RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 6: 1009-1020 (June 2026)

Protective Effects of Suberoylanilide Hydroxamic Acid and Dapagliflozin Administration on the Liver of Type 2 Diabetic Rats

Zaenah Zuhair Alamri[1], Sahar J. Melebary[1]

10.18805/IJAR.BF-2040

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) patients have hepatic disorders risk. This study investigated liver-protective effects of suberoylanilide hydroxamic acid (SAHA) and dapagliflozin (DAPA) in T2DM rats.

Methods: T2DM induced by high fat diet (HFD) and single Streptozotocin (STZ) injection (35 mg/kg i.p.). Forty rats sorted into 4 groups: NC (negative control), T2DM, T2DM+SAHA (5 mg/kg/i.p. for 8 weeks) and T2DM+DAPA (1 mg/kg/p.o. for 8 weeks). At experimental end, levels of fasting blood glucose (FBG), fasting insulin, hepatic function tests [gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total protein], lipid profiles [total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG)] measured in serum. Hepatic tissue homogenization prepared for estimating oxidative stress biomarkers [glutathione (GSH), malonaldehyde (MDA), superoxide dismutase (SOD)]. Hepatic histopathological examination made under light microscope.

Result: After 8 weeks treatment, diabetic rats showed elevated FBG, insulin, liver enzymes (GGT, ALT, AST), bilirubin, TG, TC, LDL-C, MDA, with reductions in albumin, proteins, HDL-C, GSH, SOD. Histopathological liver damage observed. SAHA and DAPA improved metabolic, oxidative stress and liver function. DAPA was effective in improving FBG and lipid profiles, while SAHA showed effective on insulin resistance and antioxidant. Histological liver improvements were more pronounced in SAHA group. SAHA and DAPA offer hepatoprotection in T2DM by improving oxidative stress, lipid metabolism and insulin sensitivity and preserving liver structure.

Key words: Dapagliflozin, Liver, Protective effects, Rats, Suberoylanilide hydroxamic acid, Type 2 diabetes mellitus.

INTRODUCTION

By 2045, there will likely be 700 million more diabetes mellitus (DM) patients worldwide than 463 million currently cases (Teo *et al.,* 2021). More than 90% of diabetic people diagnosed with type 2 diabetes mellitus (T2DM) (Salem *et al.,* 2022). Chronic hyperglycemia and disorders in fats, proteins and carbohydrates metabolism are T2DM hallmarks. There is a strong correlation between chronic hyperglycemia and complications, including liver disorders. Microvascular and macrovascular problems may result from improper T2DM therapy. Even with antidiabetic drugs available, T2DM linked to high rate of morbidity and mortality (Khan *et al.,* 2025).

Histone deacetylases (HDAC) and histone acetyltransferases (HAT) play crucial roles in cellular responses and pathological conditions. HATs promote transcriptional activation and nucleosome relaxation through histone acetylation, a key epigenetic mechanism for regulating gene expression. In contrast, HDACs are involved in transcriptional repression, chromatin condensation and de-acetylation. Recent studies suggest that HDACs may impact carbohydrate and fatty acid metabolism in diabetes mellitus (DM) by regulating gene transcription (Wang *et al.,* 2024). Classical HDACs linked to non-histone proteins deacetylation and signal transducer and activator of transcription 3 (STAT3) activation, which inhibits gluconeogenesis enzymes expression. HDAC inhibitors (HDACi) showing beneficial effects on β-cell formation, differentiation, proliferation and insulin signaling, demonstrating protective effects against cytokine-induced β-cell destruction (Lundh *et al.,* 2012). Suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor, exhibits anti-inflammatory and antioxidant properties, primarily suppressing Class II, I and IV HDACs and gaining approval for cutaneous T-cell lymphoma treatment in 2006 (Grant *et al.,* 2007). SAHA has antidiabetic effects in type 1 diabetes mellitus animal model (Cabrera *et al.,* 2013) and can prevent liver fibrosis by inhibiting HDAC2, HDAC6 and HDAC8 proteins (Hadden and Advani, 2018).

Sodium-glucose cotransporter-2 inhibitors (SGLT2is) were promising pharmaceuticals class for T2DM therapy because they improve insulin sensitivity, suppress gluconeogenesis and reduce glucose reabsorption by blocking SGLT2 protein that included in 90% of glucose reabsorption (Xu *et al.,* 2022). FDA originally authorized

[1]Department of Biological Sciences, College of Science, University of Jeddah, Saudi Arabia, P.O. Box 80327, 21589, Jeddah.

Corresponding Author: Zaenah Zuhair Alamri, Department of Biological Sciences, College of Science, University of Jeddah, Saudi Arabia, P.O. Box 80327, 21589, Jeddah.
Email: zzalamri@uj.edu.sa

How to cite this article: Alamri, Z.Z. and Melebary, S.J. (2026). Protective Effects of Suberoylanilide Hydroxamic Acid and Dapagliflozin Administration on the Liver of Type 2 Diabetic Rats. *Indian Journal of Animal Research.* **60(6):** 1009-1020. doi: 10.18805/IJAR.BF-2040.

Submitted: 03-07-2025 **Accepted:** 29-04-2026 **Online:** 12-06-2026

canagliflozin in 2013 and dapagliflozin (DAPA) in 2014 as SGLT2i (Harris and Nealy, 2020). SGLT2i improve hepatic function in T2DM patients (Bica *et al.,* 2023). SGLT2i decrease liver lipid accumulations and decrease fat mass and body weights by enhancing fatty acids utilization instead of glucose as source of energy for metabolism (Szekeres *et al*., 2021). Due to insulin-independent mechanism of SGLT2i, individuals with insulin resistance (IR) or reduced pancreatic function may benefit from this drugs family as an additional treatment (Bica *et al.,* 2023).

In particular, although previous studies have documented hyperglycemia-induced metabolic disturbances and hepatic dysfunction in STZ-induced diabetic rats, limited information exists regarding precise mechanistic relationship between T2DM-associated hyperlipidemia and progressive liver damage in this experimental model. Therefore, the current study aims to investigate whether T2DM-induced metabolic abnormalities-specifically hyperglycemia and dyslipidemia-directly contribute to hepatic structural and functional alterations. Clearly stating this hypothesis will strengthen the scientific rationale and improve the overall coherence of the introduction.

MATERIALS AND METHODS

Animals

Forty adult male Albino rats weighing 250-300 gm and aged 8 weeks obtained from Animal house at Faculty of Pharmacy, King Abdulaziz University (KAU), Jeddah, Saudi Arabia during period from 2024-2025. Rats kept in suitable laboratory status of light (12 h light/dark cycle), temperature (25±1°C) and humidity 60% with *ad libitum* access to distilled water and a rodent rat's diet for one week before experiment start for acclimatization to laboratory condition.

Chemicals and drugs

Streptozotocin (STZ) was purchased from Sigma-Aldrich (Sigma-Aldrich Co. St. Louis, Missouri, USA) and mixed in 0.1M citrate buffer (pH 4.4) for intraperitoneal administration in rats. Suberoylanilide hydroxamic acid, also from Sigma-Aldrich (Sigma-Aldrich Co. St. Louis, Missouri, USA), was mixed in a vehicle containing 10% DMSO, 45% PEG-400 and 45% saline, administered intraperitoneally. This research also conducted to study possible protective effects of histone deacetylase inhibitors (Suberoylanilide hydroxamic acid) and sodium-glucose cotransporter-2 inhibitor (dapagliflozin) *in vivo* administration on liver of T2DM rat's model and examine possible underlying mechanism of their effects. To assess functional impact of HDAC inhibition, animals treated with (5 mg/kg/i.p. for 8 weeks) SAHA (Sigma-Aldrich Co. St. Louis, Missouri, USA) (Colussi *et al.,* 2010). While HDAC inhibitor activity was not directly validated *via* Western blot for acetylated histone H3 or H4 markers in this study, the chosen dosage and time points were based on previous literature demonstrating (Colussi *et al.,* 2010) inhibition of HDACs and subsequent downstream effects at these conditions. Dapagliflozin, obtained from AstraZeneca Pharmaceutical Company (Cairo, Egypt), was prepared in a 0.5% aqueous solution of carboxymethyl cellulose (CMC) and administered orally *via* gastric lavage.

