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ORIGINAL ARTICLE

Protective Effect of Dapagliflozin against Non-alcoholic Fatty Liver in Rats AMPK/SIRT1 Pathway Activation

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a serious chronic hepatic disorder with high prevalence globally and commonly coincides with obesity and type 2 diabetes mellitus (T2DM). Dapagliflozin (Dapa), a sodium-glucose cotransporter-2 (SGLT2) inhibitor, has an antidiabetic effect through increasing urinary glucose excretion also exerts beneficial effects on metabolic system. The current work aims to assess the possible protective effect of Dapa in hepatic steatosis induced by high-fat diet (HFD) in male albino rats.

Methods: 25 male albino rats weighing 200-250 gm were randomly allocated equally into5groups; Control group, HFD group, (Dapa-A, Dapa-B, and Dapa-C groups) received daily dapagliflozin (0.5, 1, and 2 mg/kg, by oral gavage), respectively, for 12 weeks. At the end of experiment, rats were euthanized, the following parameters were measured: body weight (BW), liver weight (LW), and LW/BW ratio, parameters of insulin resistance (IR) assessment, lipid profile, liver enzymes, proinflammatory cytokine: Interleukin 1β (IL1β), profibrotic factors, AMP-activated protein kinase (AMPK), sirtuin1 (SIRT1), hepatic farnesoid X receptor (FXR) and liver-X-receptorα (LXRα), along with assessment of blood pressure.

Results: Dapa significantly decreased BW, LW, LW/BW ratio, parameters of IR, lipid profile, liver enzymes, IL1β, LXRα, mean arterial blood pressure as compared to HFD group and significantly increased AMPK, SIRT1, and FXR compared to HFD group. Best values were detected in the dose of 2mg/kg/day that were insignificantly different from control group in most of the parameters.

Conclusion: Dapa hasa potential effect against NAFLD confirmed by reduced BW, liver weight along with declined liver enzymes and lipid profile. Furthermore, Dapa treatment was associated with lowered inflammatory response and downregulation of IL1β and LXRα, along with upregulated FXR, AMPK and SIRT1 in liver tissue. The current results give additional insights for the protective potential of Dapa on NAFLD.

Keywords: NAFLD, HFD, dapagliflozin, AMPK, SIRT1

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the exaggerated hepatocytic lipid deposition. It is one of the worldwide chronic hepatic diseases, with triglycerides above 5% of liver weight and no considerable use of alcohol. Inflammation and swelling turn NAFLD into non-alcoholic steatohepatitis (NASH). NASH cases could deteriorate to fibrotic liver which may turn into cirrhosis or hepatocellular carcinoma (HCC) in 20% of them [1].

Prevalence of NAFLD exceeded quarter of the world's people and almost reach hundred percent in obese persons. Obesity, NAFLD and type 2 mellitus (T2DM) coincide with each other in prevalence [2].

The pathogenesis of NAFLD is multifactorial,some related toexcessive lipid dietary intake, reduced physical activity associated with obesity,others related to increased adipose tissue lipolysis,hepatic

fat delivery and lipogenesis with disturbed secretion and oxidation,which cause free fatty acid (FFA) accumulation and induce insulin resistance (IR). This toxic lipid accumulation initiateshepatic injury andinflammasome activation [1].

Nucleotide-binding oligomerization domain (NOD)-like receptor 3(NLRP3) is an inflammasome upregulated and activated in liver injury, which increases the level of cytokines like Interleukin-1β (IL-1β),one of the main inflammatory mediators, and is responsible for the multiplication and stimulation of hepatic stellate cells (HSCs).Therefore, inactivation of NLRP3 may relieve liver injury resulting from excessive FFAs [3].

Adenosine monophosphate-activated protein kinase (AMPK) is an energy regulator thatpreserves lipid metabolism stability and is strongly related to the whole course of NAFLD's onset and progression. Anincreased AMP/ATP ratio activates AMPK,which leads to lowering the hepatic synthesis of fat, elevating the oxidation of fatty acid, and enhancing mitochondrial function in adipose tissue[4]. Sirtuin1 (SIRT1) is a protein deacetylase, based on nicotinamide adenine dinucleotide (NAD+) level which maintains balance of energy and lipid metabolism. Inhibition of SIRT1 reduces cellular activity and is associated with accumulated lipidand mitochondrial dysfunctionhoweverits activation leads to sterol regulatory element-binding protein 1c (SREBP-1c) phosphorylation that declines hepatic fat synthesis, promotes fat breakdown, and increases fatty acid oxidation. So, targeting AMPK and SIRT1 pathways activation is a way of NAFLD treatment [5].

As critical nuclear transcription factors, Farnesoid X receptor (FXR) is a highly expressed hepatic nuclear receptor which hasa significant impact in the modulation of hepatic triglyceride metabolism,decreases inflammatory cells infiltration and plays an antifibrotic role through activation of antifibrotic gene expression in HSCs.Hepatic steatosis and hyperlipidemia were seen with FXR deficiency;however, the liver's condition could be effectively improved by activating FXR thus its activation is another target in mitigation of liver injury[6]. On the other hand, Liver-X-receptor (LXR) is a nuclear receptor that is upregulated in liver, abnormally elevated in NAFLD andmay be the key factor in hepatic lipid deposition and induce the hepatic synthesis of fatty acids so its inhibition may have a role in treatment [7].

Lifestyle modification, even surgical treatment of obesity are therapeutical methods for NAFLD as no definite drugs till now. Drugs improving insulin sensitivity like metformin and thiazolidinediones show an effect on NAFLD due to the close relation between NAFLD and T2DM [8].

Dapagliflozin (Dapa) is accepted as a recent therapy for T2DM as one of the sodium-glucose linked cotransporter-2 (SGLT2) inhibitors. The main mechanism of Dapa as a SGLT2 inhibitor is to increase renal loss of glucose by decreasing its reabsorption in the convoluted renal tubules. Dapa improves blood glucose level, helps in reducing body weight and blood pressure because of glucose and fluid loss in urine,apart from hypoglycemic effect.Dapa can have cardioprotective effects and inhibit the progression of renal complications [9]. However, the possible protective effect of Dapain NAFLD needs to be investigated.

The current work aims to assess the possible protective effect of Dapa in hepatic steatosis induced by high-fat diet (HFD) in male albino rats.

