treatment with either pioglitazone (Group C) or dapagliflozin (Group D) significantly reduced MDA levels compared with the induction group. No significant improvement was observed in the vehicle-treated group (Group E). The detailed results are presented in Table 1 and Figure 1.

Table 1: Tissue biochemical variables

Groups	Mean ±SDNRG4 ng/ml	MDA ng/ml	Reduced GSH (ng/ml)
Group A (N=8)	252.95±33.63	112.12± 20.16	1691.7± 795.2
Group B (N=8)	177.41±24.57*	163.78±33.94*	555.7±57*

Group C (N=8)	223.49±44.61 †	126.52±36.76 †	983.2±255.6* †
Group D (N=8)	236.04±26.8 †	113.3±35.4 †	997.1±282.9* †
Group E (N=8)	195.99±22.73*	161.54±31.63*	610.8±209.1*

Group A: normal control; Group B: induction; Group C: pioglitazone; Group D: dapagliflozin; Group E: vehicle. Data are expressed as mean ± SD. * $p < 0.05$ vs Group A, † $p < 0.05$ vs Group B

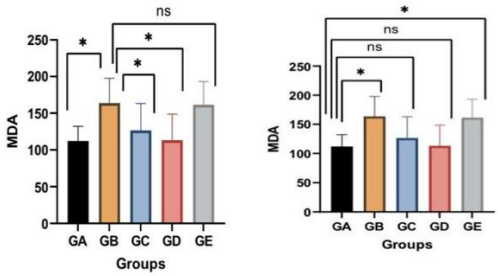

Figure 1: Effect of treatment on tissue MDA

Group A: Normal group; Group B: Induction; Group C: Pioglitazone; Group D: Dapagliflozin; Group E: Vehicle; p < 0,05. Ns: non-significant

Reduced Glutathione (GSH)

Hepatic reduced glutathione (GSH) levels were significantly decreased in the induction group compared with the control animals. Administration of pioglitazone and dapagliflozin significantly restored GSH concentrations, reflecting an improvement in antioxidant defence status. The vehicle-treated group showed no significant difference compared with the induction group. These findings are summarised in Table 1 and Figure 2.

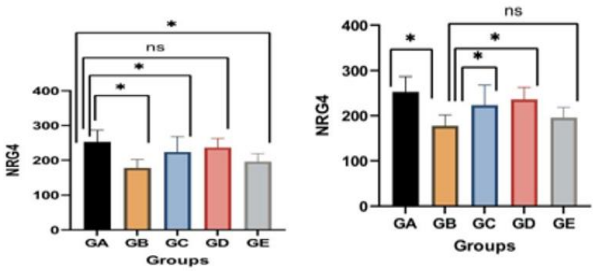

Figure 2: Effect of treatments on tissue NRG-4

Group A: normal control; Group B: induction; Group C: pioglitazone; Group D: dapagliflozin; Group E: vehicle. (*p<0.05), ns: non-significant

Neuregulin-4 (NRG4)

A significant reduction in hepatic NRG4 levels was observed in rats receiving the high-fat/high-fructose diet compared with the control group. Treatment with dapagliflozin and pioglitazone significantly increased NRG4 levels relative to the induction group, whereas administration of the vehicle alone did not produce a significant effect. The corresponding data are illustrated in Table 1 and Figure 3.

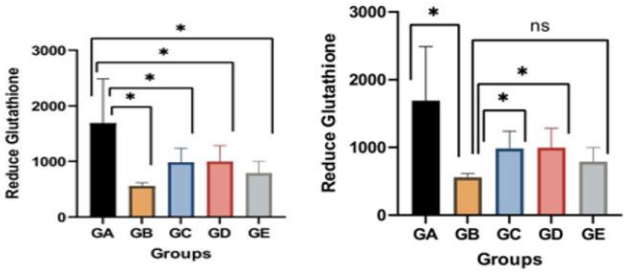

Figure 3: Effect of treatment on tissue-reduced GSH

Group A: normal control; Group B: induction; Group C: pioglitazone; Group D: dapagliflozin; Group E: vehicle. (*p<0.05), ns: non-significant

