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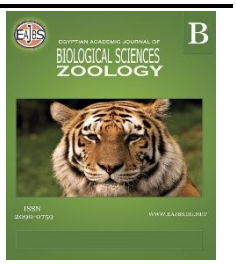

Leverage of Dapagliflozin and/or Curcumin on Liver Injury in Diabetes-Induced Male Albino Rats

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ABSTRACT

This study investigated the therapeutic effects of dapagliflozin (DAPA), a sodium-glucose co-transporter 2 (SGLT2) inhibitor, and curcumin (CUR), a bioactive compound with anti-inflammatory and antioxidant properties, on diabetic liver damage induced by a high-fat diet (HFD) and low-dose streptozotocin (STZ) in rats. Type 2 diabetes mellitus (T2DM) is associated with various hepatic complications, and this research aimed to evaluate the potential benefits of DAPA and CUR, both individually and in combination, in mitigating these effects. Thirty-six male albino rats were divided into six groups: a negative control, a CUR-only group, a diabetic untreated group (positive control), and three diabetic groups treated with DAPA, CUR, or a combination of both. After 30 days, various parameters, including fasting blood glucose (FBG), insulin, HbA1c, homeostatic model assessment of insulin resistance (HOMA-IR), beta-cell function (HOMA-B), liver enzyme activities, lipid profiles, and histopathological changes, were assessed. Results showed that the diabetic group exhibited elevated FBG, insulin resistance, HbA1c, liver enzymes, triglycerides (TG), and total cholesterol (TC), along with reduced HOMA-B, HDL, and protein profiles. Histopathological analysis revealed severe liver damage, including congested portal veins, disorganized hepatocytes, fatty degeneration, and increased caspase-3 expression. Treatment with DAPA, CUR, or their combination significantly improved metabolic and hepatic parameters. The combination therapy showing the most promising results in reducing diabetic liver complications. In conclusion, the combination of DAPA and CUR demonstrated superior efficacy compared to monotherapy in alleviating diabetic liver damage, suggesting its potential as a polytherapy regimen for managing T2DM-related hepatic complications.

INTRODUCTION

Diabetes, often known as diabetes mellitus, is a complex, long-term metabolic disorder. It is distinguished by elevated blood glucose levels brought on by the body's inefficient synthesis or use of the hormone insulin, which is necessary for glucose regulation (Abinaya *et al.,* 2020). Type 2 diabetes mellitus (T2DM) accounts for over 90% of all occurrences of diabetes (Goyal *et al.,* 2023). Numerous body organs undergo damage from chronic hyperglycemia and metabolic disorders, which can lead to serious consequences (Fowler, 2008). The liver, which is crucial for regulating the metabolism of lipids, proteins and carbohydrates, is most susceptible organ to T2DM (Shibabaw *et al.,* 2019). T2DM is the most prevalent cause of end-stage liver illnesses at the moment (Caldwell *et al.,* 1999; Al-Naemi *et al.,* 2024). Diabetes mellitus (DM) has induced several liver problems, such as cirrhosis, non-alcoholic fatty liver disease (NAFLD), fibrosis, abnormal glycogen deposition, hepatocellular carcinomas (HCCs), excessively elevated hepatic enzymes, acute liver illness

and viral hepatitis. Additionally, high levels of liver fat could increase insulin resistance and cause major metabolic complications. Hepatocytes may be destroyed by hyperglycemia with a fatty liver, increasing the morbidity and mortality rates among diabetic patients (Mohamed *et al.*, 2016).

Alanine transaminase (ALT) and aspartate aminotransferase (AST) enzymes are determined as indicators of liver health, and elevated levels are considered signs of liver damage in T2DM (Zhang *et al.,* 2023). Furthermore, hepatocyte necrosis and fibrosis are eventually accelerated by oxidative stress and inflammation brought on by fat buildup in the liver (Alonso *et al.,* 2017). A common chemical agent used to create experimental models of diabetes mellitus is streptozotocin (STZ), which destroys animal pancreatic beta cells and causes hyperglycemia. When combined with a high-fat diet, it can be administered to create an animal model of type 2 diabetes (Magalhães *et al.*, 2019). According to Furman (2015), the fat-fed/STZ rat model was utilized to create a nongenetic rat model of T2CM. T2DM is now being treated with a variety of injectable medications as well as orally given medicines that work on decreasing blood sugar levels while reducing body weight and the risk of cardiovascular disease (Rendell, 2004; Deepthi *et al.*, 2017). One of the sodium-glucose cotransporter families, sodium-glucose cotransporter-2 (SGLT2), mediates glucose reabsorption in the renal proximal tubules of the kidney. Dapagliflozin (DAPA) is a novel medication of SGLT2 inhibitors (SGLT2i) that prevents glucose reabsorption and permits glucose excretion in urine. In addition to its main function of decreasing blood glucose levels, DAPA has also been demonstrated to have hepatoprotective benefits. It improves indicators of liver damage and lowers the amount of fat in the liver (Choi *et al.,* 2018). Meanwhile, there may be several negative effects from the continued intake of pharmacological medications.

On the other hand, herbal remedies have a gentle action, are non-toxic and carry a low risk of adverse effects or contraindications (Kumari *et al.*, 2016; Shaikh and Patil, 2019). Natural substances also have the disadvantage of being less efficient (Nisar *et al.*, 2018). As a result, they are advised throughout the early stages of T2DM, during the pre-diabetic stage, and in conjunction with prescription medications (Greń and Massány, 2016). Polyphenols, also known as polyhydroxyphenols, are secondary metabolites of plants that provide defense against ultraviolet radiation or plant pathogen aggression. T2DM is one of the most studied diseases that can be affected by the activity of polyphenols. Curcumin (CUR) is one of the polyphenol groups that is derived from Turmeric (*Curcuma longa*) (Blahova *et al.,* 2021). CUR, a natural phenolic antioxidant, effectively scavenges free radicals due to its phenolic hydroxyl group (Wojcik *et al.,* 2018). It exhibits diverse biological activities, including antioxidant, antiproliferative, antineoplastic, and anti-inflammatory effects. Additionally, CUR shows therapeutic potential for conditions like diabetes, liver damage, kidney diseases, and neurodegenerative disorders (Rivera-Mancía *et al.,* 2018). Notably, it can improve T2DM by reducing blood sugar, cholesterol, oxidative stress and inflammation while enhancing insulin resistance, which is beneficial for patients with hyperlipidemia (Quispe *et al.,* 2022).

These facts led to the design of the current investigation, which examined the levels of FBG, insulin, HbA1c, HOMA-IR, HOMA-B, lipid profile and liver function estimation after DAPA and CUR treatment in diabetic animals induced by HFD/STZ. The later effect of DAPA and CUR was achieved by histological analysis using hematoxylin and eosin (H&E) staining and immunohistochemical detection of caspase-3 in the liver.

MATERIALS AND METHODS

Experimental Animals:

A total of thirty-six mature, healthy male Wistar albino rats weighing between 190 and 200 grams were split up into six groups, each of which had six rats. Before the studies started, the animals were adapted for a week.

Ethical Consideration:

The Institutional Animal Care and Use Committee (ZU-IACUC) of Zagazig University examined and approved this experimental protocol; its approval number is ZU-IACUC/1/F/22/2024.

Induction of Type 2 Diabetes mellitus (T2DM):

By combining a low dose of STZ injection that causes initial β-cell dysfunction (impairment in insulin secretion) with an HFD that produces insulin resistance (IR), this

protocol can be used to introduce an appropriate animal model that mimics all the features of T2DM in humans (Furman, 2021). Rats were given *ad libitum* HFD, which was prepared as described by Srinivasan *et al*. (2004).As a percentage of total kcal intake, HFD was established as follows: 58% fat, 25% protein, and 17% carbohydrate. Water was provided *ad libitum*. After 3 weeks, rats were fasted for 6 to 8 h before the intraperitoneal (IP) injection with one dose of streptozotocin (STZ 40 mg/kg), freshly dissolved in citrate buffer (0.1 M, pH 4) at a dose of 1ml/kg body weights (b. wt). The use of a 20% glucose drinking solution was permitted overnight to diabetic-induced rats to prevent lethal hypoglycemia. A glucometer (ACCU-CHEK) was used to measure tail vein fasting blood glucose levels, diabetes mellitus was established (Gandhi *et al.,* 2013). After completing the 4th week of the diabetes induction, the animals with stable fasting hyperglycemia (FBS ≥ 250 mg/dl) were included (Gad *et al.,* 2010). The diabetic rats were used in the different diabetic groups and were allowed to continue to feed on HFD until the experiment ended.

Materials:

1- **Streptozotocin (**STZ) and 0.1 M citrate buffer (pH 4-4.5) were purchased from Sigma–Aldrich Inc. (St. Louis, Mo, USA) from the Egyptian branch (Sigma pharma chemicals company, Cairo, Egypt). STZ solution was freshly prepared by dissolving STZ powder in cold 0.1M citrate buffer.

2- **Dapagliflozin:** 10 mg tablets (AstraZeneca Pharmaceuticals LP Mount Vernon, Indiana, USA) were obtained from a local pharmacy. Aqueous solution of DAPA was prepared and administered orally by gastric tube at a dose of 1 mg/kg/day according to Jaikumkao *et al*. (2018).

3- **Curcumin:** It was obtained as Theracurmin (60 Vegetarian capsules; the absorbed form of curcumin) extract from turmeric (*Curcuma longa* root) from Natural Factors Chemical Company in CANADA website: https://naturalfactors.com. Curcumin was dissolved in dimethyl sulfoxide (DMSO) then diluted to the appropriate volume with distilled water and orally administrated through a gastric tube at a dosage of 80 mg/kg/day according to Zhang *et al.* (2013).

Experimental Design:

The animals were split into six groups, with six rats in each group as follows:

Group 1, normal (C): normal rats with fasting blood glucose less than 110 mg/dl that intraperitoneally (IP) injected with one dose of 1.0 ml/kg of 0.1 M citrate buffer (pH 4). The animals were kept untreated and fed a regular diet.

Group 2, Curcumin group (CUR): rats received oral administration of CUR (80 mg/kg/day) for 4 weeks and were fed with a typical diet.

Group 3, Diabetic untreated group (D): served as the reference group for the corresponding diabetic-treated groups.

Group 4, Diabetic+ Dapagliflozin (D+DAPA): Dapagliflozin was orally administered daily to diabetic-induced rats at 1 mg/kg/day for 4 weeks.

Group 5, Diabetic + Curcumin (D+CUR): Curcumin was orally administered daily to diabetic-induced rats at an 80 mg/kg/day dose for 4 weeks.

Group 6, Diabetic+ Dapagliflozin+ curcumin (D+DAPA+CUR): Diabetic-induced rats were treated orally with dapagliflozin (1 mg/kg/day) followed by curcumin (80 mg/kg/day) for 4 weeks.

Collection of Samples:

A) Blood collection: The overnight fasted rats were weighed and then anesthetized with an IP of Urethane (99%, Sigma-Aldrich, Dorset, UK) at a dose of 1 g/kg (b. wt). From each rat's retro-orbital venous plexus, two samples of blood were collected. To measure the percentage of glycosylated hemoglobin (HbA1c) in total hemoglobin, one sample was combined with an anticoagulant. The serum from the second blood sample was kept at -20°C for biochemical analysis after it was coagulated at room temperature and centrifuged for 15 minutes at 4000 rpm.

B) Tissue collection: Following dissection, the tissue sample was immediately removed, perfused with phosphate buffer saline (PBS, pH 7.4), and dried with filter paper. The liver was weighed to determine its relative weight. Histological and immunohistochemical investigations were carried out on the liver organ.

Methods:

Body Weight Changes:

In the current experiment, the rat's body weights were measured weekly from the start of the experiment using a digital electrical balance (Sartorius Goettingen type 140 /AG, W. Germany).

