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Effect of dapagliflozin against NAFLD and dyslipidemia in type 2 diabetic albino rats: possible underlying mechanisms

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Abstract. – OBJECTIVE: The aim was to investigate the effect of dapagliflozin on non-alcoholic fatty liver disease and dyslipidemia in type 2 diabetic rats by studying the histopathological structure of the liver and detecting possible underlying mechanisms for this impact by evaluating the potential anti-inflammatory action of dapagliflozin.

MATERIALS AND METHODS: 100 albino rats were used in this work and divided into five equal groups: group I (Control group), group II (Control diabetic group), group III (was administered dapagliflozin, 0.75 mg/kg, p.o.), group IV (was administered dapagliflozin, 1.5 mg/kg, p.o.), and group V (was administered dapagliflozin, 3 mg/kg, p.o.).

RESULTS: In our study, the total body weight, liver weight, liver index, blood glucose level, insulin level, insulin resistance, total cholesterol, triglycerides, liver enzymes, IL-1 β, and MDA were significantly higher in the control diabetic group than the normal group. The dapagliflozin reduced all the above variables significantly in a dose-dependent manner compared to the control diabetic group (*p*-value = 0.001 for all).

CONCLUSIONS: Dapagliflozin may be a promising novel treatment strategy for treating T2DM-related non-alcoholic fatty liver disease (NAFLD), and dyslipidemia where it possesses anti-oxidative, anti-inflammatory and anti-dyslipidemic effects.

Key Words:
Dapagliflozin, T2DM, MDA, IL-1 β, GSH, Liver enzymes.

Introduction

The hallmark of non-alcoholic fatty liver disease (NAFLD) is the over-accumulation of fat in the liver[1]. The primary two forms of NAFLD are simple fatty liver and non-alcoholic steatohepatitis (NASH). People often only experience one form of these diseases because they are distinct from one another[2].

The most prevalent form of diabetes, type 2 diabetes mellitus (T2DM), is defined by elevated blood sugar levels driven mainly by insulin resistance. Numerous trials[3,4] have shown that individuals with T2DM who are obese have a higher prevalence of NAFLD. NAFLD, particularly NASH, was identified in almost 75% of T2DM patients, frequently indicating a more dire prognosis[3]. Therefore, NASH is known as a diabetic liver disease[4].

An earlier investigation by Mirea et al[5] highlighted the possibility that insulin resistance has an important role in developing NAFLD. They

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stated that activating IL-1 group cytokines (IL-1β and IL-18) is essential for developing NAFL. By promoting the deposition of cholesterol and triglyceride in the hepatic cells and the development of lipid droplets, IL-1 promotes hepatic fatty degeneration[6]. Additionally, IL-1 enhances the inflammatory process by triggering the release of IL-6[7]. Also, a substantial role of IL-18 in lipid and glucose metabolism was discovered to be linked with insulin resistance, as evidenced by considerably higher plasma IL-18 levels in these patients[8].

Sodium-glucose transporter 2 (SGLT2) inhibitors are a promising way to treat T2DM because they increase insulin sensitivity, decrease glucose production, and decrease glucose reuptake by blocking the SGLT2 protein, which is responsible for 90% of glucose reuptake[4]. Dapagliflozin was the second SGLT2 antagonist to be authorized by the FDA, coming after canagliflozin in 2013[9].

Dapagliflozin is an effective suppressant of hyperglycemia[10]. Besides inhibiting the reuptake of the glucose collected by the kidney, it was reported to enhance insulin resistance[11,12]. Based on a study conducted by Liao et al[13], elevated blood levels of lipid mobilization caused a significant rise in lipolysis rate and higher insulin sensitivity after 90 days of dapagliflozin administration. An additional investigation conducted by Joannides et al[14] revealed that giving dapagliflozin to hyperglycemic rats for 45 days reduced weight increase, blood glucose levels, and improved glucose tolerance, which was accompanied by increased insulin sensitivity.

In addition to their ability to lower blood glucose levels, SGLT2 antagonists have also been associated with improved liver function in patients with and without NAFLD who have T2DM[15,16]. SGLT2 inhibitors are therefore thought to be a factor in inhibiting the development of hepatic impairment in diabetic rats[17]. However, the specific mechanisms causing such an effect have not yet been fully identified or clearly explained[18].