The histopathological evaluation using the NASH-CRN/Kleiner staging system revealed significant structural variations across the experimental groups. The induction group (Group B) exhibited a high fibrosis score of 2.80 ±0.44, characterised by dense perisinusoidal and bridging fibrosis. Similarly, Group E (Induction + DMSO) showed a non-significant difference compared to Group B, with a score of 2.60 ±0.54 ($p > 0.05$), confirming that the vehicle (DMSO) had no therapeutic effect on hepatic scarring.
In contrast, the standard treatment group (Group C) showed a marked reduction in liver fibrosis, with a mean fibrosis score of 0.60 ± 0.54 (F1 stage). Similarly, dapagliflozin treatment (Group D) significantly ameliorated hepatic fibrosis, resulting in a mean score of 0.80 ± 0.44 (F1–F2 stage). No statistically significant difference was observed between the dapagliflozin-treated and pioglitazone-treated groups, suggesting that dapagliflozin exerted hepatoprotective effects comparable to the standard treatment. Detailed fibrosis data are presented in Table 2 and Figure 4.

Table 2: Liver Fibrosis Scores in the Experimental Groups(Liver fibrosis was assessed according to the NASH Clinical Research Network (NASH-CRN/Kleiner) fibrosis staging system.)

Group	Fibrosis Score (Mean ± SD)	Histopathological Stage
Group A (Control)	0.00 ± 0.00	Normal
Group B (HFD)	2.80 ± 0.44	F3
Group C (Pioglitazone + HFD)	0.60 ± 0.54	F1
Group D (Dapagliflozin + HFD)	0.80 ± 0.44	F1–F2
Group E (Vehicle + HFD)	2.60 ± 0.54	F3

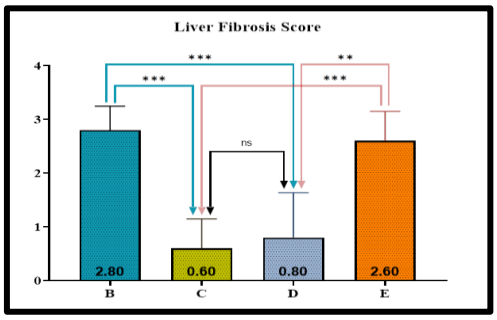

Figure 4: Comparison of Liver Fibrosis Scores
Data are presented as mean ±SD(Y-AXIS), experimental groups (x-axis).** P<0.001 and *** P<0.0001 indicate significant differences from Group B. ns (non-significant) indicates that Dapagliflozin (Group D) achieved a comparable effect to the standard treatment (Group C)

Effect of Treatments on Liver Function Tests
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly elevated in the induction group compared with the control group, indicating hepatocellular injury associated with the high-fat/high-fructose dietary model. Treatment with dapagliflozin or pioglitazone significantly reduced both ALT and AST activities relative to the induction group. In contrast, the vehicle-treated group showed no statistically significant improvement. The biochemical findings are presented in Table 3 and Figure 5.

Table 3: Serum biochemical variables in the studied groups

Groups	Mean ±SD	
	AST IU/L	ALT IU/L
Group A (N=8)	71.63± 7.41	36.22±11.95
Group B (N=8)	105.53± 24.91*	60.37±6.54*
Group C (N=8)	86.97±14.96 †	48.23±17.08* †
Group D (N=8)	74.92±8.94 †	41.73±8.12 †
Group E (N=8)	98.11±22.06*	50.72±11.71*

Group A: normal control; Group B: induction; Group C: pioglitazone; Group D: dapagliflozin; Group E: vehicle. Data are expressed as mean ± SD. * $p < 0.05$ vs Group A, † $p < 0.05$ vs Group B

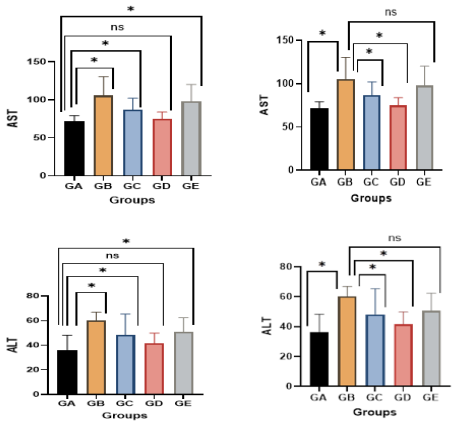

Figure 5: Effect of treatment on serum ALT and AST

Group A: normal control; Group B: induction; Group C: pioglitazone; Group D: dapagliflozin; Group E: vehicle. (*p<0.05), ns: non-significant

Histopathological Findings and Liver Fibrosis Assessment

Histopathological examination of liver sections revealed marked differences among the experimental groups. The control group (Group A) showed normal hepatic architecture with preserved hepatic lobular organisation and no evidence of steatosis or fibrosis.