MATERIALS AND METHODS

Materials:

Dapagliflozin was suppliedas powder fromAstraZeneca Egyptpharmaceutical and was suspended in saline fromE.I.P.I. Co. A.R.E., Pentobarbitalwas supplied from Sigma-Aldrich, St Louis, MO, USA.

Animals:

Twenty-five male albino rats weighing 200-250 grams were enrolled and purchased from the animal house of Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed under standard environmental conditions (22 ◦ C± 3) and 12h light-dark cycle, rats received food and water *ad-libitum*to adapt to the surroundings, animals were kept out of the study for one week before it started. The experimental protocols were approved by the Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC) "No. ZU-IACUC/3/F/5/2023"

Experimental design:

Rats were randomly allocated into five groups (5 rats each) as follows:

Group (1): Control group: rats received standard chow and saline for 12 weeks.

Group (2): HFD group: rats received high fat diet (HFD) (Casein 33.11%, Cystine 0.30%, Starch 15.21%, Dextrose 15.21%, Cellulose 5.00%,

Soybean oil 5.00%, Minerals 5.00%, Vitamins 1.00%, Colin 0.17%, and Lard 20%) and saline for 12 weeks.

Group (3): (Dapa-A): rats received HFD and dapagliflozin suspended in saline in a single daily dose (0.5 mg/kg)[10]by oral gavage for 12 weeks.

Group (4): (Dapa-B): rats received HFD and dapagliflozin suspended in saline in a singledaily dose (1 mg/kg)[10]by oral gavage for 12 weeks.

Group (5): (Dapa-C): rats received HFD and dapagliflozin suspended in saline in a single dailydose (2 mg/kg) [11]by oral gavage for 12 weeks.

At the end of experiment animals were anaesthetized by pentobarbital 75mg/kg/i.p and blood samples were collected from the retro-orbital plexus,serum was collected by centrifuging the blood samples at 4000 rpm for 15 min then it was stored at − 20 ◦C for biochemical assays. Rats were euthanized and liver tissue of each rat was dissected, body weight (BW) and liver weight (LW) was measured to estimate LW/BW ratio. Liver samples were preserved in 10% neutral buffered formalin in order to facilitate histological examination. The remaining liver tissues were promptly frozen at -80°C to facilitate homogenization and evaluation of various biochemical markers.

Methods:

Fasting blood glucose (FBG),serum insulin and IR index:

FBG concentration was evaluated using One Touch Brand strips, Blood Glucose Meter. One blood drop was collected by tail vein puncture [12].serum insulin was quantitatively measured in vitro using the RayBio Rat Insulin ELISA kit. IR was calculated by the HOMA-IR employing the formula: HOMA-IR index = [FBG (mmol/L) × fasting insulin (μ IU/ml)] / 22.5 [13].

Assessment of lipids:

Serum triglycerides(TGs) were measured usingCell Biolabs' Serum TG Quantification Kit by a coupled enzymatic reaction system [14] and free cholesterol as well as cholesterol esters were measured using an enzyme-driven process [15].

Mean arterial blood pressure (MAP) measuring by Noninvasive-blood-pressure measurement system (NIBP 250):

Rats were put in a box restraint, with their tails going through an optical sensor and cuff before being taped to the platform. The evaluation of the BP was done according to Whitesall et al. [16].

Serum liver enzymes:

Aspartate aminotransferase (AST) andAlanine aminotranferase (ALT) were assessedutilizing assay kits from Cell Biolabs uses a sequence of enzyme-driven processes to assess AST and ALT activity [17],[18].

Quantitative real time-PCR (RT-PCR) analysis:

Total RNA was extracted from the tissue employing Trizol (Invitrogen). For cDNA synthesis, a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was utilized. Mx3005P Real-Time PCR System (Agilent Stratagene) was utilizedemployingTOPreal™ qPCR 2X PreMIX (Enzynomics) according to the manufacturer's guidelines. The oligonucleotide specific primers (IL-1β, AMPK, SIRT1, Hepatic FXR, LXRα) were supplied by Sangon Biotech (Table 1). The fold change between the control and treatment groups calculated by calculation of 2 -ΔΔCt [19].

Histopathological analysis:

Hepatic tissues are processed, sectioned at 5 μm thickness, and stained with H&E in the conventional histopathological manner, and examined using light microscope to evaluate the severity of NAFLD. The scoring of NAFLD was done regarding Takahashi and Fukusato method [20]. In sections stained with Masson trichrome, the amount of collagen fibers stained dark green is measured to assess fibrosis.

Statistical analysis:

All data were gathered, tabulated, entered, verified, and analyzed utilizing SPSS (Windows version 25). The results were given as means ± standard error of mean (M±SEM). After ANOVA, the least significant difference (LSD) was employed for multiple comparisons The level of significance was adjusted at $p < 0.05$.

RESULTS

Effect of DapaonBW, LW, and LW/BW ratio.

HFD group showed significant increase in BW, LW, and LW/BW ratio compared to control group ($P<0.05$). Dapa treated groups revealed significant reduction in BW, LW, and LW/BW ratio compared to HFD group ($P<0.05$), BW was significantly ($P<0.05$)lowered in a dose dependent manner (DDM), in Dapa-C, LW was insignificantly different from control group($P<0.05$), In all treated groups LW/BW ratio was insignificantly different from control group and between each other ($P>0.05$) (Table 2).

Effect of Dapa on FBG, serum insulin levels and HOMA-IR index.

FBG, serum insulin, and HOMA-IR were significantly higherin HFD group compared to control group (P<0.05). FBG was significantly reduced in a DDM in Dapa treated groups compared to HFD group(P<0.05) and was insignificantly different from control group in Dapa-C group (P>0.05).Both serum insulin and HOMA-IR were insignificantly different from control group in Dapa-B and Dapa-C (P>0.05), but serum insulin was insignificantly varied from HFD group in Dapa-A group (Table 3).

Effect of Dapa on hyperlipidemia.

HFD group showed significant rise in both total cholesterol and TG levels compared to control group (P<0.05). Dapa exerted a significant decline in total cholesterol and TG values in all treated groups in a DDM compared to HFD group(P<0.05) however, their values were significantly elevated than that of control group (P<0.05) (Table 3).