Experimental design and induction of T2DM

Forty rats randomly sorted into 4 groups: negative control (NC) that fed by regular chow diet (23% protein, 53% carbohydrate and 5% fat) for 12 weeks. From week 4, NC group rats took 1 ml/kg daily i.p. injections of 10% DMSO, 45% PEG-400, 45% saline (vehicle of SAHA) and 1 ml/kg daily orally of CMC (vehicle of DAPA). All other rats (n= 30) fed high fat diet (HFD) (normal diet + 2.5% cholesterol + 20% saccharose + 15% lard stearin) for 11 weeks and received at 3rd week STZ injection (35 mg/kg i.p.) for T2DM induction (Ying *et al.,* 2020). Three days followed STZ injection, fasting blood glucose (FBG) levels evaluated and animals showed FBG ≥250 mg/dl considered diabetic (Ulger and Cakiroglu, 2020). For more 8 weeks, HFD gave with the following therapy. T2DM group took saline. T2DM+SAHA received SAHA injection daily (5 mg/kg/i.p. for 8 weeks) (Colussi *et al.,* 2010). T2DM+DAPA received daily dapagliflozin (1 mg/kg/p.o. for 8 weeks) (Arab *et al*., 2023).

Rats' body weights recorded at baseline and weekly using digital scale. Weight gain (%) calculated as:

$$\frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Weekly food intake measured and food efficiency ratio (FER) calculated as:

$$\frac{\text{Weight gain (g/week)}}{\text{Food intake (g/week)}} \times 100$$

At study's end, rats fasted for 12 hours and anesthetized with thiopental sodium (50 mg/kg). Blood collected from retro-orbital plexus into plain tubes, allowed to clot for 20 minutes and centrifuged at 4000 rpm for 15 minutes. Sera stored at -20°C until analysis.

Rats sacrificed by cervical dislocation. Livers removed, rinsed in cold saline, weighed and hepatic index (%) calculated as:

$$\frac{\text{Liver weight}}{\text{Final body weight}} \times 100$$

Each liver was bisected: One half fixed in 10% formalin for histopathology; other half rinsed, flash-frozen in liquid nitrogen and stored at -80°C for later homogenate preparation.

Hepatic tissue homogenate preparation

Half of liver homogenized in phosphate-buffered saline (PBS) to made 10% (w/v) homogenate utilized homogenizer (Omni International, Kennesaw, GA, USA). Homogenates further spun at 5,000 g at 4°C for 15 min. Supernatants collected, aliquoted and storage at -20° for further measurement of oxidative stress biomarkers.

Biochemical measurements

FBG levels were measured using a colorimetric kit from Abcam (catalog# ab282922). Fasting serum insulin concentrations assessed by ELISA kit from My BioSource (catalog# MBS281388) and insulin resistance was evaluated with the HOMA-IR index, calculated as [fasting insulin (µU/ml) × fasting plasma glucose (mg/dl)]/405. Liver functions such as ALT, total bilirubin, GGT, AST, total protein and albumin were estimated using colorimetric methods by available kits (Teco diagnostics, Cairo, Egypt). Serum lipid profiles including total cholesterol, triglycerides and HDL-C were measured with kits from Spectrum Diagnostics (Cairo, Egypt), with LDL-C calculated via Friedewald's formula. Additionally, malondialdehyde (MDA) (catalog# MBS268427), superoxide dismutase (SOD) (catalog# MBS036924) and glutathione (GSH) (catalog# MBS265966) were quantified in hepatic homogenates using commercial kits from MyBioSource (San Diego, CA 92195-3308, USA).

Histological examination

After left liver lobes was preserved in a 10% neutral formalin solution, sample progressively dried out in 50%-100% ethanol, cleaned in xylene and embedded into paraffin. Hematoxylin and eosin (HX and E) staining made after sections cut into 5 m thick portions. Slices of liver analyzed using light microscopy.

Statistical analysis

Results presented as mean±standard deviation (SD). Value analysis made using IBM corporation's SPSS version 22 (Armonk, NY, USA). Shapiro-Wilk test employed to determine value distributions normality. Tukey's test utilized to compare groups of normally distributed values after One-Way ANOVA used to analyze data. Kruskal Wallis and Mann Whitney tests utilized to compare groups when values were abnormally distributed (weight increase, weight index, FER, liver weights and liver indices). Statistical significance defined as a $p<0.050$.

RESULTS AND DISCUSSION

Biological parameters

In comparison to the NC group, the T2DM, T2DM+SAHA and T2DM+DAPA groups showed significant reductions in final body weight, weight gain, weight gain percentage and FER. Additionally, liver weight significantly decreased in T2DM+DAPA compared to NC ($p<0.010$), while hepatic index increased significantly in T2DM versus NC and T2DM+DAPA groups ($p< 0.001$ and $p<0.010$, respectively) (Table 1). Food intake variations across weeks among studied groups are illustrated in Fig 1.

Glycemic control

At 3rd week, FBG level significantly increased in T2DM, T2DM+SAHA and T2DM+ DAPA groups versus NC

Table 1: Comparison of initial, final body weight, weight gain, food efficiency ratio, liver weight and liver index in various studied groups.

Variables	NC group	T2DM group	T2DM+SAHA group	T2DM+DAPA group
Initial body weight (grams)	272.70±45.97	232.80±35.37	235.90±29.12	252.10±14.04
Final body weight (grams)	404.50±50.31	248.40±41.30***	287.40±56.90***	308.20±72.21**
Weight gain (grams)	131.80±55.23	15.60±36.44***	51.50±64.40**	56.10±67.21*
Weight gain (%)	32.07±11.69	5.32±12.74***	15.35±18.79 *	14.84±17.05*
Food efficiency ratio (FER) (%)	0.051±0.022	0.006±0.014***	0.020±0.024**	0.021±0.026*
Liver weight (grams)	9.76±1.16	8.75±1.09	8.67±1.33	6.98±2.18 **
Liver index (%)	2.42±0.16	3.62±0.82***	3.17±0.90	2.35±0.77##

*: Significance versus NC group; #: Significance versus T2DM. *: $P<0.050$, **: $P<0.010$, ***: $P<0.001$.

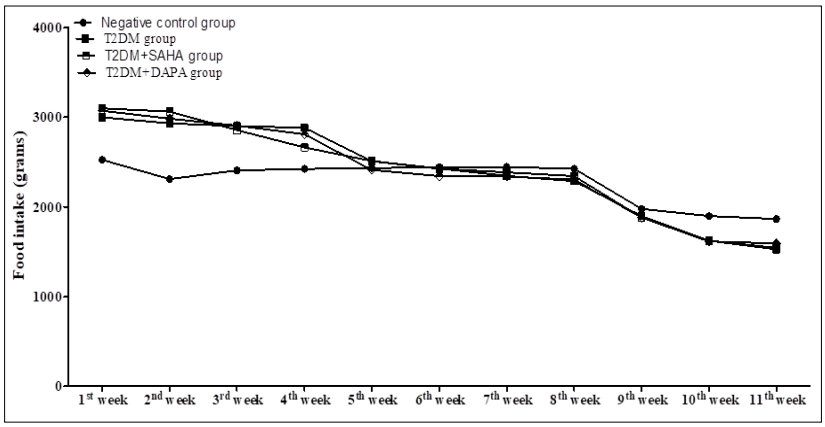


Fig 1: Food intake in different studied groups at different weeks.

(p<0.0001). At 11th week, FBG level significantly declined in T2DM+DAPA versus T2DM and T2DM+SAHA groups (p<0.001) and in T2DM+SAHA versus T2DM (p=0.029). Fasting insulin levels and HOMA-IR significantly elevated in T2DM, T2DM+SAHA and T2DM+ DAPA groups versus NC (p <0.0001) but were significantly decreased in T2DM+SAHA and T2DM+ DAPA groups versus T2DM group (p<0.0001) and in T2DM+ DAPA groups versus T2DM+SAHA (p<0.0001) (Table 2).