In contrast, rats fed the high-fat/high-fructose diet (Group B) demonstrated severe hepatic injury characterised by extensive fatty degeneration, hepatocellular damage, and marked collagen deposition. Histological evaluation using Masson's trichrome staining revealed bridging fibrosis consistent with stage F3 according to the NASH Clinical Research Network (NASH-CRN/Kleiner) fibrosis staging system.

The vehicle-treated group (Group E) exhibited histopathological features similar to those observed in the induction group, with persistent severe steatosis and advanced fibrosis, indicating that administration of 1% DMSO alone did not exert a protective effect against diet-induced liver injury.

In comparison, the pioglitazone-treated group (Group C) demonstrated substantial histological improvement, with only mild fatty changes and limited collagen deposition corresponding to stage F1 fibrosis. Likewise, the dapagliflozin-treated group (Group D) showed marked attenuation of hepatic injury, with reduction of steatosis and fibrosis compared with the induction group. The observed fibrotic changes were generally limited to mild-to-moderate collagen deposition without well-developed bridging septa.

Quantitative assessment of liver fibrosis scores further supported these observations. The induction group exhibited the highest fibrosis score, whereas both dapagliflozin and pioglitazone treatment significantly reduced fibrosis severity. No statistically significant difference was observed between the dapagliflozin-treated and pioglitazone-treated groups, suggesting that dapagliflozin achieved a hepatoprotective effect comparable to that of the standard treatment. Detailed fibrosis scores are presented in Table 2, while representative histopathological findings are illustrated in Figure 6 and the accompanying photomicrographs.

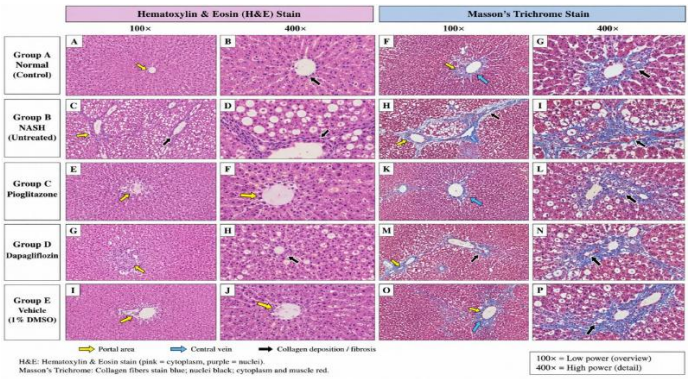

Figure 6: Photomicrographs of liver sections from the experimental groups stained with hae-matoxylin and eosin (H&E) and Masson's trichrome

The left two columns show H&E-stained sections at original magnifications of ×100 and ×400, demonstrating the general hepatic architecture and the degree of steatosis. The right two columns show Masson's trichrome-stained sections at original magnifications of ×100 and ×400, illustrating the extent of collagen deposition and hepatic fibrosis. Group A (Control) exhibited normal hepatic architecture. Group B (HFD) demonstrated severe steatosis and advanced fibrosis. Group C (Pioglitazone + HFD) showed marked histological improvement with mild residual steatosis and fibrosis. Group D (Dapagliflozin + HFD) revealed attenuation of hepatic injury with mild to moderate steatosis and fibrosis. Group E (Vehicle + HFD) displayed persistent severe steatosis and fibrosis comparable to those observed in the HFD group. Black arrows indicate fatty degeneration or collagen deposition, yellow arrows indicate central veins, and blue arrows indicate portal areas

Somatic Parameters

The chronic nutritional induction utilising a high-fat diet supplemented with 20% fructose for eight consecutive weeks induced severe metabolic stress, manifest as profound elevations in final body weight, absolute liver weight, and the calculated hepatosomatic liver index. In the present study, we demonstrated that HFD-fructose induction led to a substantial increase in total body mass and liver hypertrophy (Table 4).