Therefore, the current investigation aimed to determine dapagliflozin's effect on liver function in diabetic rats and to understand the underlying mechanisms for this impact by evaluating the potential anti-inflammatory action of dapagliflozin.

Materials and Methods

Animals

Our study included 100 albino rats. Rats were placed in stainless steel cages with mesh floors and hardwood beds. They were housed in a laboratory with a standard light/dark cycle and a constant 25°C temperature. Throughout the trial, rats had access to food and drink. Before starting the study, the rats were given two weeks to acclimate. The Guide for the Care and Use of Laboratory Animals was considered the standard by which all experimental procedures were conducted.

Experimental Methodology

Twenty rats were fed a chow diet for 15 weeks with the addition of saline from the 9th week and were considered the control group (Group 1). However, the other 80 rats were exposed to diabetogenic agents and were considered a diabetic group. To induce diabetes in the rats, they were fed a high-fat diet (HFD), which consisted of 2% cholesterol, 10% lard, and 0.3% bile, and was administered for 8 weeks. After HFD administration, rats were injected with one dose of streptozotocin (STZ) (30 mg/kg I.P).

Fasting blood glucose was assessed after one week of STZ administration, and we found that after giving STZ for a week, the level of fasting blood glucose was above 180 mg/dl. In the next 6 weeks, 80 diabetic rats were given HFD along with dapagliflozin to only 60 rats.

As regards the treatment regimen in those six weeks, the diabetic rats were divided into four groups. Group 2 (20 rats) was administered HFD only, Group 3 (20 rats) was administered HFD and dapagliflozin (0.75 mg/kg, p.o.), Group 4 (20 rats) was administered HFD and dapagliflozin (1.5 mg/kg, p.o.), Group 5 (20 rats) was administered HFD, and dapagliflozin (3 mg/kg, p.o.).

At the end of the trial, blood was drawn from the rats' tails to determine the glucose concentration in their blood. We then administered 50 mg/kg of sodium thiopental to anesthetize the rodents. After taking blood samples from the retro-orbital plexus, we let the samples coagulate for 20 minutes and then centrifuged them for 15 minutes at 4,000 rpm. The blood samples were centrifuged and then frozen at -20 degrees Celsius for later use in biochemical analysis of liver enzymes, insulin, cholesterol, and triglycerides. After that, the rats were killed *via* cervical dislocation; their livers were harvested, weighed, and cleaned in ice-cold saline.

For histopathological analysis, liver samples were fixed in 10% buffered formalin from (Al Gomhorya, Cairo, Egypt). The rest of the liver tissue was frozen at -80°C immediately to be homogenized and tested for several biochemical markers.

Liver Enzymes and Lipids Assessment

Colorimetric assay kits were used to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. At a wavelength of 505 nm, the sample's absorbance was determined. Also, the albumin, bilirubin, GGT, ALP, and PT levels were assessed. A commercially available spectrophotometric test kit determined total cholesterol and triglyceride levels in the blood. The intensity of the color was detected at 545 nm.

Insulin Resistance Assessment

Insulin levels in the blood were determined using the Insulin ELISA Kit (MBS281388). Also, insulin resistance was calculated using the following formula: HOMA-IR index = [fasting glucose (mmol/L) × fasting insulin (IU/ml)]/ 22.5.

Histopathological Examination

For histological analysis, paraffin slices (5 m thick) were cut from the preserved liver tissues and stained with hematoxylin and eosin (H&E). An experienced pathologist used the NAFLD histological scoring system to conduct the study without previous knowledge about treatments given to the rats.

The Kleiner and Matsuzawa scoring system was used to assess the severity of steatosis and steatohepatitis (Figure 1). For steatosis, 0 was defined as lipid buildup in less than 5% of hepatocytes, 1 as lipid buildup in between 5% and 33% of hepatocytes, 2 as lipid buildup in between 33% and 66% of hepato-