Liver function tests

At experimental end, AST, ALT and GGT values significantly increased in T2DM, T2DM+DAPA and T2DM+SAHA groups versus NC (p<0.001) and in T2DM versus T2DM+DAPA and T2DM+SAHA groups (p<0.001). Meanwhile, AST and ALT values significantly decreased in T2DM+DAPA than T2DM+SAHA group (p<0.001). Total bilirubin significantly elevated in T2DM versus NC, T2DM+SAHA and T2DM+DAPA (p<0.001) and in T2DM+DAPA versus NC (p<0.050). Total proteins levels declined in T2DM, T2DM+SAHA and T2DM+DAPA versus NC (p<0.001); decreased in T2DM versus T2DM+SAHA and T2DM+DAPA (p <0.001), declined in T2DM+DAPA versus T2DM+ SAHA (p<0.001). Albumin levels significantly decreased in T2DM and T2DM+DAPA versus NC (p<0.001); decreased in T2DM versus T2DM+SAHA and T2DM+DAPA (p<0.001), declined in T2DM+DAPA than T2DM+ SAHA (p<0.001) (Table 3).

Lipid profile

At experimental end, TG, total cholesterol, LDL-C values significantly elevated in T2DM, T2DM+DAPA and T2DM+SAHA groups versus NC (p<0.001) and in T2DM versus T2DM+DAPA and T2DM+SAHA groups (p<0.001). Triglyceride and total cholesterol values significantly increased in T2DM+SAHA versus T2DM+DAPA (p<0.001). HDL-C values significantly declined in T2DM, T2DM+DAPA and T2DM+SAHA groups versus NC (p<0.001) (Table 3).

Oxidative stress

At experimental end, MDA liver homogenate levels significantly elevated in T2DM, T2DM+DAPA and T2DM+SAHA groups versus NC (p<0.001) and in T2DM versus T2DM+DAPA and T2DM+SAHA groups (p<0.001). SOD and GSH liver homogenate values significantly declined in T2DM, T2DM+DAPA and T2DM+SAHA groups versus NC (p<0.001); decreased in T2DM versus T2DM+DAPA and T2DM+SAHA groups (p<0.001 and p<0.050). SOD value significantly increased in T2DM+SAHA versus T2DM+DAPA (p<0.001) (Table 4).

Histology results

Livers of healthy control rats exhibit a typical hepatic structure with hepatocyte cords extending from the central vein to the lobule's edge, forming classic lobules. Portal triads comprised of the portal vein, hepatic artery and bile ducts with cuboidal epithelium, are found at lobule corners,

Table 2: Comparison of glycemic control in various studied groups.

Variables	NC group (n= 10)	T2DM group (n= 10)	T2DM+SAHA group (n= 10)	T2DM+DAPA group (n= 10)
Blood glucose (mg/dl)				
3rd week	75.70±7.60	395.20±31.95***	403.70±48.87***	410.90±57.36***
11th week	78.90±9.98	354.90±22.57***	333.20±7.50***,#	263.70±20.82***,###, &&&
Insulin (uIU/ml)	26.80±2.30	85.40±3.06***	42.50±1.84***,###	75.30±2.11***,###, &&&
HOMA-IR	5.22±0.76	74.94±6.90***	34.97±1.85***,###	49.01±3.80***,###, &&&

*: Significance versus NC group; #: Significance versus T2DM, &: Significance versus T2DM+SAHA group. *: P<0.050, ***: P<0.001.

Table 3: Comparison of liver function tests and lipid profile in various studied groups.

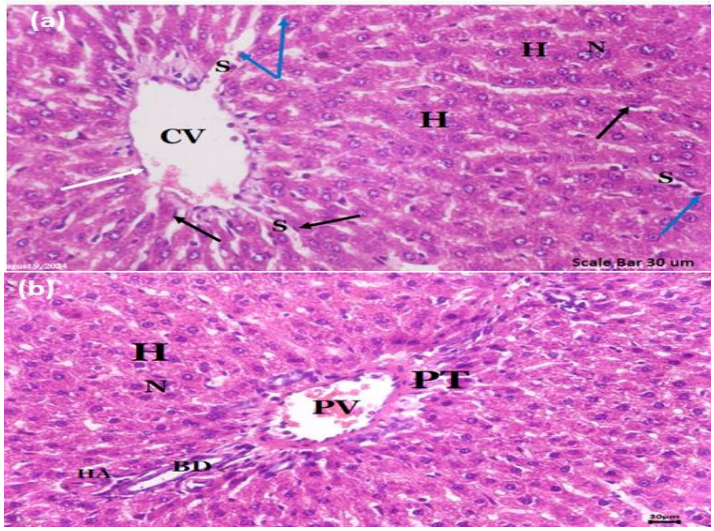
Parameters	NC group	T2DM group	T2DM+SAHA group	T2DM+DAPA group
AST (U/L)	116.10±4.06	248.80±4.32***	171.60±8.58***,###	151.50±6.77***,###,&&&
ALT (U/L)	80.70±2.31	150.60±4.27***	127.50±5.08***,###	115.40±4.86***,###,&&&
GGT (U/L)	5.98±1.58	23.30±2.41***	13.00±1.83***,###	13.90±1.29***,###
Total bilirubin (µmol/L)	5.58±0.56	8.36±0.80***	5.94±0.39###	6.38±0.41*,###
Total protein (mg/dl)	72.16±2.36	32.30±2.50***	48.60±2.91***,###	43.90±2.42***,###,&&&
Albumin (mg/dl)	45.20±2.65	23.50±1.78***	42.50±3.44###	33.90±2.42***,###,&&&
Triglyceride (mg/dl)	132.90±2.96	375.00±9.13***	314.00±12.65***,###	265.00±15.81***,###,&&&
Total cholesterol (mg/dl)	93.70±3.06	258.70±7.67***	191.00±13.29***,###	136.50±9.73***,###,&&&
LDL-C (mg/dl)	44.70±3.20	151.10±7.81***	74.90±7.17***,###	77.80±4.21***,###
HDL-C (mg/dl)	72.30±2.71	32.60±3.95***	36.10±4.12***	36.00±4.62***

*: Significance versus NC group; #: Significance versus T2DM, &: Significance versus T2DM+SAHA group. *: P<0.050, ***: P<0.001.

surrounded by loose stromal connective tissue (Fig 2a). Hepatocytes are polyhedral, often binucleated, featuring distinct nucleoli and eosinophilic cytoplasm with basophilic granules. Central vein lined by flat endothelial cells, with hepatic blood sinusoids located between endothelial cells and von Kupffer cells. Each lobule has three to six portal areas with more fibrous connective tissue and interlobular structures (Fig 2b).

Rats of diabetic group showed severe liver damage. Alterations were disorderly hepatocyte, inflammatory, degenerative, necrotic, nucleus karyolysis and hyperplastic changes. Liver showed degenerations and vacuolation of hepatocytes and some of them exhibited one large cytoplasmic vacuole. Severe infiltrative fatty changes as fat droplets occupying hepatocytes cytoplasm, pushing nucleus to periphery (Fig 3a). Hydropic degeneration of hepatocytes observed around dilated central vein. Central vein showed dilatation and congestion with leukocytic infiltrations around it (Fig 3b). There were many localized necrotic regions with extensively vacuolated hepatocyte cytoplasm and strongly stained pyknotic nuclei, together with mononuclear cells infiltration or widespread hepatic coagulative necrosis (Fig 3a,b). Portal tract showed congested and dilated portal vein and mononuclear leucocytic cell infiltrations around portal vein (Fig 3c).

Treated diabetic groups with SAHA demonstrated improvements in hepatic histological structure, including hepatocytes amelioration, reduced inflammation and leucocytic cell infiltration and necrotizing hepatocytes caused by STZ. Liver tissue maintained a normal shape with radiating hepatic cell cords and central veins (Fig 4a). Most hepatocytes were polyhedral with eosinophilic cytoplasm, basophilic granules and distinct vesicular rounded nuclei. Central vein lined with flat endothelial cells, while hepatic blood sinusoids observed between hepatocyte cords. Portal tract exhibited a normal bile duct, hepatic artery and portal vein (Fig 4b).