Table 4: Consolidated somatic indices and histopathological liver fibrosis scores across the experimental groups

Experimental Group (N=8)	Body Weight (g)	Liver Weight (g)	Liver Index (%)	Liver Fibrosis Score	Predominant Fibrotic Stage
Group A (Normal Control)	339.37 ±29.3	10.15 ±0.73	2.99%	0 ±0	Stage 0 (No Fibrosis)
Group B (HFD Induction)	455.75 ±32.1*	18.60 ±1.39*	4.08%*	2.8 ±0.44*	Stage 3 (Bridging)
Group C (Pioglitazone)	410.38 ±33.1†	15.20 ±1.12†	3.70†	0.6 ±0.54†	Stage 1a/1c (Delicate)
Group D (Dapagliflozin)	395.12 ±28.6†	13.99± 0.87†	3.54%†	0.8 ±0.83†	Stage 1b (Dense)
Group E (Vehicle Control)	450.13 ±36.5*	18.09 ±1.56*	4.01%*	2.60 ±0.54*	Stage 3 (Bridging)

Group A: normal control; Group B: induction; Group C: pioglitazone; Group D: dapagliflozin; Group E: vehicle. *: significant compared to Group A. Data are expressed as mean ± SD. * $p < 0.05$ vs Group A, † $p < 0.05$ vs Group B

Discussion

The present study investigated the effects of dapagliflozin on biochemical, oxidative stress, and histopathological alterations induced by a high-fat/high-fructose dietary model of fatty liver disease in rats. The principal findings demonstrated that dapagliflozin administration was associated with significant improvement in liver enzyme activities, restoration of antioxidant defence mechanisms, reduction of lipid peroxidation, and attenuation of hepatic fibrosis (1). In addition, treatment was accompanied by increased hepatic NRG4 levels, suggesting a potential association between NRG4 regulation and the observed hepatoprotective effects (3).

The present findings demonstrated that HFD induction significantly increased body weight and liver weight, reflecting excessive lipid accumulation and hepatic enlargement. These observations are consistent with the findings of Buettner et al., who reported that high-fat dietary models are associated with increased somatic indices and hepatic steatosis. This pathological expansion is driven by the unrestricted flux of dietary fatty acids and accelerated *de novo* lipogenesis, resulting in massive ectopic triglyceride storage within the hepatic parenchyma.

Upon therapeutic intervention, daily administration of the SGLT2 inhibitor dapagliflozin resulted in a highly significant reduction in body weight and normalisation of the liver index ($P \leq 0.05$).

Dapagliflozin-mediated renal glycosuria forces a state of negative caloric balance, thereby compelling the metabolic system to utilise peripheral and visceral fat depots for energy expenditure, which directly relieves the hepatic parenchymal volume.

Conversely, while the standard agent pioglitazone also significantly attenuated absolute liver weight and index, it exhibited a less pronounced restriction on terminal body weight compared to dapagliflozin.

The present study demonstrated that induction of fatty liver disease by a high-fat/high-fructose diet resulted in a significant increase in hepatic MDA levels together with elevated serum ALT and AST activities, indicating enhanced oxidative stress and hepatocellular injury. These findings are in agreement with the observations of (20), who reported that high-fat dietary models are associated with marked metabolic disturbances and oxidative damage. Oxidative stress plays a central role in the progression of non-alcoholic fatty liver disease through excessive production of reactive oxygen species and lipid peroxidation.

In the current study, treatment with dapagliflozin significantly reduced MDA concentrations and improved serum liver enzyme activities

compared with the untreated HFD group. These findings are consistent with those reported by (21), who demonstrated that dapagliflozin attenuated hepatic inflammation and oxidative stress in a rat model of diet-induced fatty liver disease. Moreover, (19) found that dapagliflozin possesses antioxidant properties capable of reducing oxidative tissue injury, supporting the hepatoprotective effects observed in the present work.

The observed reduction in ALT and AST activities following dapagliflozin treatment may reflect stabilisation of hepatocyte membrane integrity and attenuation of oxidative damage. Comparable reductions in hepatic fat accumulation and liver injury markers were also reported by (17), who demonstrated that dapagliflozin decreases liver fat content in patients with type 2 diabetes, and by (2), who observed beneficial effects of dapagliflozin on non-alcoholic fatty liver disease in diabetic individuals.