Organ Relative Weights:

After the dissection of the animals, liver weight was measured using the digital electrical balance, and liver relative weight was determined according to the following equation:

$$\text{Formula} = \frac{\text{Absolute liver weight}}{\text{Final Body weight}} x100$$

The Biochemical Studies:

Estimation of Fasting Blood Glucose Levels (FBG) in the Serum*:*

Following the manufacturer's instructions, a colorimetric assay kit (SPINREACT, S.A./S.A. U Ctra. Santa Coloma) was used to measure the FBG in rat serum using **Tietz's** method (1995).

Estimation of Serum Insulin Levels*:*

The serum insulin levels were determined according to Pradelles *et al.* (1990), using an enzyme-linked immunosorbent assay (ELISA) Kit (Cat. No.: RA19004R) manufactured by Bio Vendor – Laboratorní Medicína.

Estimation of Homeostasis Model Assessment Of Insulin Resistance Index (HOMA-IR) and (HOMA-β) Cell Function:

The Matthews *et al.* (1985) approach was used to estimate HOMA-IR. By applying the subsequent formula: [FBG (mg/dl) x fasting serum insulin (μU/ml)]/ 405)

The HOMA- β-cell function was calculated according to Yoon *et al. (*2016) equation:

HOMA-B% = (20 × fasting serum insulin)/ (FBG − 3.5).

Estimation of Glycosylated Hemoglobin (HbA1c):

Using Nayak and Pattabiraman's (1981) method the blood's HbA1c was calculated. Free amino groups at the N-terminus of hemoglobin A0's β-chain are glycated non-enzymatically to generate HbA1c. The blood glucose level and the HbA1c level are related. The HbA1c test demonstrates the average daily blood glucose level throughout the previous two months because glucose stays attached to the red cell during its life cycle.

Estimation of the Liver Function:

Serum total protein was estimated according to Koller and Kaplan's (1984). However, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were demonstrated by Burtis and Ashwood (1999) methods by using the colorimetric assay kits (SPINREACT, S.A./S.A. U Ctra. Santa Coloma) was used to quantify these values.

Estimation of Lipid Profile:

Triglyceride, total cholesterol and high-density lipoprotein (HDL) cholesterol were estimated according to Bucolo and David (1973), Meiattini *et al.* (1978) and Naito *and* Kaplan (1984) respectively. As recommended by the manufacturer, a colorimetric assay kit (SPINREACT, S.A./S.A. U Ctra. Santa Coloma) was used to quantify these values. Meanwhile, Low-density lipoprotein (LDL) and very low-density lipoproteins (vLDL) were assayed according to Al-Rasheed *et al.* (2016) formula:

LDL cholesterol = Total cholesterol–[(triglycerides/5) +HDL cholesterol]

vLDL cholesterol = Triglycerides/5

The Histopathological and Immunohistochemical Studies of The Hepatic Tissues:

Following the experiment's conclusion, the control and treated groups' rats were sacrificed, and the livers were removed right immediately. They were then preserved in 10% neutral formalin for a whole day, dehydrated in increasing alcohol grades, cleared in xylene, and covered with paraffin wax. Then, using the procedure described by Suvarna *et al.* (2013), sections were cut to a thickness of 5 μm and stained with hematoxylin and eosin stain (H & E). According to Eissa and Shoman (1998), apoptotic caspase-3 expression was assessed.

Morphometric Analysis:

Image Synthesis: an Olympus digital camera (Olympus LC20-Japan) placed atop an

Olympus microscope (Olympus BX-50, Tokyo, Japan) with a 1/2X picture adapter and a 40X objective was employed to digitize the slides. On an Intel® Core I3® computer, the resulting images were examined using Video Test Morphology 5.2 software (Russia), which has a built-in a specialized stain quantification and immunohistostaining evaluation technique. The system measured the area percentage of Caspase-3 positive expression.

Quantitative Immunohistochemical Analysis:

The optical density of Caspase-3 positive expression in liver tissue of the experimental groups was recorded using image Proplus 4.5.1.22 analysis software.

Statistical Analysis:

The statistical package for social sciences (SPSS) version 16 was employed to statistically examine the results obtained. Each value is indicated as mean ± standard error. After comparing means using a one-way ANOVA, Tukey's post hoc analysis was applied. $P < 0.01$ was regarded as a significant value.

RESULTS

The present study demonstrated the effects orally administrated DAPA, CUR and a combination of both on the male albino rats with type-2 diabetes mellitus induced by HFD and a low dose of STZ were demonstrated in the present study.

The Body Weight Changes:

The body weight changes in the control and the different treated groups are shown in the Figure (1). Continuous normal body weight gain in the control group during the experiment was recorded (8 weeks). However, the animals fed a high-fat diet (HFD) to induce insulin resistance revealed a marked body weight increase over the first four weeks as shown in the figure (1). From the fifth week to the completion of the experiment, the diabetic (D), D+DAPA, D+CUR and D+DAPA+CUR groups showed a gradual reduction in their body weights. Compared to the control and the untreated diabetic groups, the DAPA and/or curcumin treated groups recorded a significant decrease in the rat's body weight in the sixth week of the experiment. Meanwhile, the aforementioned groups' body weights demonstrated a significant increase compared to the untreated diabetic group which remained less than the control values.

The data in Figure (2), illustrated the effects of the DAPA and/or CUR on the relative liver weights. When compared to the control rats, the relative liver weights in the diabetic (D) group showed a significant increase ($p<0.01$). Conversely, both the D+ CUR and the D+DAPA+CUR groups exhibited a significant ($p<0.01$) reduction in relative liver weights when compared to that in the diabetic group. At the end of the experiment, DAPA alone did not enhance the liver weight in diabetic rats. However, when DAPA was combined with curcumin, the liver weights of the diabetic animals were modified and came close to the relative liver weight of the control group.

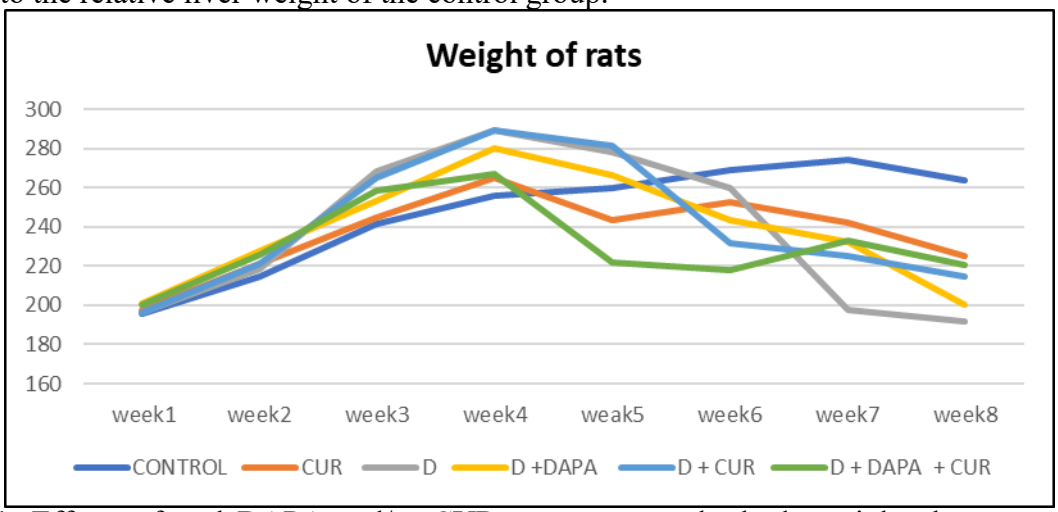

Fig. 1: Effects of oral DAPA and/or CUR treatment on the body weight changes after 8 weeks in the different experimental rats. Data represent means ± SE (n=6), respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

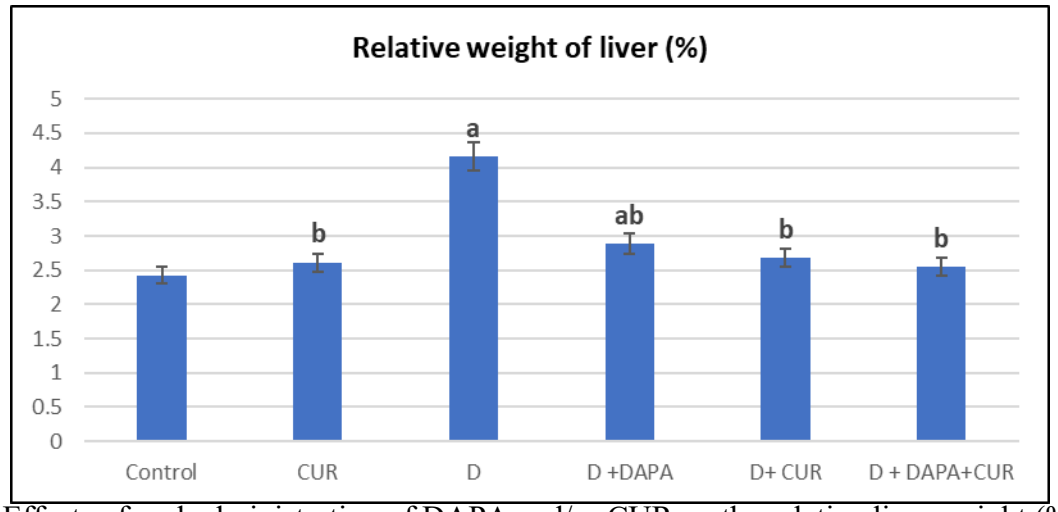

Fig. 2: Effects of oral administration of DAPA and/or CUR on the relative liver weight (%) in the different experimental rats. Data represent means ± SE (n=6). Superscripts (a) and (b) demonstrate statistical differences at ($p < 0.01$) as compared to the control and the diabetic group, respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

The Biochemical Studies:

1-The Serum Fasting Blood Glucose (FBG), Insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Beta-Cell Function (HOMA-B), and Glycated Hemoglobin (HbA1c) Values:

Initially, the non-diabetic curcumin administered group recorded non-significant changes in the previously listed parameters in contrast to the standard control rats. However, a significant ($p<0.01$) increase in the FBG, insulin, HOMA-IR and HbA1c has been demonstrated in the diabetic (D) group compared to the normal group (Figs. 3,4,5 and 6). However, the HOMA-B values recorded a significant decrease in the untreated group compared to the normal group values (figure 7). However, the diabetic rats treated with DAPA in D+ DAPA or curcumin in the D+ CUR groups showed a significant decrease ($p< 0.01$) in the FBG, insulin, HOMA-IR, and HbA1c levels as compared to their levels in the diabetic untreated animals. Nonetheless, a significant increase ($p< 0.01$) in these parameter levels was still recorded as compared to the normal group (control). The combined treatment with the DAPA and curcumin (D + DAPA + CUR) group resulted in a significant decrease in the insulin, HOMA-IR, and a significant increase in HOMA-B as compared to the untreated group with no significant change when compared to the normal control group. Meanwhile, the level of the FBG and HbA1c in the same group persisted with a significant increase at ($p< 0.01$) as compared to that of the control group.