(a) Thin walled central vein (CV) lined with flat endothelial cells (White arrow) and normal hepatocytes (H) with rounded vesicular nuclei (N) and eosinophilic cytoplasm with basophilic granules. Some cells are binucleated. Note: thin walled blood sinusoids (S) between hepatocyte cell cords lined with endothelial cells (Black arrows) and von Kupffer cells(blue arrows). (X400 HX and E). Scale bar 30 um.

(b) Portal tract (PT) contained branch of hepatic artery (HA), branch of portal vein (PV) and bile duct (BD). Hepatocytes with eosinophilic cytoplasm (H) and rounded vesicular nuclei (N) were observed. (X400 HX and E). Scale bar 30 um.

Fig 2: A photomicrograph in the control rat liver (NC) stained with HX and E stain.

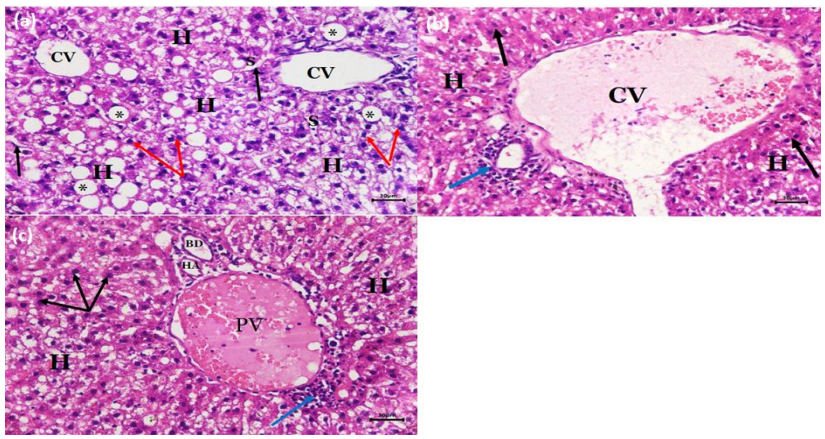
Table 4: Comparison of oxidative stress markers in various studied groups.

Parameters	NC group	T2DM group	T2DM+SAHA group	T2DM+DAPA group
MDA (nmol/ml)	9.67±3.33	23.54±2.38***	15.31±2.14***,###	17.55±1.82***,###
SOD (U/ml)	17.93±2.11	9.11±1.37***	14.40±0.71***,###	10.93±1.15***,#,&&&
GSH (U/L)	315.99±13.04	225.01±11.60***	267.27±7.25***,###	277.01±10.72***,###

*: Significance versus NC group; #: Significance versus T2DM, &: Significance versus T2DM+SAHA group. *: P<0.050, ***: P<0.001.

In diabetic rats treated with DAPA, there was a moderate improvement in liver histology, with preservation of normal hepatic shape including central veins and hepatic cell cords. Most hepatocytes were polyhedral, featuring eosinophilic cytoplasm with basophilic granules and rounded nuclei. Central vein lined with flat endothelial cells and hepatic blood sinusoids were observed between hepatic cell cords. Portal tracts maintained a normal histological structure (Fig 5a,b). However, some specimens exhibited moderate fatty infiltrative changes, characterized by smaller fat droplets in cytoplasm, loss of normal liver architecture and mononuclear cell infiltration (Fig 5c,d).

T2DM-related metabolic abnormalities may cause liver damage that lead to liver illnesses, including cirrhosis, hepatocellular carcinoma and fatty liver. Numerous

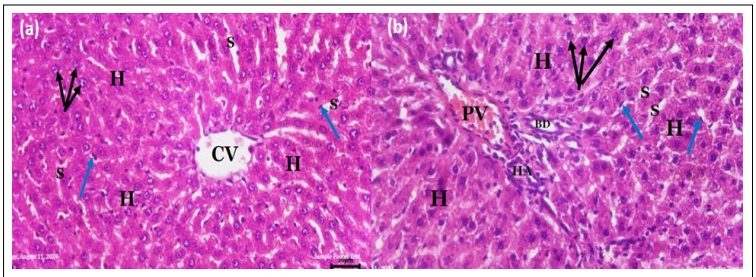


(a) Degeneration and vacuolation of hepatocytes (H) with deeply stained pyknotic nuclei (red arrows) and appearance of large one vacuole inside most hepatocytes (*) (Severe infiltrative fatty changes). Note: Blood sinusoids (S) with lining endothelial cells (Black arrows) and central vein (CV). (HX and E-X 400). Scale bar 30 um.

(b) Dilatation and congestion of central vein (CV) with mononuclear cellular infiltrations around central vein (Blue arrow). Note: Degeneration and vacuolation of hepatocytes (H) (Fatty changes) with deeply stained pyknotic nuclei (Black arrows). (HX and E-X400). Scale bar 30 um.

(c) Dilatation and congestion of portal vein (PV) associated with lymphocytic infiltrations (blue arrow) around portal vein. Degeneration and vacuolation of hepatocytes (H) with deeply stained pyknotic nuclei (Black arrows) were observed. Note: Hepatic artery (HA) and bile duct (BD) (HX and E-X 400). Scale bar 30 um.

Fig 3: Photomicrograph of T2DM group liver stained HX and E stain.



(a) Improvement in liver histological structure with normal hepatocytes (H) Exhibited eosinophilic cytoplasm and rounded vesicular nuclei (Black arrows) radiating from central vein (CV). Note: Blood sinusoids (S) Lined with flat endothelial cells (Blue arrows) (X400 HX and E). Scale bar 30 um.

(b) Normal portal vein (PV), bile duct (BD) and hepatic artery (HA). Improvement in liver histological structure with normal hepatocytes (H) Exhibited eosinophilic cytoplasm and rounded vesicular nuclei (Black arrows). Note: Blood sinusoids (S) lined with flat endothelial cells (Blue arrows) (X400 HX and E). Scale bar 30 um.

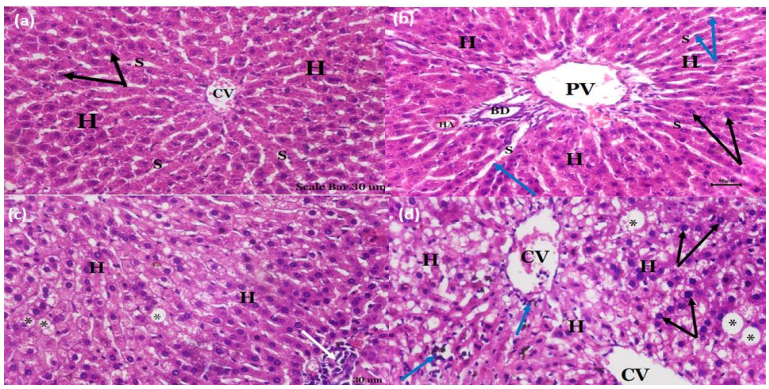
Fig 4: A photomicrograph in T2DM+SAHA group liver stained with HX and E stain.

variables, including hyperglycemia, insulin resistance, dyslipidemia, oxidative stress and inflammations, can lead to diabetic liver damage (Khajuria *et al.,* 2018).