Effect of Dapa on meanarterial blood pressure.

In HFD group, MAP was significantly higher than that of control group (p<0.05) and Dapa significantly lowered MAP in all treated groups with a graded dose effect as compared to HFD group (p<0.05) but MAP still was significantly greater than that of control group (p<0.05) as represented in (Figure 1).

Effect of Dapa on liver enzymes.

In HFD group, AST and ALT concentrations were significantly greater than that of control group (P<0.05), Dapa treated groups showed significant lowering of their levels in a DDM compared to HFD group (P<0.05) and AST level was insignificantly different from control group in Dapa-C group)((P>0.05) (Table 3).

Dapa alleviated liver inflammation and suppressed hepatic fibrosis.

HFD group showed significant elevation in IL-1β, TGF- β1 and fibronectin expression values compared to control group (p<0.05), Dapa exerted significant suppression in their expression values in all treated groups in a DDM(p<0.05) however, these levels still were significantly higher in Dapa-C groups compared to control group (p<0.05)(Table 3).

Effect of Dapa on AMPK and SIRT1 expression.

HFD group showed marked suppression of expression levels of AMPK and SIRT1 with significant difference compared to control group (p<0.05), AMPK and SIRT1 expression levels were significantly elevated in Dapa treated groups compared to HFD group (p<0.05) and their rise was in a DDM but still was significantly reduced than control group (p<0.05) (Figure 2).

Effect of Dapa in 3 dose levels on FXR expression and LXRα expression.

FXR level was significantly lowered in HFD group compared to control group (p<0.05).While LXRα expression level was significantly elevated(p<0.05). In Dapa treated groups, a significant elevation of FXR was shown compared to HFD with a graded dose effect (p<0.05) but was significantly less than that of control group (p<0.05). Dapa treatment suppressed LXRα expression as compared to HFD group (p<0.05) and in a DDM, that showed insignificant difference from control group in Dapa-C group (P>0.05) (Figure 2)

Effect of Dapa on liver histopathology:

The liver tissue of control group displayed normal hepatocytes and lobular architecture and surrounded by normal sinusoids. No ballooning of hepatocytes, steatosis or inflammatory cell infiltrations were seen. In HFD group, marked macrovesicular steatosis with hepatocytes signet ring cells, sinusoidal inflammation, portal tract with marked fibrosis, lobular inflammation with focal inflammatory cells aggregation were observed. Gradual improvements in the liver tissue histopathology were detected in Dapa treated groups; Dapa-A group showed mild improvement in hepatocytes steatosis, regression of lobular inflammation, ballooning and fibrosis of portal tract. In Dapa-B group, moderate improvement of changes was observed. Dapa-C group showed marked improvement and normal hepatocytes with no ballooning or steatosis, portal tract with no fibrosis and minimal inflammatory infiltrate were detected. (Figure 3 and 4)

Table (1): The sequences of the primers utilized in the study

gene	forward primer	reverse primer	size	accession no.
IL-1β	CACCTCTCAAGCAGAGCACAGA	ACGGGTTCCATGGTGAAGTC	81	NM_031512.2
Gapdh	GCATCTTCTTGTGCAGTGCC	GGTAACCAGGCGTCCGATAC	91	NM_017008.4
AMPK	GCGTGTGAAGATCGGACACT	TGCCACTTTATGGCCTGTCA	103	NM_023991.1
FXR-1	CCACTGACACGCCCTTTTTG	TGTTGCCGCATGGAGGATAA	97	NM_021745.1
LXR-α	GAGTCATCCGAGCCTACAGC	AAGAATCCCTTGCAGCCCTC	191	NM_031627.2
Sirt-1	GACAACCTCCTGTTGGCTGA	TGCGTGTGATGCTCTGTCAT	84	NM_001414959.1
TGF- β1	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGGC	168	NM_021578.2
MMP-9	GATCCCCAGAGCGTTACTCG	GTTGTGGAAACTCACACGCC	132	NM_031055.2
Fibronectin	GGATCCCCTCCCAGAGAAGT	GGGTGTGGAAGGGTAACCAG	188	NM_019143.2

Table (2): Effect of administration of single daily oral doses of Dapagliflozin (Dapa) 0.5 mg/kg, 1 mg/kg, and 2 mg/kg for 12 weeks in HFD-induced NAFLD in male albino rats on Liver weight and LW/BW ratio.

	Control (n=5)	HFD (n=5)	Dapa-A (n=5)	Dapa-B (n=5)	Dapa-C (n=5)
Body weight (BW) *(gm)*	**324 ± 7.16**	**416 ± 11.61**	**381 ± 3.56**	**366 ± 4.93**	**344 ± 3.19**
	A	**B**	**C**	**D**	**E**
Liver weight *(gm)*	**8.2 ± 0.57**	**12.2 ± 1.15**	**10.5 ± 0.5**	**9.6 ± 0.82**	**8.6 ± 0.82**
	A	**B**	**C**	**C**	**A**
LW/BW ratio	**0.025 ± 0.002**	**0.029 ± 0.002**	**0.028 ± 0.001**	**0.026 ± 0.002**	**0.025 ± 0.002**
	A	**B**	**A**	**A**	**A**

-Data represented as (Mean ± SE)
-Within the same row, values with different capital letters are significantly different ($p<0.05$)
- n: number of rats in each group.
-Statistical comparisons were carried out using one-way ANOVA followed by post hoc tests using LSD method.

Table (3): Effect of of Dapagliflozin (Dapa) in HFD-induced NAFLD on fasting blood glucose, insulin, HOMA-IR, total cholesterol, triglycerides, AST, ALT levels, IL- 1β, TGF- β1, and Fibronectin.