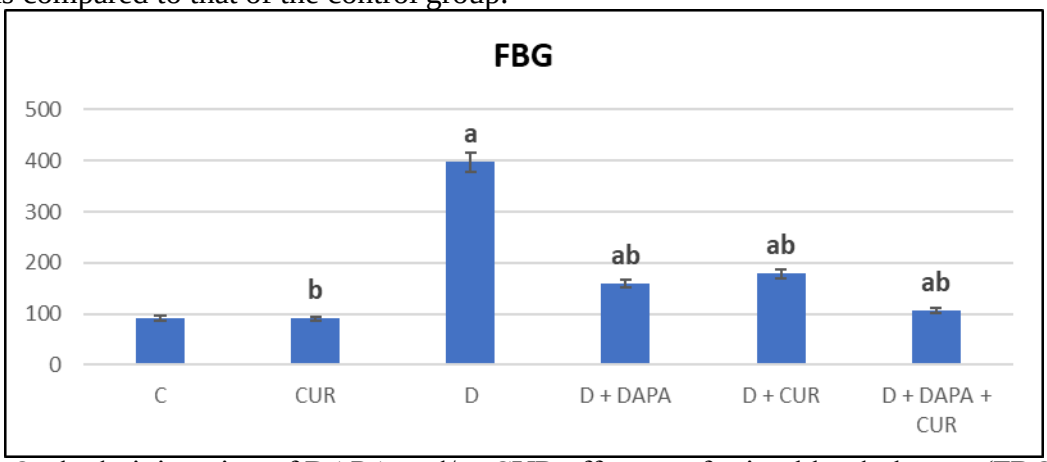

Fig. 3: Oral administration of DAPA and/or CUR effects on fasting blood glucose (FBG) in the different experimental rats. Data represent means ± SE (n=6) and the statistical difference at $p< 0.01$ represented by (a) and (b) superscripts as compared to the control and the diabetic group, respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

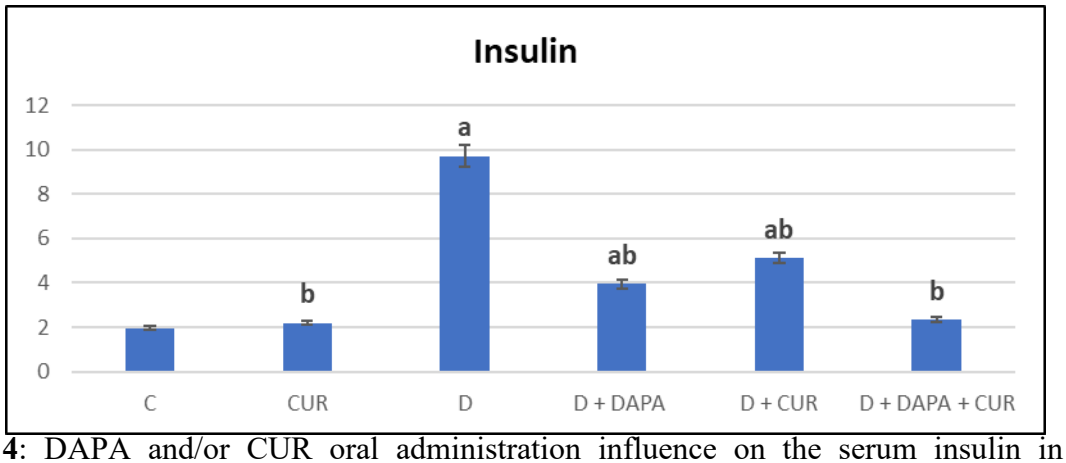

Fig. 4: DAPA and/or CUR oral administration influence on the serum insulin in the different experimental rats. Data represent means ± SE (n=6) where (a) and (b) show the statistical difference as compared to the control and the diabetic group, respectively at $p < 0.01$. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

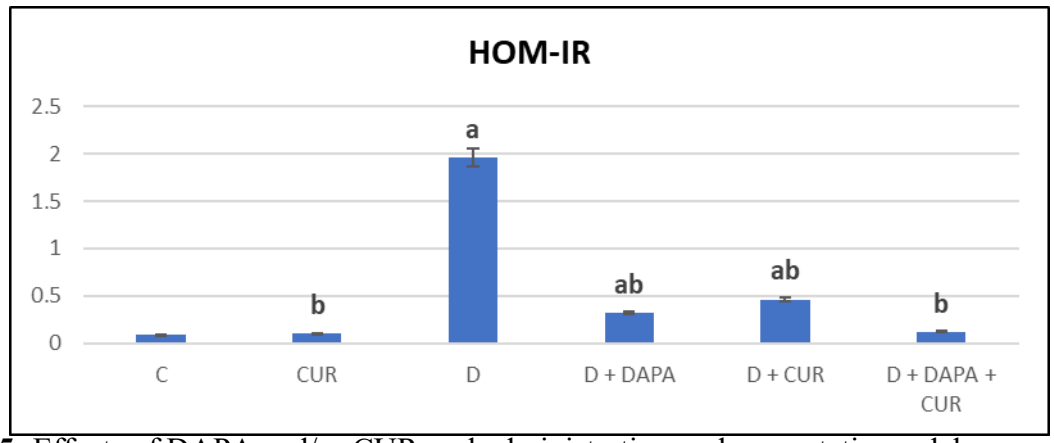

Fig. 5: Effects of DAPA and/or CUR oral administration on homeostatic model assessment-insulin resistance) HOM-IR)in the different experimental rats. Data represent means ± SE (n=6) where (a) and (b) show the statistical difference at $p < 0.01$ as compared to the control and the diabetic group, respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

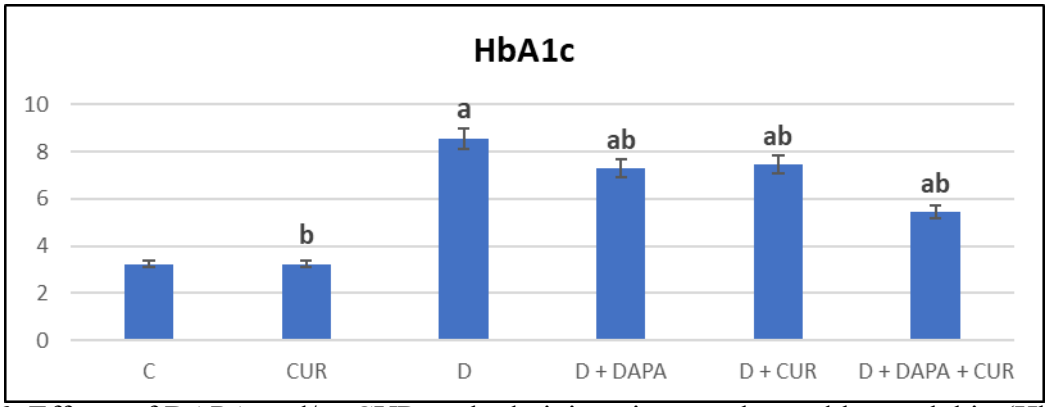

Fig. 6: Effects of DAPA and/or CUR oral administration on glycated hemoglobin (HbA1c) (% of normal Hb) in the different experimental rats. Data represent means ± SE (n=6), (a) and (b) demonstrate the statistical difference at $p < 0.01$ as compared to the control and the diabetic group, respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

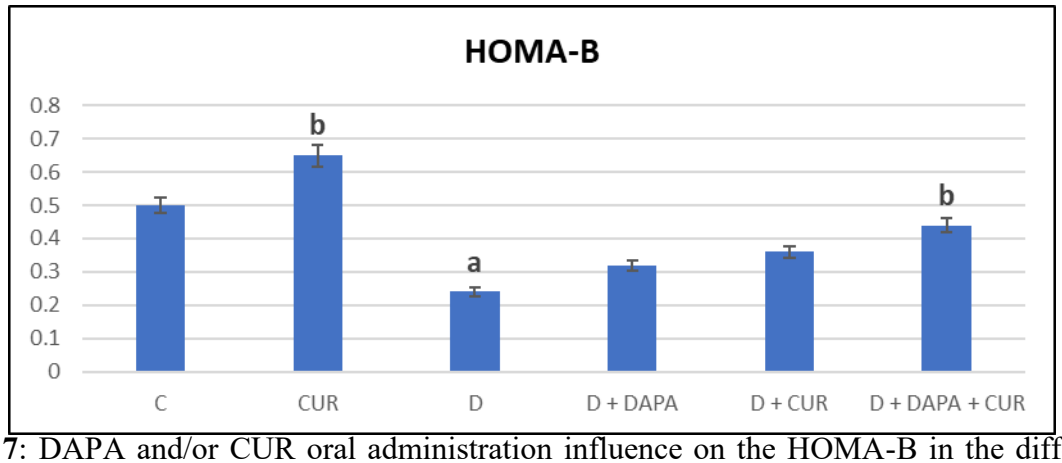

Fig. 7: DAPA and/or CUR oral administration influence on the HOMA-B in the different experimental rats. Data represent means ± SE (n=6). (a) are statistically different as compared to the control, (b) are statistically different as compared to the diabetic group at $p < 0.01$. HOMA-B: Homeostatic model assessment - β-cell function, D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

2-The Liver Functions:

Initially, the non-diabetic curcumin-administered group recorded no significant changes in the concentrations of total protein, serum albumin, or ALT and AST activities in contrast to the standard normal group values. However, the untreated (D) rats reflected significant decreases in the concentrations of total protein and albumin ($p < 0.01$) when compared to their corresponding levels in the normal group. Despite the D+DAPA, D+ CUR, and D+DAPA+ CUR being recorded to have significantly elevated ($p < 0.01$) serum levels in the total protein and albumin as compared to the diabetic group (Fig. 8**).** However, in the same groups, a significant reduction in the serum total protein and a non-significant change in the albumin levels were shown as compared to the control group. Significant elevations ($p < 0.01$) were recorded in the serum AST and ALT activities in the untreated group in contrast to that in control rats, while a significant decrease in these parameters was recorded by treatment of diabetic animals with DAPA and curcumin in the D+DAPA and D+CUR groups, respectively, in contrast to that of the untreated group at $p < 0.01$ (figure 9). As depicted in Figure (9), non-significant changes in the AST activity and a significant increase in the ALT activity were recorded in the sera of the D+DAPA+ CUR group as compared to their activities in the normal rats.

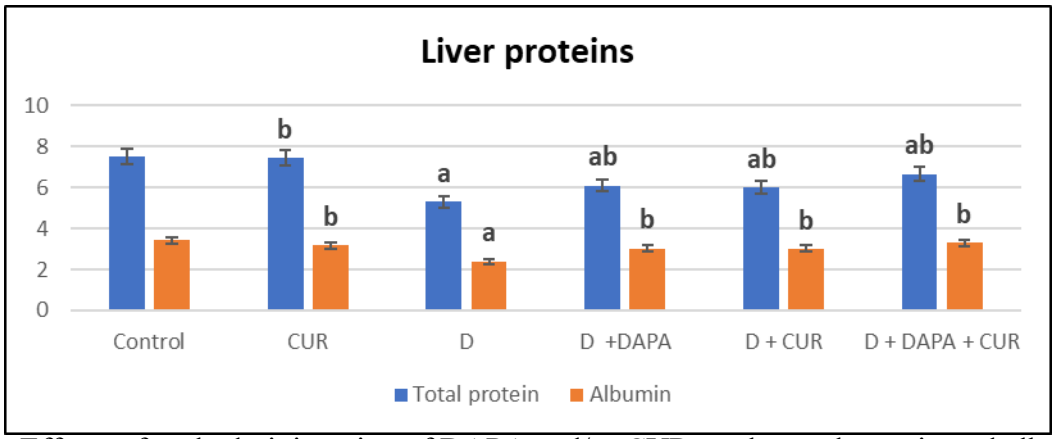

Fig. 8: Effects of oral administration of DAPA and/or CUR on the total protein and albumin levels in the different experimental rats. Data represent means ± SE (n=6) and the statistical difference at $p< 0.01$ represented by (a) and (b) superscripts as compared to the control and the diabetic group, respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