In this study, HFD/STZ utilized to induce a T2DM liver damage rat model. The results revealed that, final body weight, weight gain, weight gain percentage and FER declined in T2DM group compared to NC group. In this respect, According to Zhu *et al*. (2021) T2DM rat group's body weight dramatically dropped while their intake of food, water and urine volume significantly elevated (Zhu *et al.,* 2021). Lower body weight in T2DM is due to catabolic state of poorly controlled glycaemia. Under these conditions, lipolysis, metabolic processes and oxidative degradation of amino acids elevated; they degrade greatest energy and tissue reserves in animal, so decreasing body weights. In this study, body weights in diabetic rats were not improved by administration of SAHA or DAPA to diabetic rats, which may be caused by continuous catabolic condition. SAHA appears to have complex effects on body weight. While some metabolic alterations and adverse consequences, such as anorexia, were noted in preclinical and clinical trials, significant weight changes were not always recorded (Kelly *et al.,* 2005). This suggests that not all circumstances and dose ranges will result in a consistent or direct effect of SAHA on body weight. In the meantime, osmotic drainage brought on by the glycosuria brought on by DAPA alters body composition, resulting in a decrease in body fat and body weight loss. According to Phrueksotsai *et al*. (2021) DAPA prescription resulted in significant decline in body weight and body fats after 12 weeks of therapy, with a mean 3% decrease in baseline body weights. Furthermore, correlation analysis showed a significant positive relationship between weight loss and decrease in liver fat content (Phrueksotsai *et al*., 2021). Ahmed *et al.* (2023) found that total body weight was declined in three groups of rats that received three different DAPA doses (0.75, 1.5 and 3 mg/kg, p.o.) than negative and diabetic control groups.

Liver index in this research significantly increased in T2DM versus negative control and T2DM+DAPA groups. (Zhu *et al.,* 2021) reported that liver indices signifi-cantly increased in T2DM rat's model (Zhu *et al*., 2021). In this study, liver weight significantly decline in T2DM+DAPA versus negative control. DAPA medication significantly reduces total liver fat content in T2DM patients (Phrueksotsai *et al.,* 2021). In addition to decreased body weight, metabolic substrate shift from glucose to fatty acids and likely higher hepatic oxidation of fatty acids are other theories explaining loss of liver fat (Esterline *et al.,* 2018).

This study showed that at experimental end FBG level, fasting insulin level and HOMA-IR significantly elevated in T2DM group versus negative control as previously reported (Abdulwahab *et al.,* 2021; Zhu *et al.,* 2021). In rats, HFD causes IR (Zhu *et al.,* 2021). The results of this research



(a) Improvement in hepatic histological structure with normal hepatocytes (H) exhibited eosinophilic cytoplasm and rounded vesicular nuclei (Black arrows) radiating from central vein (CV). Note: Normal blood sinusoids (S) lined with flat endothelial cells (HX and E- X 400). Scale bar 30 um.

(b) Normal portal vein (PV), bile duct (BD) and hepatic artery (HA). Improvement in liver histological structure with normal hepatocytes (H) Exhibited eosinophilic cytoplasm and rounded vesicular nuclei (Black arrows). Note: Normal blood sinusoids (S) Lined with flat endothelial cells (Blue arrows) (HX and E-X 400). Scale bar 30 um.

(c) Some hepatocytes (H) Showed moderate fatty infiltration in their cytoplasm (*) and mononuclear cell infiltration's (White arrow) between hepatocytes (HX and E-X 400). Scale bar 30 um.

(d) In some specimens, hepatocytes (H) around central vein (CV) showed fatty infiltration in their cytoplasm (*) with deeply stained pyknotic nuclei (Black arrows) and mononuclear cell infiltration's (Blue arrows) (HX and E-X400). Scale bar 30 um.

Fig 5: A photomicrograph in T2DM+DAPA liver stained with HX and E stain.

showed that FBG, fasting insulin, HOMA-IR was significantly decreased T2DM+SAHA versus T2DM but were still significantly elevated than negative control group. Bocchi *et al.* (2019) reported that injection of SAHA (25 mg/kg i.p.) for 23 days into mice leads to improved sensitivity to insulin. Silva *et al.* (2020) reported that injection of SAHA (25-50 mg/kg i.p.) for 8 weeks into HFD fed mice leads to ameliorate insulin sensitivity. In the MG63 mesenchymal stem cell model, SAHA enhances β-cell formation and insulin production by upregulating PDX1 expression. Furthermore, SAHA pretreatment elevates β-cell markers in high glucose scenarios, suggesting an improvement in the stem cells' capacity to differentiate into insulin-producing β-cells (Elsharkawi *et al.,* 2020). Histones, regulatory proteins and transcription factors involved in glucose metabolism are deacetylated by HDACs, which play a role in insulin gene transcription regulation through histone acetylation (Dewanjee *et al.,* 2021). The results of this research revealed that therapy of diabetic rats with DAPA led to decline in FBG, fasting insulin, HOMA-IR versus T2DM but were still significantly higher than negative control group. In DAPA treated diabetic rats, FBG was significantly decline, but fasting insulin and HOMA-IR were increased versus SAHA treated diabetic group. Tang *et al.* (2017) reported that after four weeks of therapy, there was decline in blood glucose values and an increase in urine glucose excretion with DAPA. Joannides *et al.* (2017) reported that DAPA administration for 45 days into PEPCK transgenic rats caused reduced plasma glucose and insulin values due to improve IR, elevated fat and muscle glucose uptake and GLUT4 protein values and decreased adipocyte size and elevated adipocyte number, but not elevated insulin secretion (Joannides *et al.,* 2017). HFD/STZ-induced diabetic mice treated with DAPA showed improved glucose tolerance, IR and insulin secretion, with a significant elevation in insulin pancreatic content (Millar *et al.,* 2016).

Liver was affected in T2DM rat models in this study as revealed by increased ALT, AST, GGT, total bilirubin and decline in total protein and albumin versus negative control. Zhu *et al.* (2021) reported ALT and AST values elevated in T2DM rats model. T2DM+SAHA administration to diabetic rats had protective effect on liver by decreased AST, ALT, GGT, total bilirubin and significant increase in total protein and albumin. SAHA has protective effect on liver damage produced by lethal hemorrhagic shock in rats that was associated with elevated H3K9 acetylation and suppression of JNK/caspase-3 apoptotic pathway (Zhang *et al.,* 2013). SAHA administration significantly reduced AST, ALT and lactate dehydrogenate levels, increased survival rate and decreased apoptotic markers expression in liver tissues, suggesting its protective role in early hemorrhagic shock conditions (Zhao *et al.,* 2015). Wang *et al.* (2018) found that in rats carbon tetrachloride (CCl4) significantly made liver fibrosis and elevated serum values of transforming growth factor (TGF)-β1, total bilirubin, ALT, AST, laminin and procollagen type III; liver HDAC2, p-Smad2/3, HDAC6, HDAC8, α-SMA and connective tissue growth factor (CTGF) proteins; whereas Smad7 mRNA and AH3 protein levels were notably suppressed. SAHA treatment led to significantly downregulated, these liver chemistries, cytokines and liver fibrosis-related genes and mitigated hepatic fibrosis (Wang *et al.,* 2018). Abbas *et al.* (2019) reported that SAHA administration (15 mg/kg/day p.o.) for 8 weeks to rat model of autoimmune hepatitis made by Concanavalin A led to decreased in AST and ALT liver enzymes. DAPA administration to diabetic rats had protective effect on liver as revealed by decreased ALT, AST, GGT, total bilirubin and significant increase in albumin and total protein. Dapagliflozin therapy protected the liver in db/db mice, as revealed by markedly lower levels of oxidative stress and inflammatory indicators, hepatic lipid buildup and plasma ALT activity and TG levels (Tang *et al.,* 2017). DAPA markedly reduced ALT and AST serum levels and protected liver from pathologic damages in diabetic mice (Leng *et al.,* 2019). A clinical trial utilizing DAPA in T2DM patients revealed reduced values of liver damage biomarkers, as ALT, AST and GGT; combining carboxylic acids (OM-3CA) resulted in a significant decline in hepatic fat content (Eriksson *et al.,* 2018). DAPA led to ALT-lowering effect by decrease liver fat deposition by making hyperglucagonemia and ameliorate IR caused by decreased ectopic steato-sis (Ito *et al.,* 2017).