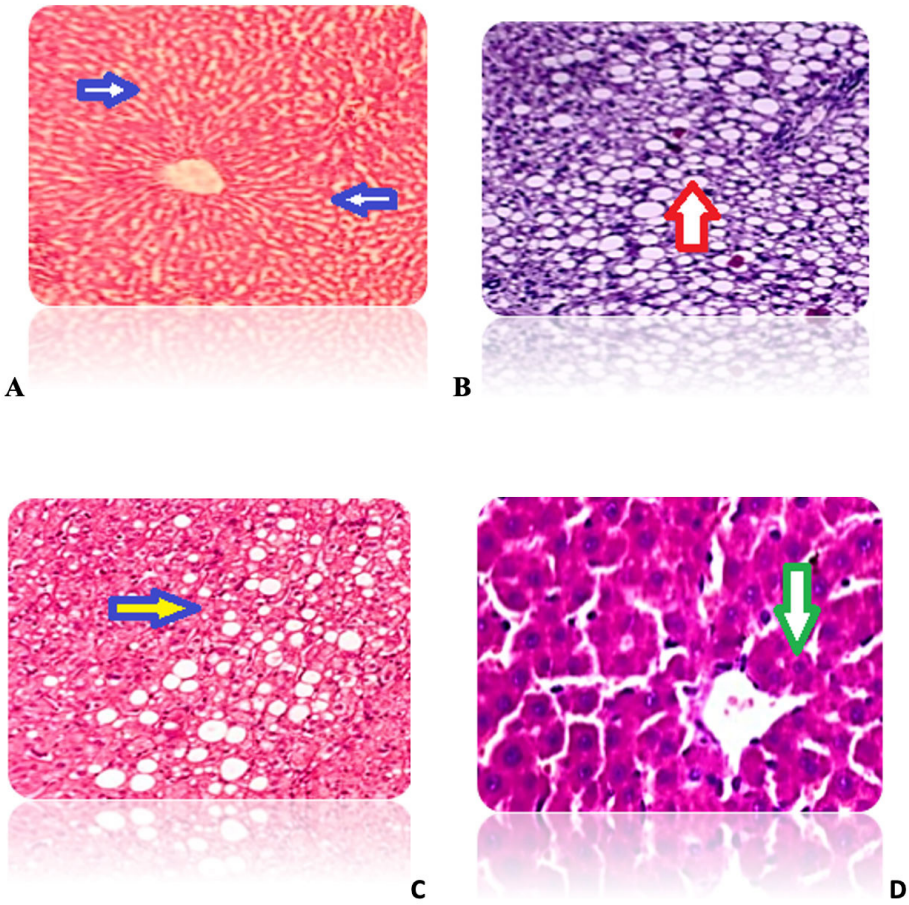

Figure 1. Light microscopic examination of liver tissues in the different study groups: photomicrograph of liver sections of the different groups using (H&E 40x magnification). **A**, Control group: section of liver tissue showed normal hepatocytes radiating around the central vein and separated by sinusoids (H&E- x100). **B**, Diabetic group: section of liver tissue showed severe infiltrative fatty changes in the form of more well-defined fat droplets occupying the cytoplasm of hepatocytes, pushing the nucleus to the periphery. Also, multiple inflammatory cells appear with loss of normal architecture of hepatocytes (H&E- x400). **C**, Diabetic on dapagliflozin 0.75 m: section of liver tissue showed mild to moderate fatty infiltrative changes where smaller well-defined fat droplets occupying the cytoplasm of hepatocytes with loss of normal architecture of liver tissue (H&E- x 400). **D**, Diabetic on dapagliflozin 1.5 and 3 m groups: section of liver tissue showed improvement of fatty infiltrative changes of the liver with the appearance of normal hepatocytes around the central vein with the appearance of normal architecture of liver tissue (H&E- x400).

cytes, and 3 as lipid buildup in more than 66% of hepatocytes. For inflammation, a score of 0 meant that there was no hepatocyte injury or inflammation, 1, a mild focal injury, 2, a noticeable or moderate injury, and 3, a severe injury or inflammation in zone 3.

Statistical Analysis

Statistical analysis was performed by SPSS statistical software, version 26, (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test initially checked the normality of continuous data. Continuous data were presented as mean and SD. The within-group comparison was done using way ANOVA test followed by Post hoc analysis to compare every 2 groups. A p-value < 0.05 was considered significant.

Results

Regarding the final body and liver weights of the rats, we found that they were significantly higher in group 2 (Control diabetic group) than in the normal group (p = 0.001). Their levels were significantly decreased in the dapagliflozin groups (groups 3, 4, and 5) in a dose-dependent manner compared to the control diabetic group (group 2) (p-value = 0.001 for all) (Table I).