	Control (n=5)	HFD (n=5)	Dapa-A (n=5)	Dapa-B (n=5)	Dapa-C (n=5)
Fasting blood glucose level *(mg/dl)*	**96.5 ± 2.34**	**215.3 ± 8.24**	**151 ± 4.27**	**129.5 ± 4.12**	**101.3 ± 3.72**
	A	**B**	**C**	**D**	**A**
Serum insulin level *(UIU/ml)*	**1.43 ± 0.037**	**2.55 ± 0.412**	**2.51 ± 0.141**	**1.76 ± 0.133**	**1.48 ± 0.129**
	A	**B**	**B**	**A**	**A**
HOMA-IR	**0.34 ± 0.017**	**1.36 ± 0.267**	**0.94 ± 0.078**	**0.56 ± 0.058**	**0.37 ± 0.046**
	A	**B**	**C**	**A**	**A**
Total cholesterol ***(mg/dl)***	**118 ± 4.73**	**176 ± 3.75**	**159 ± 1.35**	**134 ± 1.82**	**128 ± 0.83**
	A	**B**	**C**	**D**	**E**
Triglycerides	**99.3 ± 6.58**	**223.5 ± 2.11**	**166.6 ± 1.36**	**130.3 ± 1.39**	**117.1 ± 1.62**

(mg/dl)	Control (n=5)	HFD (n=5)	Dapa-A (n=5)	Dapa-B (n=5)	Dapa-C (n=5)
	A	B	C	D	E
AST *(U/l)*	**22 ± 2.1**	**96.8 ± 7.88**	**72.6 ± 2.13**	**43.7 ± 5.79**	**27.2 ± 1.09**
	A	B	C	D	A
ALT *(U/l)*	**72.9 ± 3.77**	**133.7 ± 2.79**	**128.6 ± 1.30**	**103.7 ± 2.61**	**88.7 ± 1.34**
	A	B	C	D	E
Interlukin-1β	**0.99 ± 0.04**	**6.47 ± 0.18**	**5.13 ± 0.27**	**3.76 ± 0.14**	**2.39 ± 0.17**
	A	B	C	D	E
TGF- β1	**1 ± 0.01**	**5.68 ± 0.29**	**3.74 ± 0.17**	**2.65 ± 0.23**	**1.79 ± 0.11**
	A	B	C	D	E
Fibronectin	**0.99 ± 0.01**	**5.57 ± 0.14**	**4.61 ± 0.07**	**3.33 ± 0.12**	**1.71 ± 0.16**
	A	B	C	D	E

-Data represented as (Mean ± SE)
-Within the same row, values with different capital letters are significantly different ($p<0.05$)
- n: number of rats in each group.
-Statistical comparisons were carried out using one-way ANOVA followed by post hoc tests using LSD method.

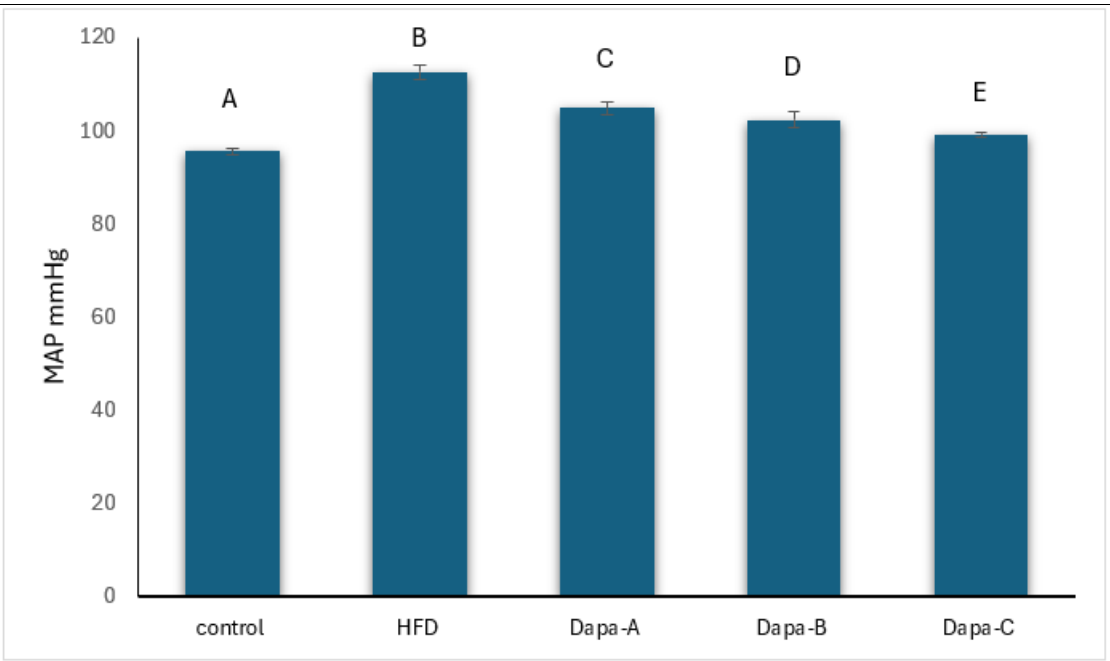
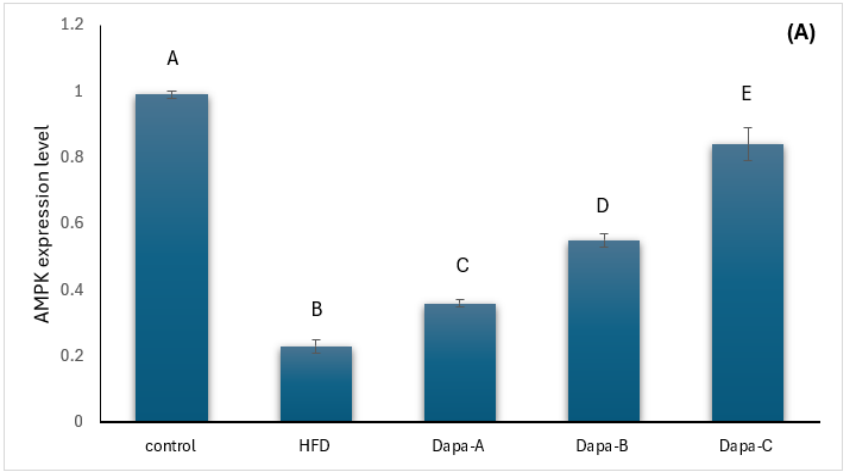
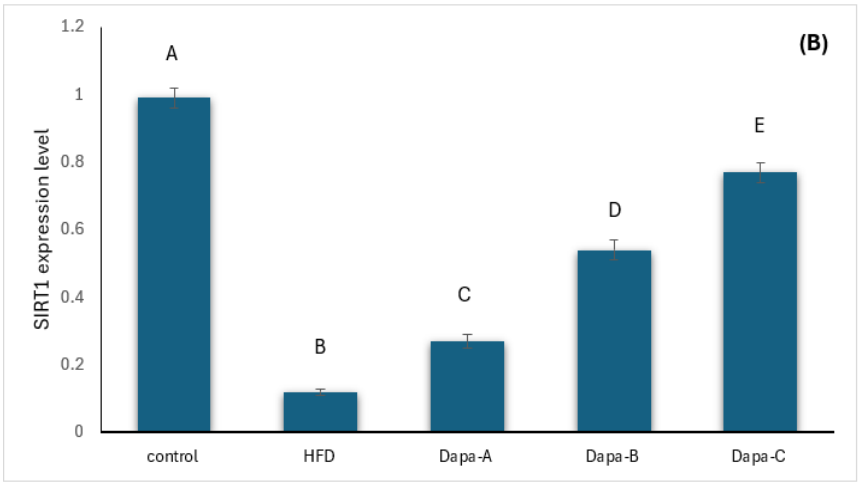