The present study demonstrated a significant reduction in hepatic reduced glutathione (GSH) levels in rats fed a high-fat/high-fructose diet compared with the control group, indicating impairment of the endogenous antioxidant defence system. Depletion of GSH is considered one of the characteristic features of oxidative stress and reflects excessive generation of reactive oxygen species during the progression of fatty liver disease. This observation agrees with the findings of (22), who highlighted the critical role of oxidative stress and antioxidant depletion in the pathogenesis of non-alcoholic fatty liver disease. Similarly, (23) reported that increased oxidative burden is associated with significant reductions in tissue GSH concentrations.

Administration of dapagliflozin significantly restored hepatic GSH levels compared with the untreated HFD group. This improvement may be attributed to the antioxidant and anti-inflammatory properties of SGLT2 inhibitors, which reduce lipid accumulation and mitochondrial oxidative injury. These findings are consistent with the work of (21), who demonstrated that dapagliflozin ameliorated oxidative stress and inflammatory changes in a rat model of diet-induced fatty liver disease. Furthermore, the reduction in oxidative damage observed after dapagliflozin treatment is supported by the findings of (19), who showed that dapagliflozin exerts protective effects against oxidative tissue injury in experimental animal models.

The simultaneous reduction in MDA levels and restoration of GSH concentrations observed in the present study suggest that dapagliflozin may improve the hepatic redox balance and preserve endogenous antioxidant defences, thereby limiting the progression of hepatic injury induced by a high-fat/high-fructose diet.

One of the most important findings of the present study was the significant reduction in hepatic Neuregulin-4 (NRG4) levels in the HFD group and their subsequent restoration following dapagliflozin treatment. NRG4 is an adipokine predominantly secreted by brown adipose tissue and has been implicated in the regulation of lipid metabolism, energy homeostasis, and inflammatory responses (9). First described NRG4 as an endocrine link between brown adipose tissue and the liver, highlighting its potential role in maintaining metabolic health.

Recent evidence provided by (11) has demonstrated that NRG4 participates in multiple physiological and pathological pathways related to hepatic lipid metabolism and inflammatory regulation. In addition, (24) suggested that NRG4 may represent a potential therapeutic target in non-alcoholic fatty liver disease because of its ability to modulate hepatic lipogenesis and metabolic dysfunction. Experimental work by (25) further showed that NRG4 promotes fuel oxidation and improves the adipokine profile, thereby protecting against diet-induced metabolic disorders.

The increase in NRG4 levels observed in the dapagliflozin-treated group in the present study was accompanied by improvements in liver enzymes, oxidative stress markers, and histopathological findings. These observations are also supported by the work of (26), who demonstrated that reduced NRG4 activity is associated with mitochondrial dysfunction and enhanced oxidative stress. Collectively, these findings suggest that restoration of NRG4 may contribute to the beneficial metabolic effects observed following dapagliflozin administration.

However, it should be emphasised that the present study evaluated changes in hepatic NRG4 levels without directly investigating the molecular pathways responsible for these effects. Therefore, while the current findings support a possible association between dapagliflozin treatment and NRG4 modulation, they do not establish a direct causal relationship. Additional mechanistic studies involving gene and protein expression analyses are required to clarify the exact role of NRG4 in mediating the hepatoprotective actions of dapagliflozin

The biochemical and oxidative stress findings observed in the present study were further supported by the histopathological examination of liver tissues. Rats fed the high-fat/high-fructose diet exhibited severe hepatic steatosis, hepatocellular degeneration, and extensive collagen deposition compared with the control group. These observations agree with the

experimental model described by Buettner et al., who reported that prolonged exposure to a high-fat diet induces characteristic metabolic and histological alterations resembling human fatty liver disease. Likewise, (27) demonstrated that excessive fructose consumption promotes hepatic lipid accumulation, insulin resistance, and progression of steatotic liver injury.

In the current study, treatment with dapagliflozin resulted in marked improvement of hepatic architecture, with attenuation of steatosis and a reduction in collagen deposition compared with the untreated HFD group. These findings are consistent with those of (21), who found that dapagliflozin ameliorated hepatic inflammation and fatty degeneration in rats receiving a high-carbohydrate/high-fat diet. In addition, the antioxidant effects described by (19) may contribute to the preservation of hepatic cellular integrity and the reduction of tissue injury observed in the present work.