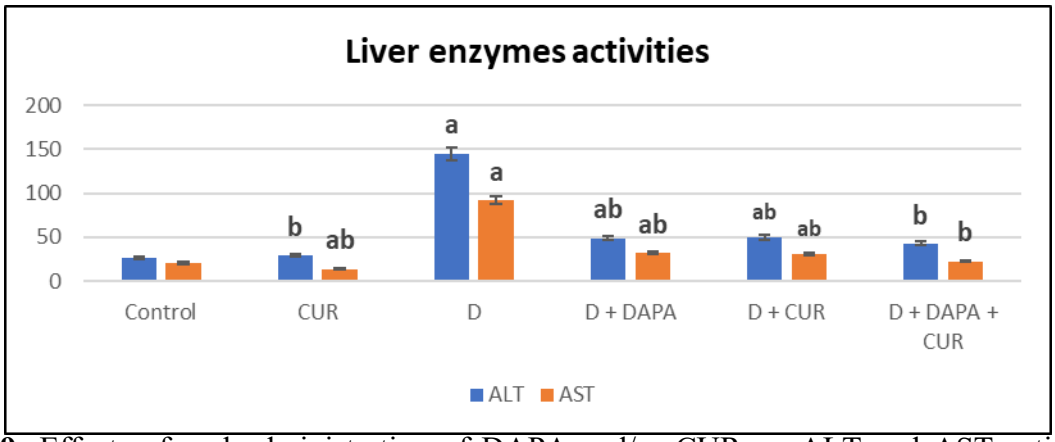

Fig. 9: Effects of oral administration of DAPA and/or CUR on ALT and AST activities (U/ml) in the different experimental rats. Data represent means ± SE (n=6) and the statistical difference at $p< 0.01$ represented by (a) and (b) superscripts as compared to the control and the diabetic group, respectively. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

3-The Lipid Profile:

The data illustrated in Table (1), revealed the changes in serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (v LDL-C) values in the different experimental groups. The TG, TC, LDL-C, and vLDL-C serum levels recorded a significant elevation ($p<0.01$) in the diabetic group than in the standard group. Meanwhile, the HDL-C values recorded a significant reduction in the same group compared to the control group. However, the D+DAPA, D+CUR, and D+DAPA + CUR groups demonstrated significant increases in serum TG, TC, LDL-C, and vLDL-C ($p < 0.01$) compared to the control rats. In contrast, the same groups showed a significant decrease in serum HDL ($p< 0.01$) compared to the control rats. Meanwhile, the D+DAPA, D+CUR, and D+DAPA + CUR groups recorded significantly reduced serum TG, TC, LDL, and vLDL ($p< 0.01$) compared to the diabetic rats. The same groups recorded significant increases in serum HDL-C ($p < 0.01$) compared to the diabetic group. Nevertheless, the animals treated with CUR recorded no significant changes in all parameters as compared to their levels in the control rats. Despite the improving effects of both DAPA and curcumin on diabetes-related physiological measurements, neither of them alone restored these measurements to the control group levels. However, using DAPA and CUR together, showed a more significant improvement.

Table 1: Dapagliflozin and/or curcumin oral administration effect on lipid profile (TG, TC, HDL-C, LDL-C, vLDL-C) in the different experimental rats.

Parameters / Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	vLDL-C (mg/dl)
Control	49.46 ± 2.76	140.89 ± 0.22	105.03 ± 0.84	25.96 ± 0.96	9.89± 0.96
Curcumin (CUR)	41.96± 0.44 ^{b}	144.55± 0.42 ^{b}	104.35± 1.32 ^{b}	31.8 ± 1.33 ^{b}	8.39± 0.08 ^{b}
Diabetic (D)	183.74 ± 4.65 ^{a}	217.45 ± 3.53 ^{a}	54.35 ± 3.62 ^{a}	125.69 ± 5.81 ^{a}	36.75± 0.93 ^{a}
D + DAPA	138.19 ± 2.86 $^{a\,b}$	194.17 ± 0.81 $^{a\,b}$	65.76 ± 0.47 $^{a\,b}$	100.77 ± 0.12 $^{a\,b}$	27.64± 0.57 ^{ab}
D+ CUR	154.28 ± 2.95 $^{a\,b}$	197.32± 0.52 $^{a\,b}$	65.53 ± 0.45 $^{a\,b}$	100.93 ± 0.16 $^{a\,b}$	30.86± 0.59 ^{ab}
D+ DAPA + CUR	132.82 ± 4.07 $^{a\,b}$	181.96 ± 0.49 ^{ab}	87.7 ± 0.74 ^{ab}	67.72 ±1.136 $^{a\,b}$	26.56± 0.81^{ab}

Data represent means ± SE (n=6). Means with (^{a}) and (^{b}) superscripts are statistically different at $p < 0.01$ compared to the control and the untreated diabetic group, respectively. DAPA, dapagliflozin; CUR, curcumin; D, diabetic; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; v LDL-C, very low-density lipoprotein cholesterol, and SE, standard error.

The Histopathological Studies:

Figure 10 (A&B), shows a control male rat liver tissue with a normal histological pattern. Typical hepatic lobules are composed of the central vein and cords of hepatocytes radiating from it and the hepatic sinusoids separate them. The hepatocytes are polyhedral cells with eosinophilic cytoplasm, and many possess large nuclei with one or more prominent nucleoli. In addition to phagocytic Kupffer cells that circulate throughout the sinusoids, endothelial cells line the sinusoids. Examined sections from the liver of curcumin-treated rats revealed nearly normal structures of the liver and maintained features of the portal area, sinusoids and hepatocytes (Figs. 11 A& B). In contrast, the liver of the diabetic untreated group showed marked hepatic injury, as indicated by highly congested hepatic portal veins containing hemolyzed blood cells, thickened walls of the hepatic artery with narrow lumen, elongated and stratified wall of the bile duct, and aggregated lymphocytes around the portal area **(**Fig. 12 A). Edematous area in and around the ruptured central veins was observed. Many hepatocytes were disorganized with pyknotic or karyolitic nuclei, hemorrhagic areas, hepatocellular micro-steatosis were seen. Also, dilated sinusoid, fatty degeneration, co-joined with moderate hepato-cellular degenerative changes, leukocytic infiltration around the congested vein was recorded in a few cases (Figs. 12 B, C &D). Liver tissues of diabetic rats treated with DAPD showed nearly normal hepatocellular architecture. The portal triad structures and the Von-Kupffer cells were morphologically normal. A part of the mild biliary reaction and some dilated sinusoids were observed (Figs. 13 A & B). Hepatic sections from diabetic rats treated with CUR revealed nearly normal hepatocytes in most parts of the sections. However, some hepatocytes were seen suffering microsteatosis and hydropic degeneration (Figs. 14 A& B). Hepatic tissues of diabetic rats that received a combination of CUR and DAPA revealed apparently normal hepatocellular components. The portal triad structures were morphologically normal (Figs. 15 A& B).

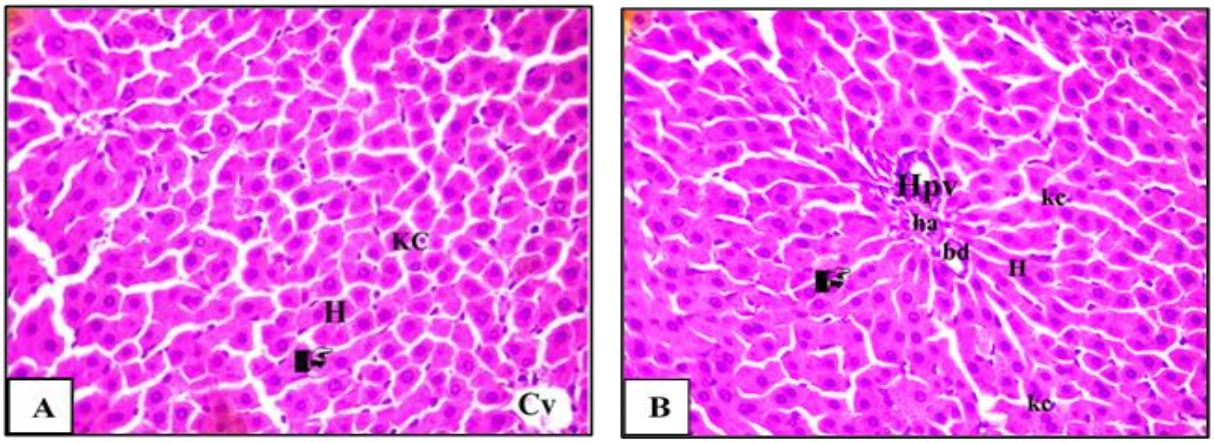

Fig. 10. Photomicrographs from hepatic tissue of the normal group showing: **A&B-** The central vein **(Cv),** cords of hepatocytes (**H)** that radiate from it and are separated from each other by blood sinusoids (**hand**). The sinusoids are lined with endothelial cells and scattered phagocytic Kupffer cells **(KC).** The portal area contains the hepatic portal vein (**Hpv),** bile ducts **(bd)** and hepatic artery (**ha).** **(A&B, H&E X 400).**

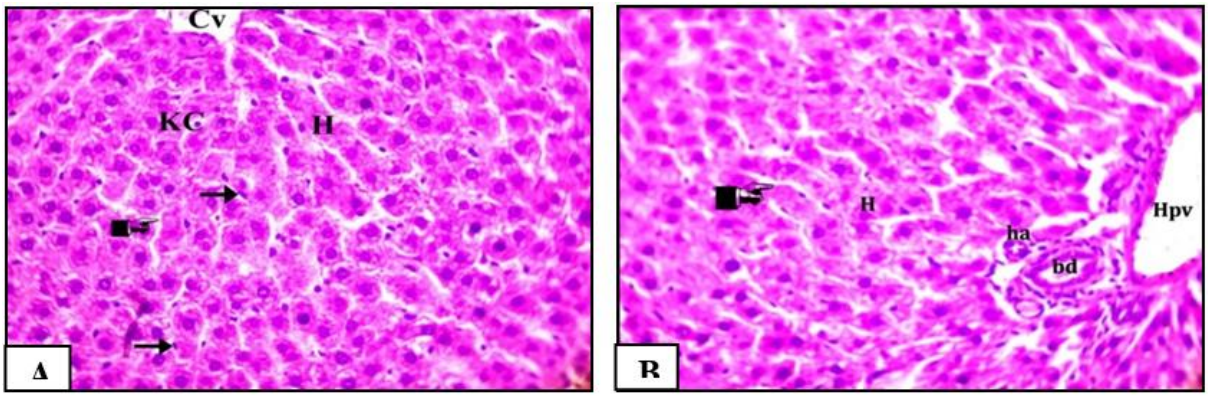

Fig. 11. Photomicrographs from liver tissue of rats treated with curcumin showing: **A&B –** Nearly normal hepatic portal area with hepatic portal vein (**Hpv),** bile ducts **(bd)** hepatic artery (h**a),** sinusoids **(hand)** and hepatocytes **(H).** Somewhat normal appearance of the central vein (**Cv**), cords of hepatocytes and Kupffer cells **(KC).** The sinusoids are lined with endothelial cells**(arrow). (A&B H&E X 400).**