Figure (1): Bar chart showing effect of administration of single daily oral doses of Dapagliflozin 0.5 mg/kg, 1 mg/kg, and 2 mg/kg for 12 weeks in HFD-induced NAFLD in male albino rats on mean arterial blood pressure (MAP).
-Data represented as (Mean ± SE)
- Bars with different capital letters are significantly different ($p<0.05$)
-Statistical comparisons were carried out using one-way ANOVA followed by post hoc tests using LSD method.





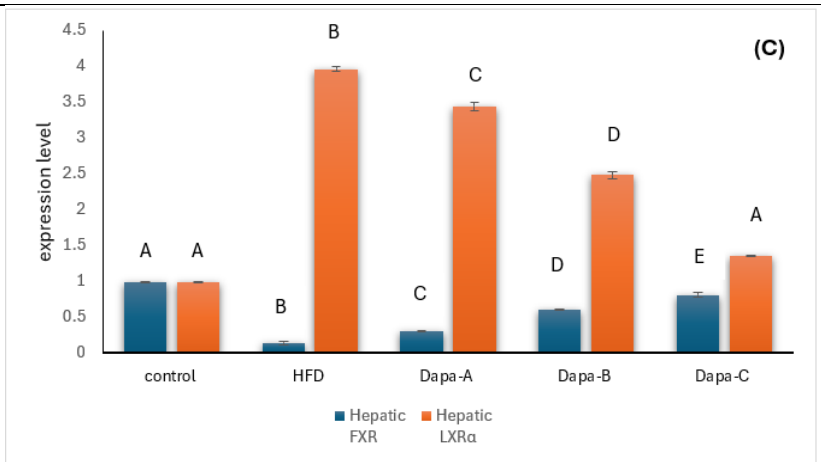


Figure (2): Bar chart showing effect of administration of single daily oral doses of Dapagliflozin 0.5 mg/kg, 1 mg/kg, and 2 mg/kg for 12 weeks in HFD-induced NAFLD in male albino rats on AMPK**(A)**, **(B)**SIRT1, **(C)**Hepatic FXR and Hepatic LXRα
-Data represented as (Mean ± SE)
- Bars with different capital letters are significantly different ($p<0.05$)
-Statistical comparisons were carried out using one-way ANOVA followed by post hoc tests using LSD method.