In terms of the liver index, which is the ratio between the liver weight and the body weight. It was significantly higher in the control diabetic group than in the normal group (p = 0.001). However, in groups 3, 4, and 5, this index was significantly decreased compared to group 2 (p = 0.001) (Table I).

In our study, the total cholesterol was 112 ± 4.7 mg/ dl in group 2, significantly higher than that of the normal group (p = 0.001). This level was significantly decreased in dapagliflozin groups to 69.4 ± 4.8 mg/dl in group 3, 48.7 ± 4.7 mg/dl in group 4, and 45 ± 2.7 mg/dl in group 5 (p = 0.001 for all). Also, the total triglyceride was higher in the control diabetic group than in other groups (p = 0.001). However, in groups 3, 4, 5 it decreased significantly (p = 0.001). The reduction of total cholesterol and triglycerides in groups 3, 4 and 5 was dose-dependent (Table II).

The level of the liver enzymes was elevated in group 2 than in group 1 and decreased in groups 3, 4, and 5 in a dapagliflozin dose-dependent manner in comparison to the other groups (p-value < 0.05) (Table II).

Regarding dapagliflozin's effect on blood glucose level and insulin levels, the blood glucose and insulin levels were elevated in the control diabetic group more than in the normal group, with a statistically significant difference between the 2 groups. A significant reduction in the blood glucose and insulin levels was noticed in the dapagliflozin groups compared to the control diabetic group (p = 0.001). Also, the HOMA-IR index was significantly decreased in groups 3, 4, and 5 compared to group 2 (p-value = 0.001) (Table III).

According to the MDA level in our study, it was elevated in the control diabetic group compared to the normal group (p = 0.001). In groups 3, 4, and 5, the MDA level was significantly decreased compared to the control diabetic group (p-value = 0.001) (Table III). Moreover, the hepatic level of GSH was

Table I. Effect of dapagliflozin on final body weight, liver weight and liver index.

Variables	Group 1 (Normal) (N=20)	Group 2 (Control diabetic) (N=20)	Group 3 (N=20)	Group 4 (N=20)	Group 5 (N=20)	p-value[a] within groups	p-value[b] between each 2 groups
Body weight (mg)	212.25 ± 8.3	317.7 ± 15.5	270.7 ± 16	250.2 ± 14.1	236 ± 11.5	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
Liver weight (mg)	0.35 ± 0.02	0.63 ± 0.03	0.43 ± 0.03	0.42 ± 0.04	0.41 ± 0.04	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
Liver index	0.1 ± 0.07	0.2 ± 0.01	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*

[a]One-way ANOVA. [b]Post hoc analysis. *significant p-value. p^1: comparison between Group 1 and Group 2. p^2: comparison between Group 2 and 3. p^3: comparison between Group 2 and 4. p^4: comparison between Group 2 and 5.

Table II. Effect of dapagliflozin on the lipid profile and Liver enzymes.

Variables	Group 1 (Control) (N=20)	Group 2 (N=20)	Group 3 (N=20)	Group 4 (N=20)	Group 5 (N=20)	*p*-value[a] within groups	*p*-value[b] between each 2 groups
Total cholesterol (mg/dl)	40 ± 1.7	112 ± 4.7	69.4 ± 4.8	48.7 ± 4.7	45 ± 2.7	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
Triglycerides (mg/dl)	38 ± 1.7	130 ± 3.45	77.5 ± 9.4	60.4 ± 4.6	48.5 ± 6.1	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
ALT level (U\L)	38.6 ± 4.03	124.4 ± 13.9	83.3 ± 8.8	64.6 ± 6.7	54.3 ± 7.7	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
AST level (U\L)	32.4 ± 4.7	102.9 ± 12.7	67 ± 8.3	42.6 ± 9.1	33.2 ± 7.4	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*

[a]One-way ANOVA. [b]Post hoc analysis. *significant *p*-value. p^1: comparison between Group 1 and Group 2. p^2: comparison between Group 2 and 3. p^3: comparison between Group 2 and 4. p^4: comparison between Group 2 and 5.

markedly decreased in the control diabetic group more than in the normal group (p = 0.001). Interestingly, dapagliflozin therapy in groups 3, 4, and 5 increased the GSH activities in a dose-dependent manner (p-value = 0.001) (Table IV).