Dyslipidemia is considered a major cardiovascular disorders risk factor and death. In this study, significant increase in serum TC, TG and LDL-C values accompanied by decline in serum HDL-C value were demonstrated in T2DM rats versus control group as others (Abdulwahab *et al.,* 2021; Zhu *et al.,* 2021). In this study, treatment of diabetic rats with SAHA led to a significant decreased in serum TG, TC and LDL-C values compared to T2DM rats but were elevated versus control group. SAHA intake did not improve levels of HDL-C. SAHA exhibits significant anti-inflammatory effects by reducing pro-inflammatory cytokines production as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and interferon gamma. These cytokines are known to influence metabolic processes, including lipid metabolism (Leoni *et al.,* 2002). HDACs inhibition by SAHA affects various signaling pathways and gene expressions related to lipid biosynthesis and metabolism as lipoxygenases. The induction of 15-lipoxygenase-1 by SAHA, correlates with significant changes in lipid metabolism (Wu *et al.,* 2015). In this study, treatment of diabetic rats with DAPA led to a significant decreased in serum TG, TC and LDL-C values versus T2DM rats but were still significantly elevated than control group. Decline in TG and TC levels were more effective that SAHA administration. Meanwhile, DAPA administration did not improve HDL-C levels. Hazem *et al.* (2022) reported lower liver weights and decreased serum values of TG and TC in rats received DAPA (Hazem *et al.,* 2022). Ahmed *et al.* (2023) reported that TC and TG were decreased in three groups that received three different DAPA doses (0.75, 1.5 and

3 mg/kg, p.o.) for 6 weeks more than negative and diabetic control groups. This reduction in TC and TG may be attributed to the total reduction in body weight or may be explained by shift of metabolic substrate from glucose to fatty acids.

The development of T2DM and its consequences, as well as IR, are significantly affected by oxidative stress. Reactive oxygen species (ROS) produced by dyslipidemia and hyperglycemia may damage live cells and certain cell membrane receptors, which may lead to damage to organs including liver. The results of this research showed elevation of MDA and decrease in SOD and GSH in diabetic rats versus negative control group. Researches revealed that tissue homogenate GSH concentrations in STZ-induced diabetic rats significantly decline versus negative control rats (Tanbek *et al.,* 2022; Rao *et al.,* 2023). Decline GSH and SOD values in diabetic rats caused by its increased consumption that is required to relieve oxidative stress. Zhu *et al.* (2021) revealed that oxidative stress and inflammation significantly elevated in hepatic homogenates of T2DM rats. Hazem *et al.* (2022) revealed that diabetic group had much lower values of GSH, SOD and catalase and significantly greater amounts of MDA. In this study, diabetic rats that received SAHA showed significant decrease in MDA but elevation of SOD and GSH hepatic homogenate levels versus T2DM rats. Bakhdar *et al.* (2023) reported that SAHA administration to Wistar rats (15 mg/kg/day i.p.) for 28 days led to pancreatic protection *via* anti-inflammatory and antioxidant actions. SAHA reduce hepatic cellular injury and ROS production in lipopolysaccharide (LPS)-induced liver damage. It enhanced the antioxidant enzyme GSH and inhibited apoptotic signaling pathways, suggesting its potential in alleviating inflammatory liver conditions (Zhao *et al.,* 2015). In this study, diabetic rats that received DAPA showed significant decrease in MDA but elevation of SOD and GSH hepatic homogenate levels versus T2DM rats. The effect of T2DM+SAHA in elevation of SOD hepatic homogenate level was better than T2DM+DAPA. Hazem *et al.* (2022) provides evidence for hepatoprotective effects of DAPA, as seen by the dose-dependent increases in antioxidant enzymes SOD, catalase activity and GSH and decline in MDA levels. In Kashiwagi study, SOD activity was significantly improved after 8 weeks of diabetic group treatment with insulin and dapagliflozin (Kashiwagi, 2001). The results of this research suggest that dapagliflozin significantly improved antioxidant status in diabetic rats. The treatment's antihyperglycemic impact, which lessens the load of oxidative stress, might be the cause of this improvement.

In this study, diabetic rats showed severe liver damage. The most alterations in diabetic control rat liver were disorderly hepatocyte, inflammatory, degenerative, necrotic, nucleus karyolysis and hyperplastic changes. These findings are in accord with Ahmed *et al.* (2023) who found that in diabetic, liver severe infiltrative fatty changes in form of more well-defined fat droplets occupying cytoplasm of hepatocytes, pushing nucleus to periphery. Also, multiple inflammatory cells appear with loss of normal architecture of hepatocytes. Al-Ani *et al.* (2009) observed that in STZ-diabetic mice showed sever congestion, necrotic foci, hydropic changes, fatty changes in hepatocytes and aggregation of lymphocytes between hepatocytes. Furthermore, additional research documented damage to liver cells, sinusoidal dilatation in central venous region, elevated cell apoptosis and lipid droplets in liver cells of diabetic mice (Rodriguez *et al.,* 2018). By activating NF-B that stimulates pro-apoptotic genes activity in liver cells and increases ROS creation, hyperglycemia circumstances aggravate liver damage by inducing oxidative stress and inflammatory conditions (Bae *et al.,* 2023).

The results of this study showed T2DM+SAHA had improvement in liver histological structure. Amelioration of hepatocytes and alleviate inflammation, leucocytic cell infiltration, necrotizing hepatocytes produced by STZ. Chen *et al.* (2015) assessed the protective effects of SAHA against LPS-induced hepatic damage through histological analysis. Their findings indicated that LPS exposure markedly heightened the inflammatory response in the murine liver, evidenced by increased inflammatory cell infiltration and hepatocyte necrosis and apoptosis. In contrast to the LPS-only group, liver architecture was maintained in mice treated with SAHA post-LPS injection. SAHA inhibited ROS production and mitigated the decline of the antioxidant enzyme glutathione due to STZ, while also reducing hepatic apoptosis linked to STZ. Additionally, SAHA blocked the activation of key kinases involved in the inflammatory and apoptotic processes (Chen *et al*., 2015). This study demonstrate that SAHA mitigate hepatotoxicity caused by STZ and imply that a novel therapeutic approach for treating STZ-induced inflammatory conditions could involve blocking upstream processes necessary for function of apoptotic signal-regulating kinase-1. Bocchi *et al.* (2019) found that SAHA therapy can reduce oxidative damage in cardiomyocytes, potentially reversing initial functional impairments. Diabetic cardiomyocytes displayed a decrease in metabolic activity, including NAD(P)H dehydrogenase and ROS levels, suggesting a link between ROS generation and cellular metabolism. SAHA appears to restore redox signaling in diabetic cells, crucial for maintaining cardiomyocyte homeostasis and the heart's response to stress.

The present research showed that diabetic rat received DAPA showed in some specimens, moderate improvement in liver histological structure. On the other hand, some specimens still showed moderate fatty infiltrative changes with droplets of fats occupying hepatocytes cytoplasm and loss of normal architecture of liver tissue accompanying mononuclear cell infiltrations. Our data agree with the results of Ahmed *et al.* (2023) who noticed that diabetics on DAPA (0.75 mg/kg), section of rat liver tissue showed mild to moderate fatty infiltrative changes where smaller well-defined fat droplets occupying hepatic cytoplasm with loss of liver tissue normal architecture. Diabetic rats on

DAPA 1.5 and 3 mg/kg groups: section of rat hepatic tissue revealed improvement of fatty infiltrative alteration with normal hepatocytes around central vein and normal liver tissue architecture. Hazem *et al.* (2022) observed that DAPA represent a viable approach to protect liver versus diabetes-induced steatohepatitis *via* suppressing oxidative stress, fibrosis progression and inflammation thus conserving hepatic functions and structure.

CONCLUSION

This research confirms that SGLT2 inhibition *via* DAPA and HDAC inhibition by SAHA decrease blood glucose level and protective in slowing liver damage progression in T2DM rats by improve IR and lipid profile and reduced tissue oxidative stress. DAPA effect was better than SAHA in decreasing fasting blood glucose level and improving lipid profile; while SAHA was better than DAPA in improving insulin secretion and IR and increased antioxidant and improvement of hepatic tissue damage. These findings suggest that selective SGLT2i and HDACi could be utilized in combination with other oral anti-diabetes drugs and insulin to further improve glycemic control and reverse organ damage in T2DM.