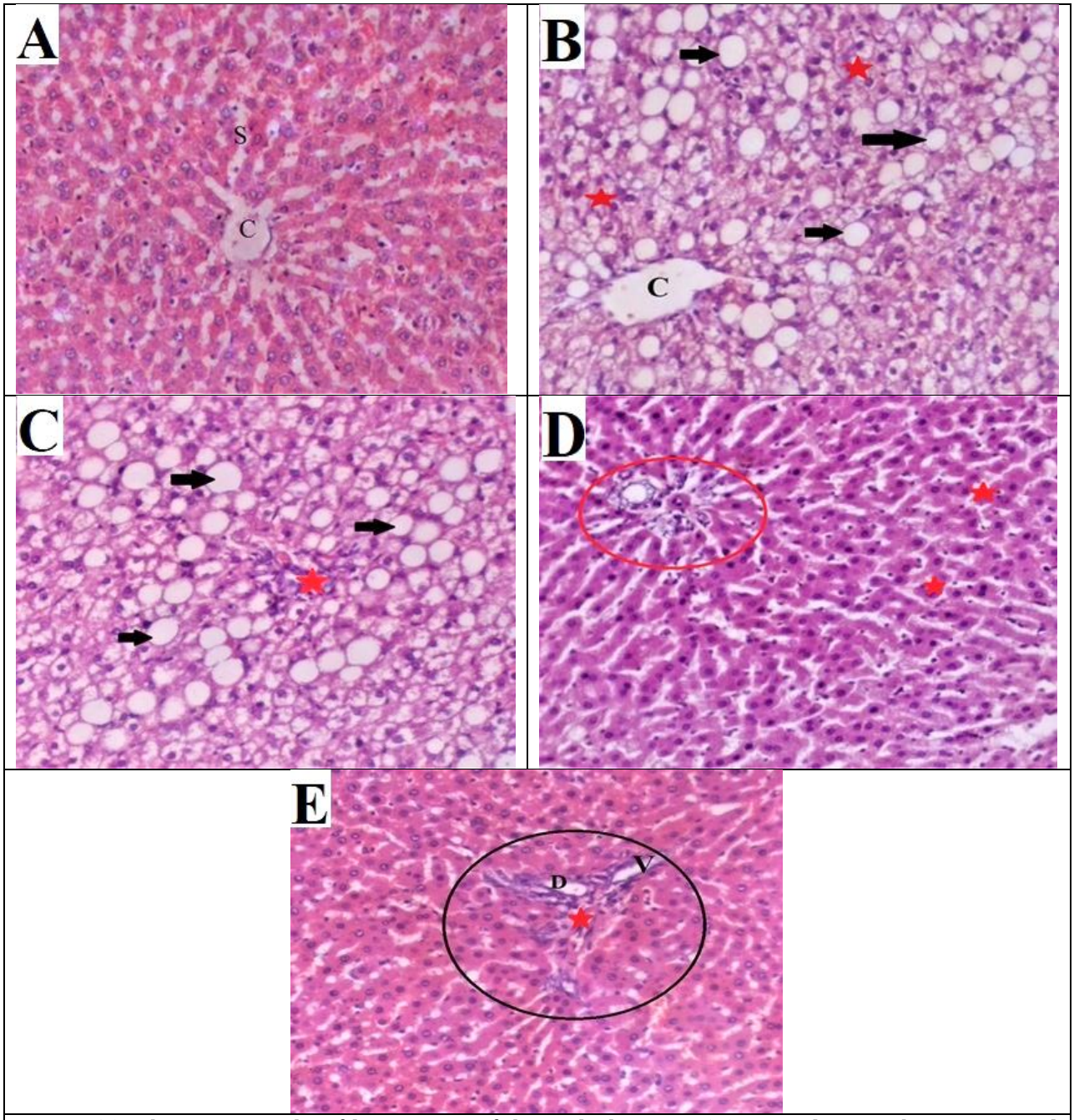


Figure (3): Photomicrographs of liver tissues of the studied groups. (A) Control group showing Normal hepatocytes, normal sinusoids (S) with central vein (C). (B) HFD group showing marked macro vesicular steatosis (score3) (black arrows) with hepatocytes signet ring cells, sinusoidal inflammation (red star).(C) Dapa-A group showing regression of lobular inflammation (red star) with foci of hepatocellular steatosis (black arrows) showing no improvement. (D) Dapa-B group showing portal tract (upper left) (red circle) with diffuse mild sinusoidal inflammatory infiltrate (red star) surrounded mild hepatocellular ballooning and degeneration with dark nuclei but no steatosis. (E) Dapa-C group showing portal tract with no fibrosis (circle) and minimal inflammatory infiltrate (red star) with patent portal vessels (V) and bile duct (D) surrounded with normal hepatocytes. (H&Ex100)

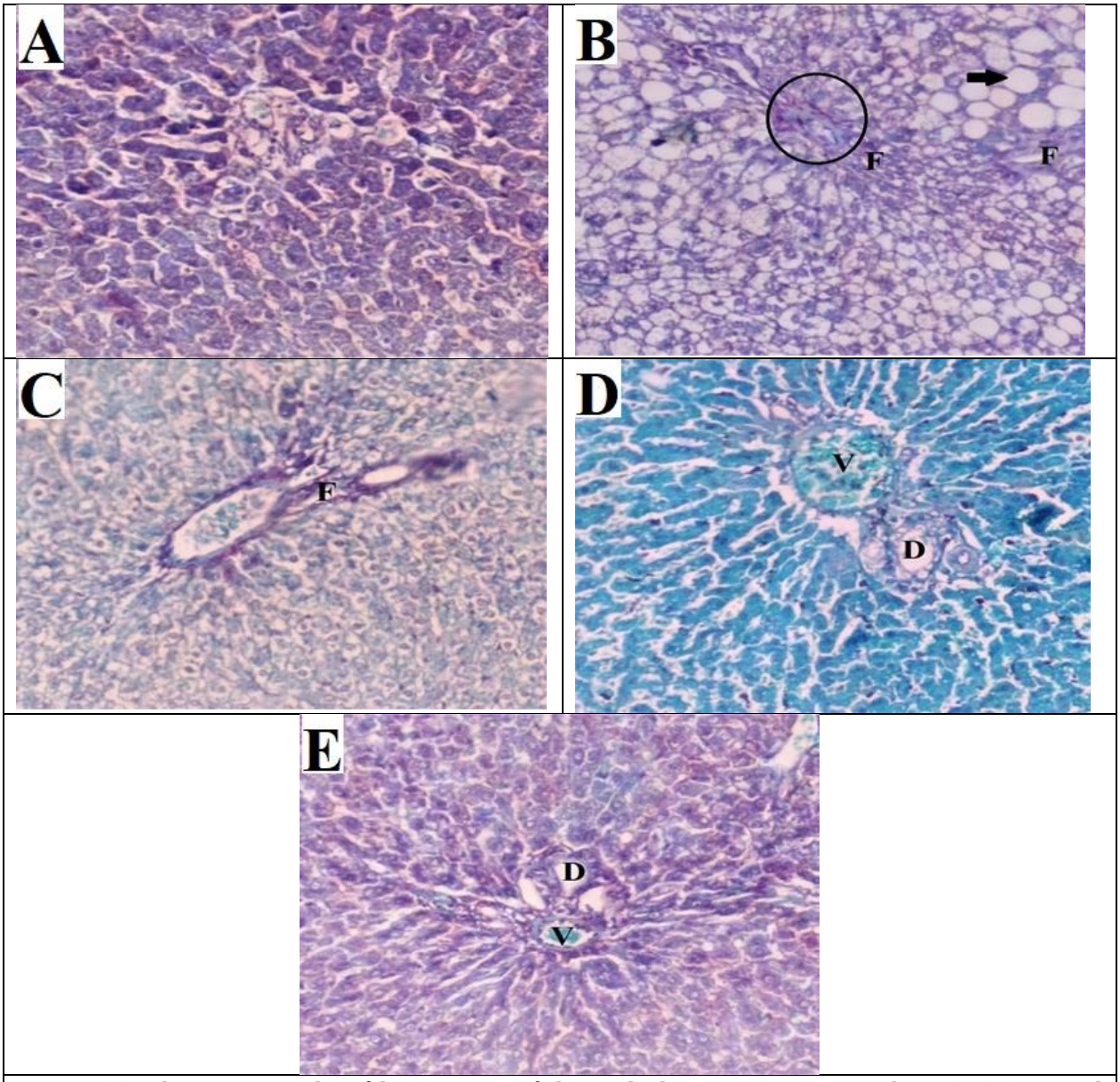


Figure (4): Photomicrographs of liver tissues of the studied groups (Masson trichrome). (A) control group showing normal hepatocytes, normal sinusoids with portal tract with no fibrosis. (B) HFD group showing two portal tracts (circles) with severe and bridging fibrosis (Porto-portal fibrosis) could be seen highlighted by Masson stain (F). The surrounding hepatocytes show severe macro vesicular steatosis (black arrow). (C) Dapa-A group portal tract showing mild to moderate fibrosis (F) highlighted by masson stain surrounded by hepatocellular ballooning and mild steatosis. (D) Dapa-B group portal tract showing improvement of fibrosis with nearly patent portal vessels (V) and bile ducts (D) surrounded by hepatocytes with mild ballooning and no steatosis. (E) Dapa-C group portal tract showing full improvement of fibrosis with patent portal vessels (V) and bile ducts (D) surrounded by normal hepatocytes with no ballooning or steatosis.