Our study gave evidence of the fact that inflammation is considered one of the main primary characteristics of steatohepatitis. We found a significant elevation of IL-1 β in the Control diabetic group compared to the other groups (p-value = 0.001). Amazingly, the dapagliflozin decreased the IL-1 β in a dose-dependent manner in groups 3, 4, and 5 compared to group 2 (p-value = 0.001) (Table IV and V).

Table III. Effect of dapagliflozin on the blood glucose, serum insulin, HOMA-IR index, and MDA.

Variables	Group 1 (Control) (N=20)	Group 2 (N=20)	Group 3 (N=20)	Group 4 (N=20)	Group 5 (N=20)	*p*-value[a] within groups	*p*-value[b] between each 2 groups
Blood glucose (mg/dl)	81 ± 9.7	288.8 ± 57.37	151.9 ± 16.4	146.3 ± 17.9	123.6 ± 20.8	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
Serum insulin (µu/ml)	1.1 ± 0.1	4.9 ± 0.46	4.1 ± 0.46	3.2 ± 0.46	2.5 0.61	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
HOMA-IR index	0.23 ± 0.03	3.5 ± 0.75	1.5 ± 0.28	1.1 ± 0.23	0.78 ± 0.23	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
MDA (ng/g tissue)	3.6 ± .4	22.5 ± 2.8	17.3 ± 1.5	13.2 ± 2.2	10 ± 2.1	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*

[a]One-way ANOVA. [b]Post hoc analysis. *significant *p*-value. p^1: comparison between Group 1 and Group 2. p^2: comparison between Group 2 and 3. p^3: comparison between Group 2 and 4. p^4: comparison between Group 2 and 5.

Table IV. Effect of dapagliflozin on the GSH, and hepatic levels of IL-1 β.

Variables	Group 1 (Control) (N=20)	Group 2 (N=20)	Group 3 (N=20)	Group 4 (N=20)	Group 5 (N=20)	*p*-value[a] within groups	*p*-value[b] between each 2 groups
GSH (pg/g tissue)	32 ± 1.2	5.2 ± .9	15.2 ± 1.6	19.2 ± 1.8	26.2 ± 1.7	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*
Hepatic levels of IL-1 β (Pg/ml)	10.9 ± .7	44.4 ± 2.3	34.4 ± 2.9	27.4 ± 2.5	20.7 ± 3.9	0.001*	p^1 = 0.001* p^2 = 0.001* p^3 = 0.001* p^4 = 0.001*

[a]One-way ANOVA. [b]Post hoc analysis. *significant *p*-value. p^1: comparison between Group 1 and Group 2. p^2: comparison between Group 2 and 3. p^3: comparison between Group 2 and 4. p^4: comparison between Group 2 and 5.

Discussion

Diabetes mellitus (DM) is a metabolic disease characterized by elevated blood glucose levels. It is considered one of the top ten primary causes of death worldwide. A significant health and financial burden is being placed on society by rising incidence and prevalence[19,20]. In Egypt, diabetes has a prevalence of 15.2% among adults[20].

Lipid abnormalities are common in diabetic patients' event with good diabetic control, especially in those with type 2 DM. It was reported that 30-60% of type 2 diabetic patients have dyslipidemia. They have an increased serum level of VIDL, LDL, and triglycerides. Also, they had a decrease in the serum level of HDL-C, which led to the loss of its anti-inflammatory and anti-oxidant effects[21].

Lipid abnormalities in diabetes include high blood pressure, smoking, poor physical activities, insulin resistance (IR), adipose tissue, inflammation, and other factors[22,23]. In patients with IR, the liver will lose the inhibitory effect of insulin on the synthesis of VLDL, increasing its serum level. Also, patients with tissue lipases had decreased activities of the tissue enzymes, particularly lipoprotein lipase, which is responsible for the

Table V. Effect of dapagliflozin on the steatosis and inflammation incidence.