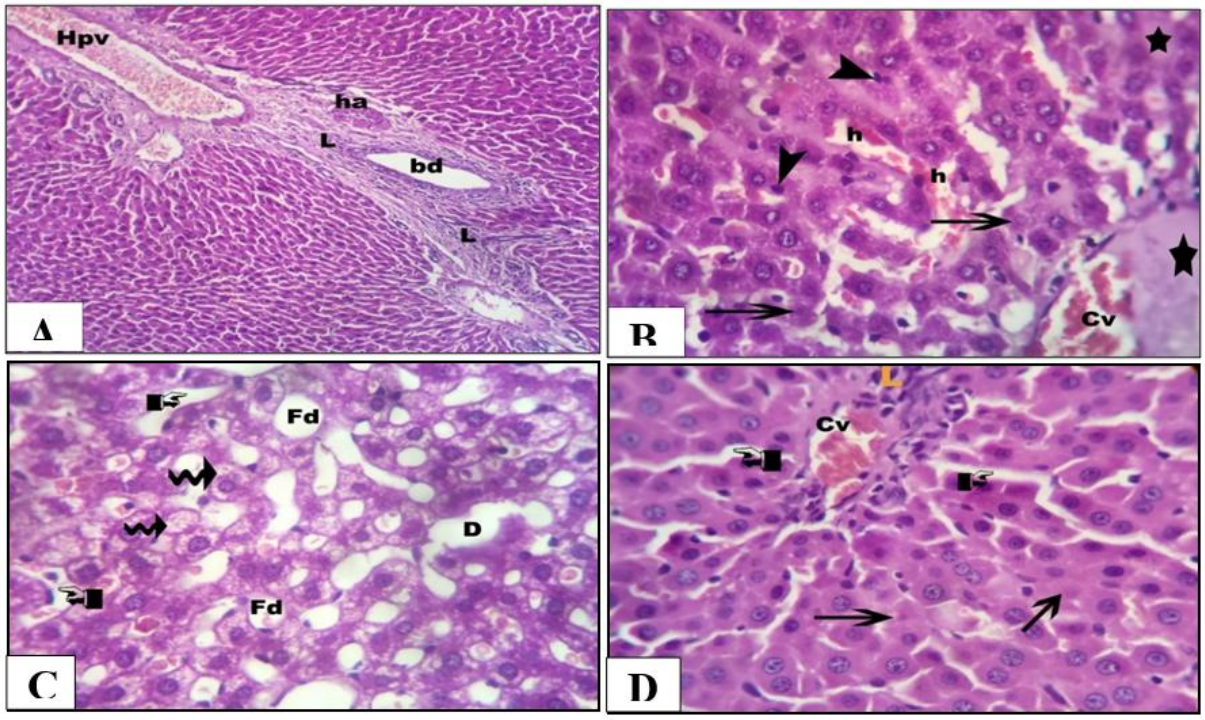

Fig. 12. Photomicrographs from sections of liver tissues of diabetic rats showing: **A:** severe congestion in the hepatic portal vein **(Hpv**) which includes hemolyzed blood cells inside it, thickened wall of the branch of the hepatic artery **(ha)** with elongated, narrow lumen and stratified wall of the bile duct **(bd)** and aggregated lymphocytes**(L)** in and around the portal area. **B:** edematous areas (**stars)** in and around the ruptured central veins **(Cv**), many liver cells with pyknotic (**head arrow**) or karyolitic **(arrows)** nuclei and many hemorrhagic **(h)** areas. **C:** disorganized hepatic strands with fat droplets **(Fd),** microvascular steatosis **(corrugated arrows),** dilated sinusoid **(hand)** and degenerated areas**(D). D:** leukocytic infiltration **(L)** around the congested central vein **(Cv),** dilated sinusoid **(hand)** and karyolitic **(→)** nuclei. **A (H& E X 200) B, C & D (H& E X 400).**

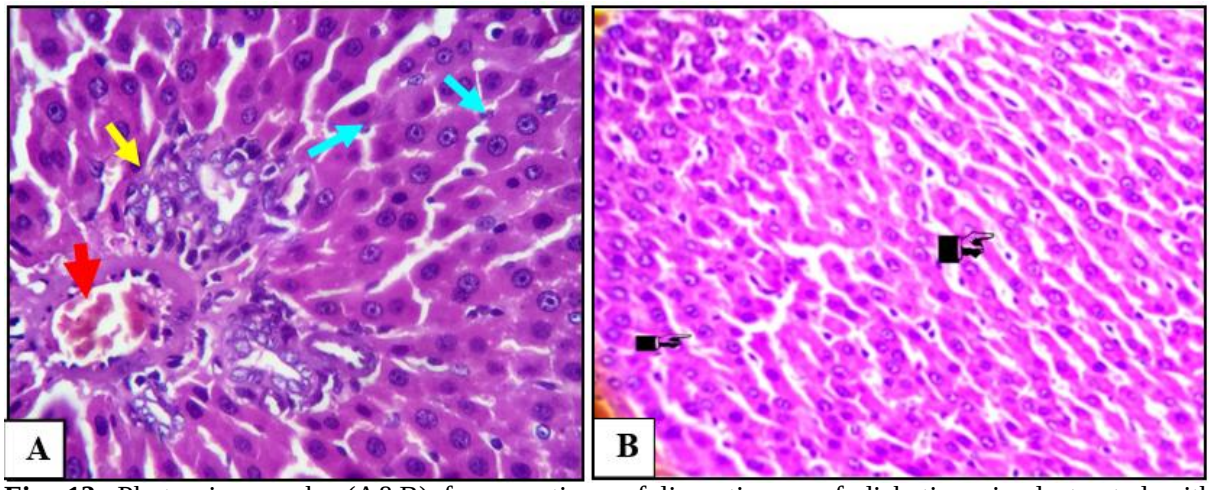

Fig. 13. Photomicrographs (A&B) from sections of liver tissues of diabetic animals treated with DAPA showing relatively normal hepatocellular components (**light blue arrows).** The portal triad structures appear morphologically nearly normal (**red arrows**), a part of mild biliary reaction (**yellow arrow),** only seen in this group and some sinusoids are dilated (**hand**). **A& B (H&E X 400).**

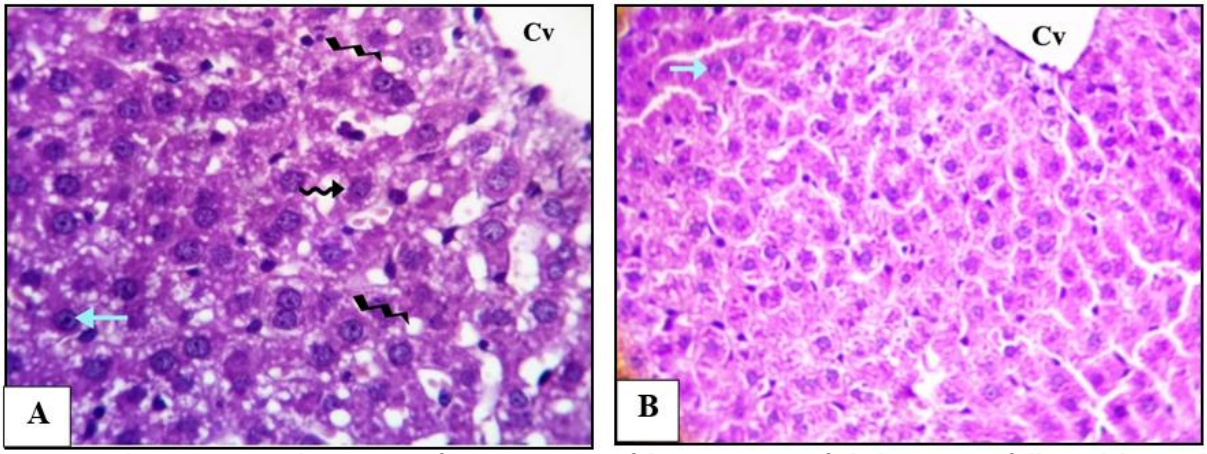

Fig. 14. Photomicrographs (A&B) from sections of liver tissues of diabetic rats followed by oral treatment with CUR showing relatively normal liver cells in most parts of the sections (**light blue arrow);** however, some hepatocytes are seen suffering micro-steatosis (**corrugated arrow)** and hydropic degeneration (**corrugated line).** The central areas were nearly normal in most areas of investigation **(Cv). A&B (H &E X 400**).

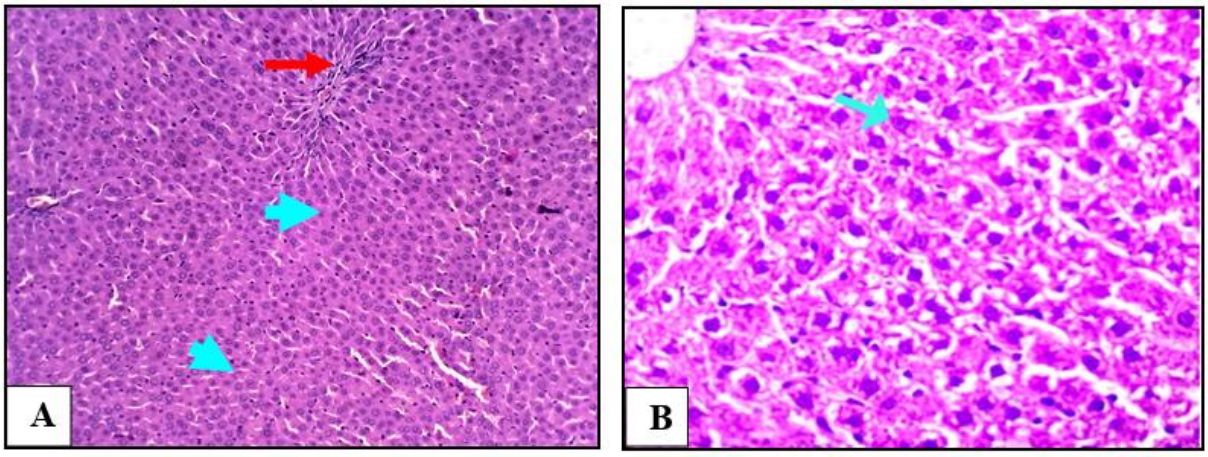

Fig. 15. Photomicrographs (A&B) from sections of liver tissues of diabetic animals that received a combination of CUR and DAPA showing normal hepatocellular architecture **(light blue arrows**). The portal triad structures appear morphologically nearly normal (**red arrows). A (H& E X 200), B (H&E X 400**)

The Immunohistochemical Studies:

As indicated in Figure 16, investigated immunestained hepatic tissue sections from the control-free group exhibited a negative cytoplasmic and/or nuclear staining reaction against caspase 3 monoclonal antibodies (Fig. 16 A). Meanwhile, the CUR-treated group showed weak immunoreactivity for caspase-3 (Fig. 16 B). The untreated group had a noticeably elevated positive caspase-3 expression level (Fig. 16 C). In contrast to the diabetic group, immunostained liver cells from D+ DAPA, D+ CUR& DAPA+ CUR demonstrated a significant reduction in caspase-3 immunoreactivity in comparison with the diabetic group.
(Figs. 16 D, E &F).