Disclaimers

The opinions and findings presented in this article are those of the authors alone and may not be representative of those of the organizations with which they are affiliated. Although the writers are in charge of information's correctness and comprehensiveness, they disclaim all obligations for any losses, whether direct or indirect, that may arise from using this content.

Informed consent

The experimental procedure made according to ARRIVE (Animal Research Reporting of *In Vivo* Experiments) and approved by Ethical Committee of King Abdulaziz University, Jeddah, Saudi Arabia.

Conflict of interest

Authors declare that they had no competing interests. This study is not funded.

REFERENCES

Ahmed, S.W.M., Soliman, A., Ahmed Amer, A.E., El Shahat, R.M., Amin, M.M., Taha, R.S., Awad, M.M.Y., Abdel, H.A.M. and El-Sayed, M.S. *et al*. (2023). Effect of dapagliflozin against NAFLD and dyslipidemia in type 2 diabetic albino rats: Possible underlying mechanisms. *Eur. Rev. Med. Pharmacol. Sci.* **27(17):** 8101-8109.

Abbas, A., Ramadan, W., Abdel Kawy, H. and Alhaddad, A. (2019). Hepatoprotective Effects of suberoylanilide hydroxamic acid (A histone deacetylase inhibitor) in immunological liver injury in rats. *Journal of Pharmaceutical Research International.* **30(3):** 1-15.

Abdulwahab, D.A., El-Missiry, M.A., Shabana, S., Othman, A.I. and Amer, M.E. (2021). Melatonin protects the heart and pancreas by improving glucose homeostasis, oxidative stress, inflammation and apoptosis in T2DM-induced rats. *Heliyon*. **7(3):** e06474.

Al-Ani, I., Al-Mishadani, N., Muslih, R.K. and Hamoodi, S.R. (2009). Histological liver changes in streptozotocin induced diabetic mice. *Med. J. Malaysia.* **8(1):** 1-4.

Arab, H.H., Fikry, E.M., Alsufyani, S.E., Ashour, A.M., El-Sheikh, A.A.K., Darwish, H.W., Al-Hossaini, A.M., Saad, M.A., Al-Shorbagy, M.Y. and Eid, A.H. (2023). Stimulation of autophagy by dapagliflozin mitigates cadmium-induced testicular dysfunction in rats: The role of AMPK/mTOR and SIRT1/Nrf2/HO-1 pathways. *Pharmaceuticals (Basel).* **16(7):** 1006.

Bae, C.S., Lee, Y. and Ahn, T. (2023). Therapeutic treatments for diabetes mellitus-induced liver injury by regulating oxidative stress and inflammation. *Appl. Microsc.* **53(1):** 4.

Bakhdar, F.A., Abdel, K.H.S., Magadmi, R.M., El-Kordy, E.A. and Alamri, A.S. (2023). Effect of histone deacetylase inhibitor (Vorinostat) on new-onset diabetes induced by tacrolimus. *J. Taibah Univ Med. Sci.* **18(1):** 9-18.

Bica, I.C., Stoica, R.A., Salmen, T., Janez, A., Volcansek, S., Popovic, D., Muzurovic, E., Rizzo, M. and Stoian, A.P. (2023). The effects of sodium-glucose cotransporter 2-inhibitors on steatosis and fibrosis in patients with non-alcoholic fatty liver disease or steatohepatitis and type 2 diabetes: A systematic review of randomized controlled trials. *Medicina* (*Kaunas*). **59(6):** 1136.

Bocchi, L., Motta, B.M., Savi, M., Vilella, R., Meraviglia, V., Rizzi, F., Galati, S., Buschini, A., Lazzaretti, M., Pramstaller, P.P., Rossini, A. and Stilli, D. (2019). The histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) restores cardiomyocyte contractility in a rat model of early diabetes. *Int. J. Mol. Sci.* **20(8):** 1873.

Cabrera, S.M., Colvin, S.C., Tersey, S.A., Maier, B., Nadler, J.L. and Mirmira, R.G. (2013). Effects of combination therapy with dipeptidyl peptidase-IV and histone deacetylase inhibitors in the non-obese diabetic mouse model of type 1 diabetes. *Clin. Exp. Immunol.* **172(3):** 375-382.

Chen, Y., Du, J., Zhao, Y. T., Zhang, L., Lv, G., Zhuang, S., Qin, G. and Zhao, T.C. (2015). Histone deacetylase (HDAC) inhibition improves myocardial function and prevents cardiac remodeling in diabetic mice. *Cardiovasc Diabetol.* **7(14):** 99.

Colussi, C., Berni, R., Rosati, J., Straino, S., Vitale, S., Spallotta, F., Baruffi, S., Bocchi, L., Delucchi, F., Rossi, S., Savi, M., Rotili, D. *et al.* (2010). The histone deacetylase inhibitor suberoylanilide hydroxamic acid reduces cardiac arrhythmias in dystrophic mice. *Cardiovasc Res.* **87(1):** 73-82.

Dewanjee, S., Vallamkondu, J., Kalra, R.S., Chakraborty, P., Gangopadhyay, M., Sahu, R., Medala, V., John, A., Reddy, P.H., De Feo, V. and Kandimalla, R. (2021). The emerging role of HDACs: Pathology and therapeutic targets in diabetes mellitus. *Cells.* **10(6):** 1340.

Elsharkawi, I., Parambath, D., Saber-Ayad, M., Khan, A.A. and El-Serafi, A.T. (2020). Exploring the effect of epigenetic modifiers on developing insulin-secreting cells. *Hum Cell.* **33(1):** 1-9.

Eriksson, J.W., Lundkvist, P., Jansson, P. A., Johansson, L., Kvarnstrom, M., Moris, L., Miliotis, T., Forsberg, G.B., Riserus, U., Lind, L. and Oscarsson, J. (2018). Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: A double-blind randomised placebo-controlled study. *Diabetologia.* **61(9):** 1923-1934.

Esterline, R.L., Vaag, A., Oscarsson, J. and Vora, J. (2018). Mechanisms in endocrinology: SGLT2 inhibitors: Clinical benefits by restoration of normal diurnal metabolism? *Eur. J. Endocrinol.* **178(4):** R113-R125.

Grant, S., Easley, C. and Kirkpatrick, P. (2007). Vorinostat. *Nat Rev. Drug Discov.* **6(1):** 21-22.

Hadden, M.J. and Advani, A. (2018). Histone deacetylase inhibitors and diabetic kidney disease. *Int. J. Mol. Sci.* **19(9):** 2630.

Harris, K. and Nealy, K.L. (2020). Cardiorenal outcomes in type 2 diabetes. *Consensus Statement.* **43:** S98-S110.

Hazem, R.M., Ibrahim, A.Z., Ali, D.A. and Moustafa, Y.M. (2022). Dapagliflozin improves steatohepatitis in diabetic rats *via* inhibition of oxidative stress and inflammation. *Int. Immunopharmacol.* **104:** 108503.

Ito, D., Shimizu, S., Inoue, K., Saito, D., Yanagisawa, M., Inukai, K., Akiyama, Y., Morimoto, Y., Noda, M. and Shimada, A. (2017). Comparison of ipragliflozin and pioglitazone effects on nonalcoholic fatty liver disease in patients with type 2 diabetes: A randomized, 24-week, open-label, active-controlled trial. *Diabetes Care.* **40(10):** 1364-1372.

Joannides, C.N., Mangiafico, S.P., Waters, M.F., Lamont, B.J. and Andrikopoulos, S. (2017). Dapagliflozin improves insulin resistance and glucose intolerance in a novel transgenic rat model of chronic glucose overproduction and glucose toxicity. *Diabetes Obes Metab.* **19(8):** 1135-1146.

Kashiwagi, A. (2001). Complications of diabetes mellitus and oxidative stress. *Japan Medical Association Journal*. **44(12):** 521-528.

Kelly, W.K., O'Connor, O.A., Krug, L.M., Chiao, J.H., Heaney, M., Curley, T., MacGregore-Cortelli, B., Tong, W., Secrist, J.P. *et al.* (2005). Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J. Clin. Oncol.* **23(17):** 3923-3931.