DISCUSSION

The main characteristic of NAFLD pathogenesis is the abnormal function of hepatic cells. This promotes hepatic de novo lipid production while increasing IR, creating a vicious cycle. Loss of balance between lipid synthesis, lysis, and uptake resulted from impaired insulin signaling that increased lipolysis in adipocytes and increased hepatic delivery of FFA, causing lipids to build up in hepatocytes, which in turn caused hepatic steatosis that may turn into NASH, cirrhosis, and HCC [21].

In this work we assessed the possible protective effect of Dapa against HFD induced NAFLD in

male albino rats and the possible underlying mechanisms.

The present work showed that administration of HFD induced NAFLD manifested as increased liver aminotransferases also hyperlipidemia along with increased liver weight. Moreover HFD induced NAFLD in the form of hepatocyte ballooning, lobular inflammation, steatosis, and liver fibrosis, which raised the NAFLD activity score, Dapa significantly reduced NAFLD activity score and fibrosis indicating the ability of Dapa to alleviate NAFLD which came in line with a previous study by Thongnak et al.[22] whorevealedthat Dapa may mitigate hepatic steatosis and reduce lipid buildup in the liver which were related to the up-regulation of genes involved in lipid metabolism, such as peroxisome proliferator-activated receptor alpha (PPARα), PPARgamma coactivator-1α (PGC-1α), and alpha carnitine palmitoyltransferase I (CpT1α) in the liver, thus stimulating lipolysis, biosynthesis of mitochondria, and fatty acid β oxidation.

The current study showed a remarkable elevation in BW of HFD group compared to control group, these findings agreed with Chang et al. [23] who observed increased BW, hyperglycemia, hyperinsulinemia, and hyperlipidemia in high fat fed rats. Saravanan & Pari [24] study explained that HFD can contribute to obesity developing that coincides with IR, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, the failure of hepatocytes to respond to insulin causes inadequate glucose consumption and ATP production that leads to additional food consumption, and increased body weight.

In the present study Dapa administration significantly reduced BW with best results observed in the dose of 2mg/kg/day. These findings agreed with Wang et al. [25] who revealed the potential effects of Dapa on nephropathy and hepatic steatosis in obese mice that were associated with reduced body weight and explained it as a result of the SGLT2 inhibitor-induced drop in glucose levels, which led to lowered adipose mass and improved fat consumption.

This study showed a significant elevation in LW. and LW. /BW ratio of HFD group compared to the control group, Dapa administration remarkably lowered LWfurthermore it significantly reduced LW/BW ratio. Our findings were consistent with ElMahdy et al. [26] reported that administering Dapa resulted in a considerable reduction in the LW index. This could be explained by the adipose tissue usage for energy generation that Dapa is thought to produce due to its induction of glucosuria. Our findings also revealed the effectiveness of Dapa in lowering FBG through decreasing glucotoxicityand enhancing insulinsensitivity. that agreed with Thongnak et al. [22] observed that Dapa enhanced insulin sensitivity in rats with IR induced to consume HFD and high fructose, while also potentially lowering plasma insulin levels. This was achieved by maintaining β-cell mass and function and minimizing cellular damage caused by glucotoxicity.

Regarding lipid profile this study showed significant rise in total cholesterol and TGs of HFD group compared to control group, Dapa administration significantly decreased total cholesterol and TGs concentrations. In line with our results regarding cholesterol and TGs, Hazem et al. [27] observed decreased hepatic weight, serum cholesterol and TG by Dapa. Thongnak et al. [22] explained that loss of calories due to glycosuria caused the substrate to be used for lipids instead of carbohydrates. Dapa may thereby correct the disturbed lipid profiles and decrease body weight and fat mass. Zhao et al., [28] revealed that Dapa notably reversed dyslipidemia and hepatic steatosis in db/db mice and the blood lipids were improved particularly TG, total cholesterol, and LDL levels suggesting that Dapa can alleviate the hepatic-related pathological alterations of NAFLD.

Regarding blood pressure findings, the present study agreed with Cremonese et al. [29] who showed spontaneous arterial hypertension in NAFLD, and obesity induced model.

In our study, Dapa administration significantly reduced MAP with bestresults observed in the dose of 2mg/kg/day. These findings support the protective effect of Dapain amelioration of the elevated blood pressure which were consistent with Saleh et al. [30] who found that Dapahad profound cardiovascular protection with amelioration of the elevated blood pressure in streptozotocin and HFD rat model which could be due to improved dyslipidemia and promotion of osmotic diuresis.

Our findings regarding ALT and AST coincide with the results obtained from previous study, Jin et al. [31] reported reduced ALT and AST levels in both serum and hepatic tissues in addition, Hazem et al. [27] observed elevated transaminases levels in control DM group which were reduced with Dapatreatment reflecting the hepatoprotective effect of Dapa that was explained by its improvement of the metabolic balance also SGLT2 inhibitors may

improve liver function by raising adiponectin and insulin sensitivity.

In a trial to assess the possible mechanism of the protective effect of Dapa, this study exhibited a significant rise in IL1β in HFD group compared to control group, this agreed with Ko et al. [32] who observed increased expression of IL-1β with activated NLRP3 inflammasome asinduction of inflammation activates inflammasomes that activate IL-1β that is involved in all stages of NAFLD. Dapaadministration showed significant reduction of IL-1β expression levels in a DDM with best results detected in the dose of 2mg/kg/day. These findings confirmed the anti-inflammatory impact of Dapa that plays a role in alleviating hepatic steatosis. Hazem et al. [27] demonstrated the strong hepatoprotective effect of Dapa by reducing IL-1β and NF-κB expression-induced tissue inflammation. This was explained by the drug's capacity to increase AMPK threonine 172 phosphorylation, which proved to modulate several pathways that have been shown to suppress NF-κB activity in human endothelium and to have anti-atherogenic and anti-inflammatory actions in blood vessels.