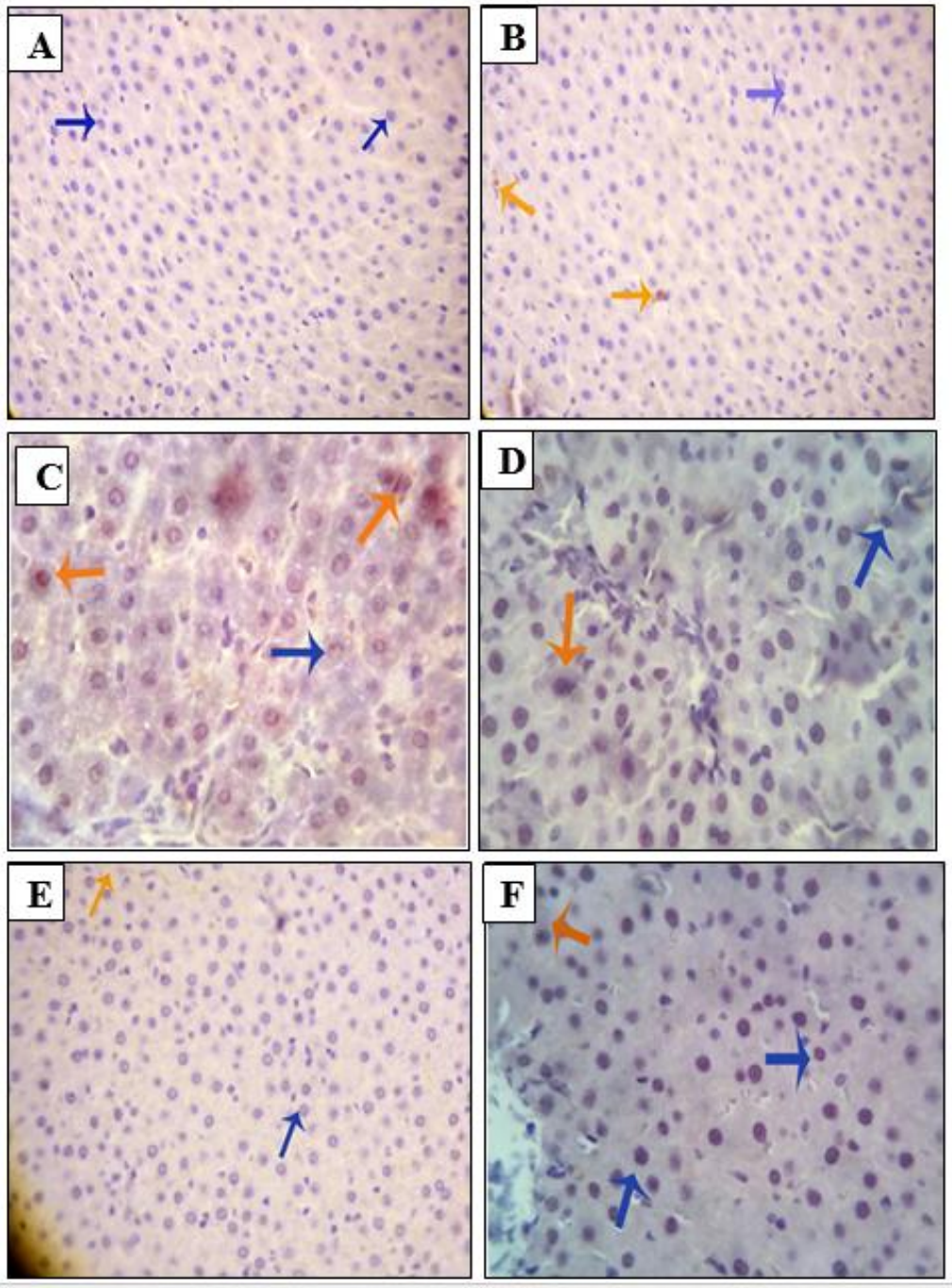

Fig. 16 (A- F). Photomicrographs from sections of liver tissues of different experimental groups immunohistochemically stained for caspase-3**,** showing brownish cytoplasmic expression of varying intensities. Positive cells are marked by orange arrows and negative cells by blue arrows. **A**: control, **B**: CUR, **C**: Diabetic(D), **D**: D+ DAPA, **E**: D+ CUR, **F**: D+ DAPA+ CUR. **(Caspase-3 antibody X 400)**

Quantitative Immune-Histochemical Measurement:

The findings displayed in Figure (17), demonstrated variations in the mean region of caspase-3 immunohistochemical expression in the liver of the various experimental animals. When compared to the standard group, the morphometric examination of the immunoreactive region for caspase-3 in the non-diabetic rats treated with CUR showed a significant increase. However, the rats in the diabetes group displayed a significant elevation compared to those in the control group. This elevation was mitigated in the D+DAPA, D+CUR, and D+DAPA+CUR groups. The immuno-reactive area for caspase-3 in this group was considerably reduced in comparison to the diabetes group.

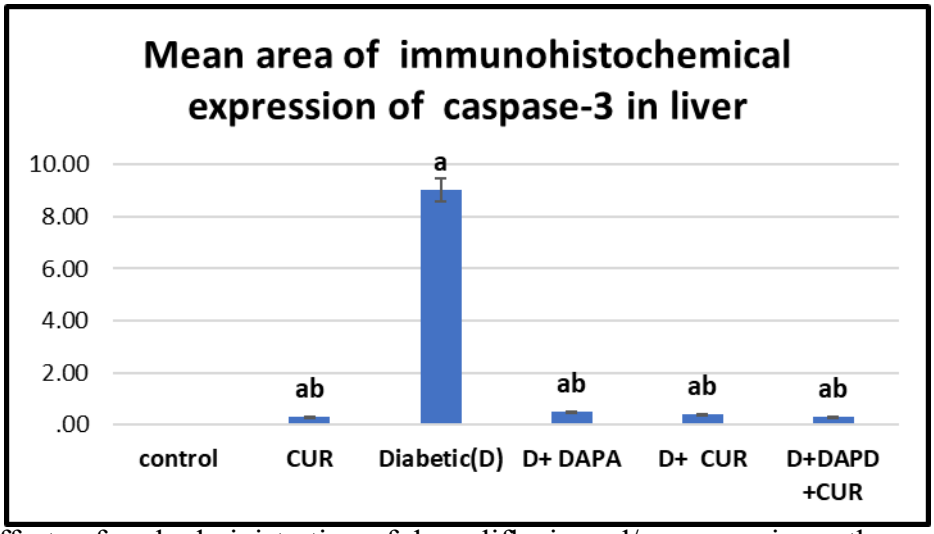

Fig. 17: Effects of oral administration of dapagliflozin and/or curcumin on the mean area of immunohistochemical expression of caspase-3 in the liver of the different experimental rats. Data represent means ± SE (n=6). (a) means are statistically different as compared to the control, (b) are statistically different compared to the diabetic group, $p < 0.01$. D: diabetic, DAPA: dapagliflozin, CUR: curcumin.

DISCUSSION

Diabetes mellitus is a considered a chronic metabolic condition marked by impairments in the metabolism of carbohydrates, lipids, proteins and high blood sugar levels (Zarrinkalam *et al.*, 2018). Type 2 diabetes mellitus (T2DM) is a complicated, heterogeneous and polygenic disease (Ghatan *et al.*, 2024). It can be defined by a reduction in insulin action, also known as insulin resistance, and pancreatic β-cell malfunction, or an inability of β-cells to release enough insulin (Srinivasan *et al.,* 2005 and Guo *et al.*, 2018). A new family of glucose-lowering medications for T2DM was released including the sodium-glucose co-transporter 2 (SGLT2) inhibitor (Wright *et al.,* 2011). SGLT2 inhibitors are considered a novel medication with non-insulin dependent action for T2DM treatment (Boran *et al.*, 2023). Dapagliflozin (DAPA) is a medication that is an effective in treating people with T2DM (Ferrannini *et al.*, 2010 and Ptaszynska *et al.*, 2014). In December 2012, it was established in clinical practice in the UK (McGovern *et al.,* 2014). DAPA is the first known as the first SGLT2 inhibitor medication used for T2DM treatment after its approval in 2014 (Boran *et al.*, 2023). Regarding T2DM remedy, DAPA is either used as a monotherapy given orally once daily (for individuals experiencing intolerance to metformin) or in conjunction with other hypoglycemic medications that lowers blood sugar such insulin, sulfonylureas and metformin (Tsushima *et al.*, 2021). In the same context, the DECLARE–TIMI 58 trial found that DAPA lowered acute renal injury and risk of death owing to kidney disorder (Baker and Perazella, 2020). In 2021, McMurray *et al.* also demonstrated a great decrease in kidney failure and a decrease cardiac problem with DAPA treatment in chronic kidney disease individuals. The DAPA works by preventing the kidneys from reabsorbing glucose and increasing urine glucose excretion, this reduces the risk of severe hypoglycemia and plasma glucose levels (Maksud *et al.*, 2024).

Several oral hypoglycemic medications had unfavorable side effects while

demonstrating anti-diabetic efficacy through various mechanisms of action. Therefore, using of medicinal plants has become the primary key factor of all available therapies due to ease of availability and low cost (Amalra *et al.,* 2017). Natural chemical compounds are becoming a more popular alternative to traditional therapeutic approaches in medicine due to its safety, effectiveness in clinical settings, and low side effect rate. As the primary active component, curcumin is a lipophilic polyphenol molecule. It is formed from the dried rhizomes of turmeric (*Curcuma longa*) (Naghdi *et al.*, 2022). It has strong hypoglycemic, anti-inflammatory, neuroprotective, anticancer, antioxidant, and anti-metastatic efficacies (Amalra *et al.,* 2017). In addition, curcumin has a wide range of pharmacological actions against many of chronic illnesses, including T2DM, Alzheimer's disease and liver injury prevention (Nelson *et al.,* 2017). Its structure contains many chemically active keto/enol groups which provide antioxidant capabilities to this molecule (Asadi *et al.,* 2019).

The present study established the T2DM model in rats by initially inducing insulin resistance through high-fat diet (HFD) feeding and then injecting a low dose of streptozotocin (STZ) to cause moderate β-cell dysfunction without affecting insulin production (Guo *et al.,* 2018). The metabolic features and the natural development of type 2 diabetes in humans, from insulin resistance to β-cell loss, are identical in this model (Watts *et al.,* 2005; Shatwan *et al.,* 2013). The study also targeted to evaluate the therapeutic capabilities of DAPA and/or CUR in mitigating diabetic complications in the T2DM rat model induced by HFD/STZ. It specifically investigated the effect of DAPA and/or CUR on blood glucose, insulin, HbA1c, liver functions and protein and lipid profiles. Furthermore, the liver tissue was subjected to histopathological and immunohistochemical investigations.

Body Weight Changes:

Laboratory animals' body weight can be used as an objective measure of pain and discomfort and is believed to indicate animal suffering. Therefore, determining the animals' body weights is essential for general health monitoring (Talbot *et al*., 2020) as the animals' body weight can impact the variables measured in animal research (Jackson *et al*., 2017). The body weight of the CUR nondiabetic group recorded a significant reduction compared with that of the control rats, which is in line with the results of Ghosh *et al.* (2015). Curcumin has been shown to reduce body weight through multiple mechanisms, including enhancing energy expenditure, modulating lipid metabolism and improving insulin sensitivity. Studies suggest that curcumin can suppress adipogenesis (fat cell formation) and promote lipolysis (fat breakdown), leading to a decrease in body weight. Additionally, curcumin possesses anti-inflammatory and antioxidative properties that may counteract obesity-related inflammation and metabolic dysfunction, contributing to weight reduction. In line with a recent study and meta-analysis by Huang *et al.* (2023), taking supplements of curcumin may assist individuals in reducing their body weight and alleviating issues associated with obesity. According to a different study by Wang *et al.* (2024), curcumin extract's anti-inflammatory properties can enhance β-cell activity and reduce insulin resistance, which may help individuals with T2DM to lose weight. Meanwhile, no significant change in the relative liver weight was reported in CUR treated group compared to the liver of normal rats. Abnormal metabolic processes of fatty acids and glucose are hallmarks of T2DM. Common symptoms of this disease are increased requirements for food and water consumption (Bibak *et al*., 2014). Following the STZ injection, the rats in this study tended to eat and drink more (data not shown). The decreased leptin receptor activation in the hypothalamus with relatively low insulin levels may account for increased food consumption in diabetic animals (Lee *et al*., 1994). The control rats in this study showed a constant body weight elevation. However, the diabetic rats showed a gradual body weight decrease, most likely resulting from enhanced fat metabolism and decreased glucose metabolism (Rossmeisl *et al*., 2003). Over time, body weight in the diabetic groups continued to decrease, particularly when accounting for hyperglycemia and insulin resistance. This pattern mirrored the early stages of type 2 diabetes mellitus (T2DM) progression, consistent with diabetes induced by a 40 mg/kg dose of STZ. In the same context, Daisy and Saipriya (2012) confirmed a significant decrease in male rats' body weight after the STZ injection, and this might also be attributable to dehydration and fat breakdown or might be due to increased muscle and tissue protein loss as confirmed by Qiang *et al.* (2014) and Guo *et al.* (2018). In parallel, there was a significant elevation in the liver relative weight of the diabetic rats compared to the normal control rat's liver relative weight which may be in line with the conclusions of Zafar and Naqvi (2010). They demonstrated the

increment (hypertrophy) in the diabetic liver relative weight which could be explained by elevated triglyceride accumulation increasing fatty acids flow into the liver cells.