Pioglitazone was included in the present study as a reference treatment because of its established beneficial effects in experimental fatty liver disease. The significant histopathological improvement observed in the pioglitazone-treated group is consistent with the findings of (18), who demonstrated that pioglitazone reduces hepatic steatosis and improves metabolic disturbances in a rat model of diet-induced non-alcoholic fatty liver disease. Similarly, (2) and Latva-Rasku et al. reported that therapies targeting metabolic dysfunction can reduce hepatic fat accumulation and improve liver-related outcomes.

Interestingly, the degree of histopathological improvement observed following dapagliflozin treatment was comparable to that produced by pioglitazone. Both treatments were associated with reductions in steatosis, oxidative stress, and fibrosis scores relative to the induction group, suggesting that dapagliflozin may provide hepatoprotective effects similar to those of the standard comparator. Although the NASH Clinical Research Network (NASH-CRN/Kleiner) fibrosis staging system was originally developed for human histology, it was applied here as a semi-quantitative scoring tool to enable consistent comparative assessment of fibrosis severity across experimental groups in this rodent model

However, because the present study was conducted in an experimental animal model, further preclinical and clinical investigations are necessary before these findings can be translated into routine clinical practice.

Overall, the concordance between the biochemical parameters, oxidative stress markers, hepatic NRG4 levels, and histopathological observations strengthens the validity of the present findings. The combined evidence suggests that dapagliflozin may mitigate diet-induced liver injury through modulation of metabolic and oxidative pathways, while the observed changes in NRG4 levels may represent an associated mechanism that warrants further investigation. The growing emphasis on regionally relevant and translational biomedical research further highlights the importance of investigating novel therapeutic targets for metabolic diseases and their complications (28).

Study Limitations

The present study has several limitations. First, the molecular mechanisms underlying NRG4 regulation were not directly explored using gene or protein expression techniques such as qPCR or Western blotting. Second, a formal assessment of insulin resistance and additional metabolic parameters was not performed. Finally, although the findings provide promising preclinical evidence, further experimental and clinical studies are required before these observations can be translated into clinical practice.

Conclusion

The findings of the present study demonstrate that dapagliflozin administration was associated with significant improvement in biochemical, oxidative stress, and histopathological alterations induced by a high-fat/high-fructose diet in rats. Treatment reduced liver enzyme activities, attenuated lipid peroxidation, enhanced antioxidant defence, and was accompanied by increased hepatic NRG4 levels.

The observed findings are consistent with a potential role of NRG4 in the hepatoprotective effects of dapagliflozin; however, the underlying molecular mechanisms were not directly investigated in the present study and require further experimental validation. Therefore, the current results should be interpreted as supportive rather than conclusive evidence of NRG4 involvement.

List of Abbreviations

ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
DMSO: Dimethyl Sulfoxide
ELISA: Enzyme-Linked Immunosorbent Assay
GSH: Reduced Glutathione
H&E: Haematoxylin and Eosin
HFD: High-Fat Diet
MASH: Metabolic Dysfunction-Associated Steatohepatitis
MASLD: Metabolic Dysfunction-Associated Steatotic Liver Disease
MDA: Malondialdehyde

NRG4: Neuregulin-4
PBS: Phosphate Buffered Saline
PPAR-γ: Peroxisome Proliferator-Activated Receptor Gamma
SD: Standard Deviation
SGLT2: Sodium-Glucose Cotransporter-2

Declarations

Ethics Approval and Consent to Participate

All experimental procedures involving laboratory animals were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Ethics Committee of the College of Medicine, University of Babylon, Iraq (Ref. No. 4329, Date: 6/7/2025).

Consent for Publication

All authors have given their explicit consent for the publication of this work under the Creative Commons Attribution Non-Commercial 4.0 International license.

Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

AHRA worked on the conceptualisation, methodology, investigation, data curation, formal analysis, writing the original draft preparation, and visualisation.
AFA worked on the supervision, validation, writing, review, editing, and final approval of the manuscript.
Both authors have read and approved the final manuscript.

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