Khajuria, P., Raghuwanshi, P., Rastogi, A., Koul, A., Zargar, R. and Kour, S. (2018). Hepatoprotective effect of seabuckthorn leaf extract in streptozotocin induced diabetes mellitus in wistar rats. *Indian Journal of Animal Research*. **52(12):** 1745-1750. doi: 10.18805/ijar.B-3439.

Khan, N., Kakar, A., Rabbani, I. and Naseem, M. (2025). Quercetin verses metformin, comparative efficacy on the lipid metabolism gene expression in different organs of diabetic wistar rats. *Indian Journal of Animal Research.* **60(3):** 439-446. doi: 10.18805/IJAR.BF-1968.

Leng, W., Wu, M., Pan, H., Lei, X., Chen, L., Wu, Q., Ouyang, X. and Liang, Z. (2019). The SGLT2 inhibitor dapagliflozin attenuates the activity of ROS-NLRP3 inflammasome axis in steatohepatitis with diabetes mellitus. *Ann. Transl. Med.* **7(18):** 429.

Leoni, F., Zaliani, A., Bertolini, G., Porro, G., Pagani, P., Pozzi, P., Dona, G., Fossati, G., Sozzani, S., Azam, T., Bufler, P., Fantuzzi, G., *et al.* (2002). The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties *via* suppression of cytokines. *Proc. Natl. Acad. Sci. USA.* **99(5):** 2995-3000.

Lundh, M., Christensen, D.P., Damgaard Nielsen, M., Richardson, S.J., Dahllof, M.S., Skovgaard, T., Berthelsen, J., Dinarello, C.A., Stevenazzi, A., Mascagni, P., Grunnet, L.G., Morgan, N.G. and Mandrup-Poulsen, T. (2012). Histone deacetylases 1 and 3 but not 2 mediate cytokine-induced beta cell apoptosis in INS-1 cells and dispersed primary islets from rats and are differentially regulated in the islets of type 1 diabetic children. *Diabetologia.* **55(9):** 2421-2431.

Millar, P.J., Pathak, V., Moffett, R.C., Pathak, N.M., Bjourson, A.J., O'Kane, M.J., Flatt, P.R. and Gault, V.A. (2016). Beneficial metabolic actions of a stable GIP agonist following pre-treatment with a SGLT2 inhibitor in high fat fed diabetic mice. *Mol. Cell. Endocrinol.* **420:** 37-45.

Phrueksotsai, S., Pinyopornpanish, K., Euathrongchit, J., Leerapun, A., Phrommintikul, A., Buranapin, S., Chattipakorn, N. and Thongsawat, S. (2021). The effects of dapagliflozin on hepatic and visceral fat in type 2 diabetes patients with non-alcoholic fatty liver disease. *J. Gastroenterol Hepatol.* 3**6(10):** 2952-2959.

Rao, K.V., Rani, M.U., Reddy, A.G., Kumar, B.A., Sree, K., Rajender, B. and Hanuman, D. (2023). Bovine lactoferrin mitigates oxidative stress and inflammation in CCL_4 and high-fat diet-induced NAFLD in C57BL/6 mice. *Indian Journal of Animal Research.* 1-9. doi: 10.18805/IJAR.B-5327.

Rodriguez, V., Plavnik, L. and Tolosa de Talamoni, N. (2018). Naringin attenuates liver damage in streptozotocin-induced diabetic rats. *Biomed Pharmacother*. **105:** 95-102.

Salem, I.S., El Mageed, N.M.A. and El Rahman, H. (2022). Effect of green coffee bean extract on rats suffering from diabetes. *Journal of Pharmaceutical Negative Results.* **13(9):** 8130-8143.

Silva, G., Ferraresi, C., de Almeida, R.T., Motta, M.L., Paixao, T., Ottone, V. O., Fonseca, I.A., Oliveira, M.X., Rocha-Vieira, E., Dias-Peixoto, M.F., Esteves, E.A., Coimbra, C.C., Amorim, F.T. and Magalhaes, F.C. (2020). Insulin resistance is improved in high-fat fed mice by photobiomodulation therapy at 630 nm. *J. Biophotonics.* **13(3):** e201960140.

Szekeres, Z., Toth, K. and Szabados, E. (2021). The effects of SGLT2 inhibitors on lipid metabolism. *Metabolites.* **11(2):** 87.

Tanbek, K., Ozerol, E., Yilmaz, U., Yilmaz, N., Gul, M. and Colak, C. (2022). Alpha lipoic acid decreases neuronal damage on brain tissue of STZ-induced diabetic rats. *Physiol Behav*. **248:** 113727.

Tang, L., Wu, Y., Tian, M., Sjostrom, C.D., Johansson, U., Peng, X.R., Smith, D.M. and Huang, Y. (2017). Dapagliflozin slows the progression of the renal and liver fibrosis associated with type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab.* **313(5):** E563-E576.

Teo, Z.L., Tham, Y.C., Yu, M., Chee, M.L., Rim, T.H., Cheung, N., Bikbov, M.M., Wang, Y.X., Tang, Y., Lu, Y. *et al.* (2021). Global Prevalence of diabetic retinopathy and projection of burden through 2045: Systematic Review and meta-analysis. *Ophthalmology*. **128(11):** 1580-1591.

Ulger, T.G. and Cakiroglu, F.P. (2020). The effects of onion (*Allium cepa* L.) dried by different heat treatments on plasma lipid profile and fasting blood glucose level in diabetic rats. *Avicenna J. Phytomed.* **10(4):** 325-333.

Wang, L., Bai, Y., Cao, Z., Guo, Z., Lian, Y., Liu, P., Zeng, Y., Lyu, W. and Chen, Q. (2024). Histone deacetylases and inhibitors in diabetes mellitus and its complications. *Biomed Pharmacother.* **177:** 117010.

Wang, Y., Zhao, L., Jiao, F.Z., Zhang, W.B., Chen, Q. and Gong, Z.J. (2018). Histone deacetylase inhibitor suberoylanilide hydroxamic acid alleviates liver fibrosis by suppressing the transforming growth factor-beta1 signal pathway. *Hepatobiliary Pancreat Dis. Int.* **17(5):** 423-429.

Wu, Q., Cheng, Z., Zhu, J., Xu, W., Peng, X., Chen, C., Li, W., Wang, F., Cao, L., Yi, X., Wu, Z., Li, J. and Fan, P. (2015). Suberoylanilide hydroxamic acid treatment reveals crosstalks among proteome, ubiquitylome and acetylome in non-small cell lung cancer A549 cell line. *Sci. Rep.* **5(1):** 9520.

Xu, B., Li, S., Kang, B. and Zhou, J. (2022). The current role of sodium-glucose cotransporter 2 inhibitors in type 2 diabetes mellitus management. *Cardiovasc Diabetol.* **21(1):** 83.

Ying, X., Chen, X., Wang, T., Zheng, W., Chen, L. and Xu, Y. (2020). Possible osteoprotective effects of myricetin in STZ induced diabetic osteoporosis in rats. *Eur. J. Pharmacol.* **866:** 172805.

Zhang, Y., Geng, Z., Xu, P. and Gao, X. (2013). Effect of suberoylanilide hydroxamic acid on liver injury induced by lethal hemorrhagic shock in rats first entering high altitude. *Chinese Journal of Anesthesiology*. pp. 477-480.

Zhao, Y., Zhou, P., Liu, B., Bambakidis, T., Mazitschek, R., Alam, H.B. and Li, Y. (2015). Protective effect of suberoylanilide hydroxamic acid against lipopolysaccharide-induced liver damage in rodents. *J. Surg. Res.* **194(2):** 544-550.

Zhu, Y., Su, Y., Zhang, J., Zhang, Y., Li, Y., Han, Y., Dong, X., Li, W. and Li, W. (2021). Astragaloside IV alleviates liver injury in type 2 diabetes due to promotion of AMPK/mTOR mediated autophagy. *Mol. Med. Rep.* **23(6):** 1-15.