Treatment with dapagliflozin in diabetic rats demonstrated a curative effect represented by increased body weight compared to the untreated animals in accordance with Boran *et al*. (2023). Meanwhile, the present study showed a significant reduction in the relative weight of the liver after treatment with DAPA in the D+ DAPA compared with the untreated diabetic animals. This aligns with the results of Zhao *et al.* (2023), who attributed that DAPA medication reduced liver weight and liver index, which stopped the development of T2DM with the enlarged liver, suggesting that DAPA has a protective effect. The study found that treating diabetic rats with curcumin increased their body weights compared to untreated diabetic rats and restored liver weight to normal levels. These results align with previous research by Dadgar *et al.* (2021), which demonstrated that diabetes reduces body weight in rats, and curcumin administration (80 mg/kg diet) counteracted this effect. This suggests curcumin may help prevent diabetes-related weight loss. Machado *et al.* (2022) also recorded increased body weight in diabetic animals treated with curcumin compared to diabetic rats. In the meantime, Ghosh *et al.* (2015) concluded that curcumin medication protects against diabetes-induced body weight loss by preventing the STZ growth-inhibiting effect that the beneficial effect of curcumin could stop. On the contrary, a study by Ding *et al.* (2016) found a reduction in body weight in diabetic animals treated with CUR. The curcumin decreased body-weight gain from increased oxygen consumption, respiratory quotient and energy expenditure in mice. Notably, in monotherapy, the treatment effect of curcumin was more efficient than DAPA on body weight and liver relative weight. However, the combined treatment showed the most relevant results either on body or liver-relative weight.

1-The Serum Fasting Blood Glucose (FBG), Insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Beta-Cell Function (HOMA-B), and Glycated Haemoglobin (HbA1c) Values:

In the present work, several parameters have been included for screening and monitoring glucose homeostasis disturbances in T2DM. Fasting blood glucose levels were determined to reflect the severity of diabetes mellitus (Bamagous *et al*., 2018), however, HbA1c indicates the average blood glucose levels for the last two to three months (Kala *et al.*, 2012). Insulin is also assessed as a key factor in controlling liver glycogen metabolism through the regulation of glycogenesis (Ojha *et al*., 2019). In the same context, pancreatic beta cell dysfunction and insulin resistance are measured as important factors in the pathophysiology of diabetes. The homeostasis model of assessment (HOMA) has been used to determine insulin resistance (HOMA-IR) as well as β-cell function (HOMA-β) of the pancreas as an indicator of insulin secretory activity. Standardized formulas were used for estimating HOMA-IR and HOMA-β based on fasting glucose and insulin levels (Sung *et al.*, 2010).

The administration of curcumin in the nondiabetic group of the present study, showed no significant change in the levels of FBG, insulin, HOMA-IR, HOMA-B and HbA1C as compared to that in the control rats. These findings might be pertinent to those of Lee *et al*. (2023) who demonstrated the safety of high bioavailability curcumin and promoted its consumption for potential health advantages.

In the present study, diabetic rats developed hyperglycemia, hyperinsulinemia, insulin resistance (HOMA-IR) and elevated HbA1C which agreed with Srinivasan *et al.* (2005). However, administering DAPA or CUR to diabetic rats in the D+ DAPA and D+ CUR groups revealed a significant reduction in the aforementioned parameters compared to those in the untreated (D) group. According to DAPA, Padda *et al.* (2022) imputed this reduction to blocking SGLT2 by dapagliflozin, which results in decreased systemic glucose reabsorption and promotes glucose filtration through the kidneys and into the urine for excretion from the body. Concerning CUR action, Barriga-S´anchez *et al.* (2024) attributed its hypoglycemic effect, at least partially, to the inhibition of the key carbohydrate hydrolyzing enzyme α-glucosidase as demonstrated in an *in vitr*o assay. According to Mohammadi *et al.* (2021) showed that curcumin acts through the regulation of incretins, α-glucosidase, amylase, dipeptidyl peptidase-4 (DPP-4) enzymes as well as peroxisome proliferator-activated receptor gamma (PPARγ) that reduce glucose levels. However, Kato *et al.* (2017) and Alli-Oluwafuyi *et al*. (2019) demonstrated that CUR enhances glucagon-like peptide-1 (GLP-1) secretion, which contribute to its antidiabetic actions.

In the current investigation, the treatment with DAPA improved insulin sensitivity and HOMA-IR as accordant with Macdonald *et al.* (2010) and Millar *et al*. (2017), which returned the cause of these improvements in insulin sensitivity and reduced glucose toxicity. Consistent with previous studies, dapagliflozin also improved beta cell function which could be because of better metabolic regulation and a reversal of beta cell glucotoxicity (Millar *et al.,* 2017). In the present investigation, the curcumin's HOMA-IR enhancing action is in line with Na et al. (2011) findings that demonstrated the curcumin enhancement activity of muscle sensitivity to insulin signalling, which encouraged muscle glucose uptake. However, Balakumar *et al.* (2023) showed that curcumin fights insulin resistance by increasing circulating adiponectin and irisin levels, activating PPARγ and inhibiting Notch1 signalling. Furthermore, curcumin improved beta cell function as compared to the diabetic animals, agreeing with Abdulmalek *et al.* (2021), Al-Saud (2020), and another study by Best *et al.* (2007) who demonstrated that curcumin enhances the pancreatic beta cells' electrical activity, which is followed by depolarization of the membrane, insulin release, and glucose uptake. Meanwhile, Pari and Murugan (2007) reported that curcumin protects the pancreatic beta cells from oxidative damage, thereby preserving the insulin production level. Interestingly, both DAPA+ curcumin treated diabetic group showed better antidiabetic effect represented by markedly reduced FBG, insulin, HOMA-IR, and HbA1c and improved HOMA-levels more than curcumin or DAPA treated groups alone.

2- Liver Function:

The liver performs several vital tasks, and any widespread illness of the organ has an equal impact on most or all of these activities (Constable *et al.*, 2016). It is an important organ for protein synthesis, degradation, and detoxification (Lee and Kim, 2019), as well as the detoxification of free radicals (Rezaei-Kelishadi *et al*., 2017). Furthermore, the liver is one of the first organs to be impacted by oxidative stress brought on by hyperglycemia, which can destroy the liver's insulin-sensitive components (Bugianesi *et al.,* 2005; Palsamy *et al.,* 2010). Liver fibrosis, cirrhosis, nonalcoholic fatty liver disease (NAFLD) and liver inflammation are the most common liver illnesses linked to T2DM that lead to liver malfunction (Leiter *et al.,* 2016 and Daverey and Agrawal, 2018). Accordingly, a decrease in total protein content and hypoalbuminemia may be a helpful indicator of the extent of hepatic cell injury. The diabetic (D) groups significantly had lower levels of total protein and albumin demonstrating the extent of the hepatic injury and are in line with the results of Mohammed *et al.* (2015), who attributed the reduction in total protein and albumin to the increased excretion by the kidney (nephropathy) or the decrease in production of both total protein and albumin. The stipulated restoration of total protein and albumin in the sera of rats treated with DAPA in the D+ DAPA or curcumin in the D+ CUR groups as compared to the diabetic group has been revealed. Compared with the monotherapies, the combination treatment elicited a more significant increase in the total protein and albumin levels. Similar results were observed in a study by Pari and Murugan (2007), who showed that protein synthesis is decreased in all tissues due to reduced production of alkaline phosphatase that may be responsible for decreased plasma protein and albumin in diabetes. Meanwhile, diabetic rats treated with curcumin and /or DAPA returned albumin levels to near-control results. Alterations in serum transaminase activities, or liver enzymes, are reliable indicators of hepatic dysfunction that is more prevalent in diabetes (Singh *et al.,* 2001). In 2018, Akhtar *et al*. also confirmed that increased serum ALT and AST activities are greatly connected with metabolic disorders, IR and DM. The data of this study showed serum significant increases of AST and ALT activities in the untreated diabetic rats compared to the normal rats. These results come in consistent with the previous study of Zafar *et al.* (2009), who demonstrated that animals treated with STZ had noticeably higher liver activities of the aminotransferases ALT and AST. The cellular damage brought on by STZ-induced hyperglycemia in the liver could be the reason for this elevation in aminotransferase activities. On the contrary, the treatment with DAPA reported a significant reduction in liver enzymes compared to the untreated animals. This comes in line with Yabiku *et al.* (2020) who reported that DAPA would be a useful medication for people with T2DM because it displayed both ascites-resolving actions and glucose-lowering in HFD-fed animals, without having negative impacts on hemodynamics, even in the presence of liver fibrosis. Meanwhile, Choi *et al.* (2018) thought that the recovery of metabolic imbalance by regulating body weight and lipid composition, as explained by the

mechanism of SGLT2, maybe the reason for the liver function improvement**.**

In the current study, the treatment with curcumin reported a significant reduction in the ALT and AST activities in the D+ CUR group as compared to the diabetic (D) group which coincides with the previous study by Abdulmalek *et al.* (2021). However, Xia *et al.* (2020) revealed that rats treated with STZ and a high-lipid diet have functional liver impairment revealed by higher AST and ALT levels, which are repaired by curcumin as a result of its benefits including anti-tumor, anti-inflammatory, and anti-lipid peroxidation properties (Goel *et al.,* 2008). However, in this study, the nondiabetic group treated with CUR showed no significant change in the level of ALT activity when compared with the normal animals. However, there was a significant decrease in the level of AST activity as compared to the normal group. This is in line with the findings of Palma *et al.* (2014), who showed that giving curcumin supplements to healthy, non-diabetic rats did not alter the structure of their liver tissue. This is advantageous since it indicates that the spice does not harm the liver.

Of particular note is the observation that DAPA+ CUR combination therapy for diabetic rats recorded a non-significant change in albumin, AST and ALT activities and a significant decrease in total protein concentration when compared with that of the control group. Meanwhile, the combination D+ DAPA+ CUR group had greater efficacy than DAPA or CUR alone for attenuating liver function.

Lipid Profile:

Dyslipidemia (Serum lipid abnormalities) serves as the main risk factor for diabetes mellitus due to insulin deficiency or resistance (Reaven and Greenfield, 1981; Taskinen, 2003). A high triglyceride (TG) concentration, low HDL cholesterol and increased concentration of small dense LDL-cholesterol particles are distinctive features of diabetic dyslipidemia (Guilherme *et al.,* 2008 and Mooradian, 2009). These features are greatly represented in the lipid profile of the untreated diabetic group in the present study which is also harmonious with Podrini *et al.* (2013), Guo *et al.* (2018) and Hasnat *et al*. (2019) lipid profiling observations. Reaven (2005) demonstrated that elevated TG levels are closely correlated with insulin resistance which was recorded in this study. In the same trend, Abbate and Brunzell (1990) and Hirano (2018) attributed hypertriglyceridemia to the overproduction of liver TG-rich lipoproteins, due to augmented portal flux of free fatty acids (FFA) levels, thereby stimulating VLDL production. In the insulin-resistant state, VLDL-TG removal impairment may be another factor that promotes hypertriglyceridemia. The removal defect is mainly caused by reduced activity of muscle lipoprotein lipase (LPL) and decreased adipose tissue due to insulin resistance (Hirano, 2018). Treating diabetes with oral DAPA in the current study corrects most of the hypertriglyceridemia, hypercholesterolemia and some of the decreased HDL cholesterol. This is consistent Saleh *et al.* (2020), Kralova *et al.* (2021) and Xue *et al.* (2023). Furthermore, Bolinder *et al.* (2012) exhibited a significant reduction in fat mass across DAPA treatments in rats. This finding was further supported by a significant decrease in adipose tissue mass. Curcumin (CUR) treated diabetic rats significantly decreased serum total cholesterol, triglyceride, LDL and vLDL and significantly enhanced serum HDL-C compared to the diabetic group. This is consistent with Shamsi-Goushki *et al.* (2020) findings that the activation of the peroxisome proliferator-activated receptor (PPARα), which elevates CUR lipoprotein lipase activity, is likely responsible for the positive effects of CUR on lipid profiles (Poolsup *et al.,* 2019). Several investigations have demonstrated that curcumin's pharmacological actions on modifying the lipid profile in diabetes mellitus are due to suppression of nuclear factor- kappa B (NF-kB) and modulation of several transcription factors and cytokines (Zhang *et al.*, 2013; Mantzorou *et al.,* 2018). Both DAPA and CUR diabetic-treated groups revealed a significant hypolipidemic impact, and the combined treated group showed more response. DAPA, an SGLT2 inhibitor, impacts the fatty acid oxidation process and the modulation of important molecules in lipid production and transport (Szekeres *et al.,* 2021). The presence of many bioactive chemicals, including ferulic acid and vanillic acid, is responsible for the process of curcumin lipid reduction. Denovo lipogenesis is mostly influenced by curcumin's other anti-hyperlipidemic actions, including inhibiting fatty acid synthase and sterol regulatory element-binding protein -1/2. Additionally, curcumin inhibits the enzyme HMGCOA reductase, which reduces cholesterol synthesis (Saleem *et al.,* 2025).