Variables	Group 1 (Control) (N=20)	Group 2 (N=20)	Group 3 (N=20)	Group 4 (N=20)	Group 5 (N=20)	*p*-value[a] within groups
STEATOSIS						
Lipid accumulation in < 5% of hepatocytes	20 (100%)	0 (0%)	(0%)	(0%)	(0%)	
Lipid buildup in between 5% and 33% of hepatocytes	(0%)	(0%)	2 (10%)	14 (70%)	16 (80%)	0.001*
Lipid buildup in between 33% and 66% of hepatocytes	(0%)	20 (100%)	15 (75%)	4 (20%)	4 (20%)	
Lipid buildup in more than 66% of hepatocytes	(0%)	(0%)	3 (15 %)	2 (10%)	(0%)	
INFLAMMATION						
No hepatocyte injury or inflammation	20 (100%)	(0%)	(0%)	(0%)	(0%)	
A mild focal injury	(0%)	(0%)	5 (25%)	13 (65%)	15 (75%)	0.001*
A noticeable or moderate injury	(0%)	20 (100%)	15 (75%)	7 (35%)	5 (25%)	

[a]Chi-square test. *Significant at $p < 0.05$.

clearance of the VLDL. Also, the IR decreased the intestinal absorption of free fatty acid, thus enhancing the lipolysis to compensate for the deficiency in the FFA level resulting in the elevation of its serum level and enhancing the liver production of triglycerides[22].

In our study, we induced liver steatosis in four groups of rats by increasing the serum level of total triglycerides, and cholesterol. IR was found to be high after steatosis induction, which agrees with Paschos and Paletas[24], who reported that most non-alcoholic fatty liver disease patients have IR.

Dyslipidemia is considered a major risk factor for cardiovascular diseases resulting in myocardial infarction, sudden cardiac arrest, and death[25]. Moreover, diabetic dyslipidemia may result in liver injury, inducing the development of non-alcoholic steatohepatitis (NASH)[26]. So, during the treatment of diabetes, avoiding liver injury is crucial.

In our study, we proposed that sodium-glucose transporter 2 (SGLT2) inhibitors, a new oral hypoglycemic drug, have a big role in improving diabetic-induced dyslipidemia, steatosis of the liver, and NASH in rats.

Regarding the lipid profile, total cholesterol and triglycerides were decreased in the three groups (group 3, 4, and 5) who received three different doses of dapagliflozin more than in groups 1 and 2. Also, the total body weight was decreased in groups 3, 4, and 5 more than in groups 1 and 2. This reduction in the total cholesterol and triglycerides may be attributed to the total reduction in body weight or may be explained by the shift of the metabolic substrate from glucose to fatty acids[27,28]. This result was in accordance with the findings of Hazem et al[26].

As regards the liver enzymes, they were elevated after steatosis induction. However, their level was significantly decreased in groups 3, 4, and 5 more than in groups 1 and 2. This is due to the fact that dapagliflozin has a hepatoprotective effect, thus controlling the level of ALT and AST[29]. This agrees with the findings of Sattar et al[30], who reported that empagliflozin decreased liver enzyme levels in patients with type 2 diabetes.

In terms of MDA level, its level was increased in our NASH models, which agrees with the results of Dal et al[31] and Zelber-Sagi et al[32]. NASH and NAFLD patients have increased lipid peroxidation, oxidative stress, and inflammation, and are also associated with decreased antioxidants. This excess lipid peroxidation leads to the development of multiple pre-inflammatory products, and the MDA is considered one of the most prevalent products[32]. The level of MDA was significantly decreased by dapagliflozin, which agrees with the findings of Hazem et al[26].

As regards the IL-1β, it increases the accumulation of triglycerides and cholesterol in the liver enhancing the development of steatosis and NASH[33]. In our study, the IL-1β decreased significantly in groups 3, 4, and 5, which received the dapagliflozin compared to the control diabetic group 2.

Conclusions

Dapagliflozin may be a promising novel treatment strategy for treating T2DM-related NAFLD and dyslipidemia where it possesses anti-oxidative, anti-inflammatory, and anti-dyslipidemic effects.

Ethics Approval

Approval of the study was obtained from the Institutional Review Board (IRB), Damietta Faculty of Medicine, Al-Azhar University, and the research is acceptable according to the guidelines and declaration of Helsinki and our committee standard operating procedure guidelines (Acceptance number: DFM-IRB 00012367 – 23-02-005)

Informed Consent

Not applicable.

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Availability of Data and Materials

All data and materials are fully presented in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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