This study showed upregulation in the expression of profibrotic factor TGF-β1and extracellular matrix accumulation of fibronectin of HFD group compared to control group. In accordance with our findings Moore et al. [33] who respectively demonstrated the effect of chronic hepatic inflammation on induction of fibrogenesis that increased levels of TGF-β1 and TIMP1, elevated ECM protein fibronectin and increased levels of expression of MMP9 and SMα-actin in HFD induced NAFLD model. In order to attract additional macrophages and leukocytes to the liver and exacerbate inflammation and collagen deposition, activated HSCs continually produce TGFβ1 and TNFα [34].

In this study Dapashowed a notable decline in the expression level of TGF-β1 and fibronectin with best results observed in the dose of 2mg/kg/day suggesting that Dapa can alleviate the process of hepatic fibrosis that came in line with Shaaban et al. [35], and Xue et al. [36] who observed Dapa antifibrotic effect on heart, kidney, and liver respectively.

The current study showed a substantialdecline in the expression of both AMPK and SIRT1 of HFD group compared to control, which came in agreement with these findings, Shen et al. [37] observed thatSIRT1 and AMPK repressed activityby the elevated amounts of fatty acid linked to HFD administrationled to decreased fatty acid consumption and aberrant hepatic lipid deposition. Our results indicate that the hepatoprotective effect of Dapa could be mediated through AMPK and SIRT1 activation. In line with our study several studies supported our results: Li et al. [38] reported AMPK activation has been shown to have a positive effect on hepatic steatosis, and Dapa has also been shown to phosphorylate the lipogenesis enzyme Acetyl Co-A carboxylase (ACC) and enhance the fatty acid oxidation enzyme Acyl-coenzyme A oxidase. Dapawas observed enhancingPGC1α andp-AMPK/AMPK ratio and downregulating SREBP-1c, fatty acid synthetase (FAS), which explained that it reduces Complex I of the respiratory chain, leading toelevated AMP/ATP ratio that can stimulate AMPK through phosphorylating Thr172 residue. Phosphorylated AMPK inhibits ACC activity and increases carnitine palmitoyltransferase I (CPT1) and PGC1α expression, increases fatty acid oxidation, and lowers lipid production by downregulation of SREBP-1c and reduces proteins such as FAS[22].

Regarding the expression levels of FXR and LXR as nuclear transcription factors related to hepatic lipogenesis the current study came in line with Li et al. [39] showed that the HFD treatment resulted in a considerable LXRα upregulation, while the phase of steatohepatitis and fibrosis showed a downregulation of FXR, indicating a negative association between FXR expression and fibrosis.In the liver of db/db mice, Dapa may upregulate FXR and reduce LXRα in addition to inhibiting DNL-related enzymes.FXR can strongly inducesmall heterodimer partners (SHP) and then suppress LXRα function to obstruct SREBP-1c expression, therefore decreasing hepatic lipogenesis [40].

CONCLUSION

This study highlighted the potential hepato-protective effect of dapagliflozin against NAFLD. Dapa potential effect was confirmed by reduction of BW, liver weight along with improved liver enzymes and lipid profile along with histopathological results. Furthermore, Dapa treatment was associated with lowered inflammatory response and downregulation of IL1β and LXR, Dapa also upregulated FXR, AMPK and SIRT1 in liver tissue. The current results give additional insights for the protective potential of Dapa on NAFLD and suggest Dapa use for treatment of NAFLD.

Conflict of interest

No potential conflict of interest was reported by the authors.

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Figure legends

Figure (1): Bar chart showing effect of administration of single daily oral doses of Dapagliflozin 0.5 mg/kg, 1 mg/kg, and 2 mg/kg for 12 weeks in HFD-induced NAFLD in male albino rats on mean arterial blood pressure (MAP).

-Data represented as (Mean ± SE)

- values with different capital letters are significantly different ($p<0.05$)

-Statistical comparisons were carried out using one-way ANOVA followed by post hoc tests usingLSD method.

Figure (2): Bar chart showing effect of administration of single daily oral doses of Dapagliflozin 0.5 mg/kg, 1 mg/kg, and 2 mg/kg for 12 weeks in HFD-induced NAFLD in male albino rats on AMPK (A), (B) SIRT1, (C) Hepatic FXR and Hepatic LXRα
-Data represented as (Mean ± SE)
- values with different capital letters are significantly different ($p<0.05$)
-Statistical comparisons were carried out using one-way ANOVA followed by post hoc tests using LSD method.
Figure (3): Photomicrographs of liver tissues of the studied groups. (A) Control group showing Normal hepatocytes, normal sinusoids (S) with central vein (C). (B) HFD group showing marked macro vesicular steatosis (score3) (black arrows) with hepatocytes signet ring cells, sinusoidal inflammation (red star). (C) Dapa-A group showing regression of lobular inflammation (red star) with foci of hepatocellular steatosis (black arrows) showing no improvement. (D) Dapa-B group showing portal tract (upper left) (red circle) with diffuse mild sinusoidal inflammatory infiltrate (red star) surrounded mild hepatocellular ballooning and degeneration with dark nuclei but no steatosis. (E) Dapa-C group showing portal tract with no fibrosis (circle) and minimal inflammatory infiltrate (red star) with patent portal vessels (V) and bile duct (D) surrounded with normal hepatocytes. (H&Ex100)
Figure (4): Photomicrographs of liver tissues of the studied groups (Masson trichrome). (A) control group showing normal hepatocytes, normal sinusoids with portal tract with no fibrosis. (B) HFD group showing two portal tracts (circles) with severe and bridging fibrosis (Porto-portal fibrosis) could be seen highlighted by Masson stain (F). The surrounding hepatocytes show severe macro vesicular steashMtosis (black arrow). (C) Dapa-A group portal tract showing mild to moderate fibrosis (F) highlighted by masson stain surrounded by hepatocellular ballooning and mild steatosis. (D) Dapa-B group portal tract showing improvement of fibrosis with nearly patent portal vessels (V) and bile ducts (D) surrounded by hepatocytes with mild ballooning and no steatosis. (E) Dapa-C group portal tract showing full improvement of fibrosis with patent portal vessels (V) and bile ducts (D) surrounded by normal hepatocytes with no ballooning or steatosis.

Citation

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