Histological and Immunohistochemical Studies in The Hepatic Tissues:

The largest and most intricate internal organ in the body is the liver. It performs

numerous essential tasks, including secretion, storage, metabolism and transforming of harmful compounds into beneficial chemicals (Bisht *et al.,* 2011). Many diverse histological changes in the liver associated with diabetes are commonly observed (Bril *et al*., 2017; de Vries *et al*., 2021 and Nogueira and Cusi, K., 2024). The study found that the liver tissue of the HFD/STZ-induced diabetic group exhibited significant hepatic injury, characterized by congested hepatic portal veins containing hemolyzed blood cells, thickened walls of hepatic artery branches with narrowed lumens, distorted and elongated bile duct walls with increased proliferation, and aggregated lymphocytes around portal and central areas with hemorrhagic regions. Edematous area in and around the ruptured central veins. Many hepatocytes were disorganized with pyknotic or karyolitic nuclei, hepato-cellular micro-steatosis was seen, dilated sinusoid, fatty degeneration, co-joined with a moderate hepato-cellular degenerative changes, leukocytic infiltration around the congested vein and accidental cellular brownish pigmentation was recorded in a few cases. The present results agree with the work done by Alamri and Melebary (2024), who demonstrated that diabetic rats' livers had significant liver damage. The most notable changes were disorderly hepatocytes, inflammatory cells, fatty and hydropic degeneration, necrosis, nucleus karyolysis and hyperplastic modifications were the most notable changes. Inflammation and oxidative stress are diabetes complication parameters that cause hepatic damage. Chronic hyperglycemia raises the risk of organ dysfunction, particularly diabetic nephropathy and liver disorders and enhances the overall oxidative state (El-Serag *et al.,* 2004). DM is an important factor of death for diabetics and is thought to be one of the most common forms of liver failure (Zhang *et al.,* 2012). Wickramasinghe *et al.* (2024) revealed that hydropic hepatocyte degradation and mild lobular inflammation are among the histological alterations seen in the liver tissue of rats fed a high-fat diet and given STZ. Previous studies indicate fatty livers and histological changes including lipid accumulation and lobular inflammation in HFD-fed STZ-induced rat models (Guo *et al.,* 2018; Dwivedi and Jena, 2020).

In the current study, the livers of normal rats treated with CUR revealed apparently normal structures of the liver with keeping features of the portal area, sinusoids and hepatocytes, this agreement with Ghosh *et al.* (2015) who showed improvement in liver morphology after CUR treatment. A healthy diet and other lifestyle changes are important therapeutic approaches for preventing and treating chronic, such as diabetes. The action of endogenous antioxidants in reducing the detrimental effects of oxidative stress can be supported by dietary antioxidants such as polyphenols, flavonoids (flavones, flavonols, theaflavins, catechins, proanthocyanidins, flavanones, anthocyanidins, and isoflavones), non-flavonoids (phenolic acids, stilbenes, and lignans), minerals (Se, Mn, Zn, Cu, and Fe), and antioxidant vitamins (C, E, A, and carotenoids) (Zujko and Witkowska, 2023).

In the current study, the diabetic animals treated with DAPA illustrated normal hepatocellular architecture. The portal triad structures and the Von-Kupffer cells were morphologically normal. A part of mild biliary rection and some dilated sinusoids were recorded.

The results of the current study are in line with those described previously by Alamri and Melebary, (2024) they noted that a diabetic animals treated with DAPA exhibited an average improvement in liver histological structure. Similar to the control group, the portal tracts displayed normal histological structure. Some specimens continued to exhibit moderate fatty infiltrative alterations, with mononuclear cell infiltrations and loss of the usual architecture of liver tissue, along with smaller, well-defined fat droplets filling the hepatic cytoplasm. According to Tang *et al.* (2017), administering DAPA to diabetic rats had a protective impact on the liver, as seen by a large increase in albumin and total protein and a decrease in ALT, AST, GGT, and total bilirubin. According to Hassan *et al.* (2024) observed that DAPA's anti-inflammatory, anti-fibrotic, and antioxidant properties produced positive results. Interleukin 1 beta (IL-1β) and tumor necrosis factor -alpha (TNF-α) were significantly reduced in the liver homogenate content of rats treated with DAPA.

In the current study, the liver of diabetic animals treated with curcumin revealed normal hepatocytes in most parts of the sections; however, some hepatocytes were seen suffering micro-steatosis and hydropic degeneration. The portal tirades were normal in most areas of investigation, with a mild vascular dilatation. This is in accordance with the results of Ghosh *et al.* (2015) who stated that rats treated with curcumin had significantly improved liver shape based on histological examinations of different liver parts. According to Ozkaptan

et al. (2024), in the livers of the diabetic group treated with curcumin, apoptotic changes in hepatocytes improved and the apoptotic index value decreased compared to diabetic rats. In addition, curcumin improved the levels of antioxidant enzymes and is a powerful antioxidant by biochemical analysis. Curcumin has been shown to directly interact with several intracellular signalling molecules (Gupta *et al.,* 2011). Its ameliorative effects have been indicated to be mediated through the modulation of multiple cells signalling molecules like cyclooxygenase (COX)- 2, apoptotic proteins and nuclear factor- kappa B (NF-κB) (Dhillon *et al.,* 2008), Single transducer and activator of transcription 3 (STAT3), interleukin [IL]-1 (Kim *et al.,* 2011), malondialdehyde (MDA), glutathione (GSH) (Kalpravidh *et al.,* 2010) and endothelin-1, C-reactive protein (CRP) (Holt *et al.,* 2005), Most importantly, it has been demonstrated that curcumin can directly scavenge reactive oxygen species (ROS) (Gupta *et al.,* 2013).

In the current study, the hepatic tissues of diabetic rats that received a combination of DAPA and CUR showed normal hepatocellular components. The hepatic sinusoids, the portal triads structures and the Von-Kupffer cells were morphologically normal. Ameliorated levels of AST, ALT, total protein, albumin, TC, TG, HDL, LDL and vLDL of rats of the group supplemented with DAPA and CUR indicated the synergistic protective effect of DAPA+ CUR and its capacity to scavenge free radicals brought on by elevated blood sugar levels.

Regarding the immunohistochemical observations in the liver tissue, the mean area of caspase-3 immunoreactivity indicated a substantial increase in the diabetic group compared to the normal group. This finding aligns with the results of El-Sherbiny *et al.* (2022). They showed that the incidence of apoptosis was confirmed by significantly elevating the genetic and protein levels of caspase-3 expression in the liver in connection with the DM-associated elevated oxidative status and increased hepatic expression of TNF-α in the DM group. According to Erdogan *et al.* (2023) demonstrated that hepatocytes from liver tissue of diabetic rats exhibited caspase-3 positivity when immunohistochemical staining was examined under light microscopy. Reactive oxygen radicals have been proposed as an autocatalytic mechanism that can lead to programmed cell death (Jones *et al.,* 2000). It has been proposed that reactive oxygen radicals participate in the apoptotic cell death of hepatocytes and endothelial cells in the liver (Jaeschke, 2000).

In the present study, the immunohistochemical analysis of liver tissue revealed a significant decrease in the mean area of caspase-3 immunoreactivity in the animals treated with DAPA compared to the diabetic group. According to El-Sherbiny *et al.* (2022) there was an important increase in apoptosis in the hepatic tissue of diabetic animals. This was indicated by a significant increase in tissue cleaved caspase-3 immunostaining compared to the normal group. As compared to the untreated animals, the giving of the DAPA to diabetic rats resulted in a significant decrease in the amount of cleaved caspase-3 immunostaining in the hepatic organ.

In the current study, the immunohistochemical investigation of the liver tissue, the mean area of caspase-3 immunoreactivity reported a significant reduction in the diabetic rats treated with CUR when compared to the diabetic group. This is in line with Afrin *et al.* (2015); they noted that apoptotic signalling proteins such as cleaved caspase-3 were significantly elevated in the diabetic rats, but curcumin treatment prevented this alteration. Curcumin improves diabetic rats with streptozotocin-induced liver damage via regulating endoplasmic reticulum stress-induced apoptosis.

Ozkaptan *et al.* (2024) showed that curcumin reduced the apoptotic index in hepatocytes, which increased with DM in the liver. The first cellular response to the challenge of elevated glucose in diabetes is the production of ROS, which rapidly causes apoptotic cell death (Park *et al.,* 2001). It is well known that curcumin has strong anti-inflammatory and antioxidant properties. Curcumin is at least ten times more potent than vitamin E as an antioxidant. Its main effects on eliminating free radicals and/or avoiding lipid peroxidation have been thought to mediate its antioxidant activity. Furthermore, antioxidants like curcumin have been reported to have anti-diabetic properties that may be associated with their ability to preserve the glucose transporter. However, the curcumin pill might not be enough to act as an ant-diabetic (Hashish and Kamal 2015).

In the current study, the immunohistochemical observations of the liver tissue and the mean area of caspase-3 immunoreactivity recorded a significant reduction in the diabetic

animals treated with both DAPA and CUR when compared to the diabetic untreated group. Ameliorated levels of FBG and insulin in the serum of rats in the group supplemented with DAPA and CUR indicated the protective effect of their synergistic effect and its ability to reduce apoptosis caused by hyperglycemia.

Conclusion:

Overall, the current investigation discovered that DAPA, CUR, or both improved physiological and histopathological alterations, increased serum total protein and albumin, decreased liver enzyme activities and enhanced lipid profiles to counteract the hepatic impairment induced by HFD and STZ (diabetic effects). Additionally, DAPA and CUR together to enhance most of earlier parameters. These findings provide a strong evidence of the DAPA and CUR combination's beneficial effects in treating diabetic hepatic impairment**.** The study revealed that DAPA treatment as an antidiabetic drug alone was less effective than using the combination of DAPA and CUR.

Declarations:

Ethical Approval: This study does not contain any studies with human participants or animals performed by any of the authors.

Competing interests: The authors declare that there is no conflict of interest.

Author's Contributions: Fatma M. El-Deeb, carried out field execution to all experiment stages, collect blood samples and field data and contributed in wrote this article. Ahkam M. El Gendy wrote this article, helped in biochemical analysis, contributed in drafting the manuscript and revision. Rasha A. wrote this article and performed the statistical analysis of the results, contributed in drafting the manuscript and revision. Hemmat M. Abdelhafez wrote this article and contributed in drafting the manuscript and revision and performed the histo-morphological and immunohistochemical parameters. Responsible for paper idea; Ahkam M. El Gendy, Rasha A. and Fatma M. El-Deeb. All authors approved the final manuscript.

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