Original Article

The Possible Protective Effect of Dapagliflozin and Moringa Alone or in Combination on the Liver of Type 2 Diabetic Albino Rats: A Biochemichal, Histological and Immunohistochemical Study

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) has injurious impact on liver due to high levels of blood sugar, fats, oxidative stress, and an increased incidence of inflammation. These factors can consequently lead to various liver disorders.

Aim of the Work: To ascertain impact of T2DM on histological structure of liver molecular and metabolic dysfunction and the potential protective function of dapagliflozin and moringa.

Materials and Methods: Fifty adult male albino rats were separated into five groups: control group, non-treated diabetic group, diabetic treated with dapagliflozin group, diabetic treated withMoringa oleifera extract group and diabetic treated with dapagliflozin plus Moringa oleifera extract group. After 4 weeks, blood samples were taken via cardiac puncture for biochemical parameters evaluation expression of miRNA 34a, Secretagogin, sirtulin genes also liver samples were processed for histological and immunohistochemical studies.

Results: Diabetes mellitus induced impairment on liver function with disturbed liver architecture. Administration of Dapagliflozin and Moringa alone in group III & IV revealed non-significant decrease in mirRNA34a and secretagogin expression with moderate improvement in biochemical and histological finding. Otherwise, after treatment of diabetic rats by Dapagliflozin plus Moringa in group V, mirRNA34a and secretagogin expression levels reduced significantly and sirtulin elevated compared to non- treated diabetic rats. The improvement shown in liver function indicators, oxidative stress markers and lipid, glucose levels. There is also an improvement in histopathological findings in group V with a significant decrease in main area distribution of collagen fibers, positive caspase 3 cytoplasmic immunoreactivity and positive PCNA nuclear immunoreactivity in compared to untreated diabetic group.

Conclusion: Administration of dapagliflozin plus moringa oleifera extract significantly ameliorated the hepatic alteration induced by diabetes mellitus type 2 in rats.

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Key Words: Dapagliflozin; liver; moringa; type 2 Diabetes.

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INTRODUCTION

Insulin resistance (IR) and beta-cell dysfunction are main causes of chronic hyperglycemia, that chiefs to progress of Type 2 diabetes mellitus (T2DM)[1]. Throughout disease's progression, there is an initial compensatory increase in insulin secretion. Diabetic liver inflammation and Non-alcoholic fatty liver disease (NAFLD) result in fibrosis, that may develop to liver cirrhosis, hepatosclerosis, liver failure, and hepatocellular carcinoma[2].

Dapagliflozin is one of sodium-glucose transporter 2 (SGLT2) antagonist that elevates insulin sensitivity, lowers glucose production, and reduces glucose reuptake by kidneys[3]. Previous studies have reported effect of dapagliflozin in suppressing hyperglycemia and enhancing insulin sensitivity[4]. Moreover, SGLT2 has been shown to improve liver function and inhibit progression of hepatic complications in diabetic rats[5].

Moringa leaves contain numerous vital minerals and vitamins. Some studies suggest that moringa has insulin-like proteins that help lower blood sugar levels. Moringa oleifera exhibits hepatoprotective, hypolipidemic, anti-inflammatory, anti-atherosclerotic, and antioxidant effects. It also can prevent release of reactive oxygen species[6].

MicroRNAs (miRNAs) are endogenous, small, non-coding RNA entities, exert their influence by regulating

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expression of specific target genes[7]. MicroRNAs sequences that respond to metabolic stressors within beta cells and islets underscore their critical role and potential as biomarkers for tracking disease progression in individuals with diabetes. Through analytical approaches, networks of genes influenced by these miRNA sequences have been identified, presenting novel avenues for therapeutic intervention aimed at preserving beta-cell function and potentially postponing onset of T2DM[8].

Secretagogin (SCGN) is a calcium-binding protein that plays a role in insulin secretion within pancreas. Despite SCGN is not produced in parallel with insulin, research has indicated that plasma levels of SCGN are higher in individuals with T2DM[9].

Sirtuin 1 (SIRT1), an enzyme that requires nicotinamide adenine dinucleotide (NAD) for its activity, is vital in regulating various cellular processes, comprise apoptosis, ageing, energy metabolism, cell cycle regulation, and cell migration[10].

This study aims to evaluate impact of dapagliflozin and Moringa oleifera each alone or in compination on hepatic structure and function in diabetic albino rats, it also emphasizes the prognostic significance of liver-derived microRNAs in progression of metabolic disorders and elucidating associated molecular and metabolic pathways.

MATERIAL AND METHODS

Drugs:

Alloxan monohydrate, acquired from Sigma-Aldrich Chemical Company (St. Louis, MA, USA), was dissolved in a 0.9% NaCl solution and given through a single injection of alloxan intraperitoneally (IP) at a dosage of 140 mg/kg B.W following a fast overnight.

Dapagliflozin: DAPAGLIF 10mg 14 film coated tablets obtained from Future Pharmaceutical Industries for Sanofi Egypt. The tablet was dissolved in 100ml distilled water.

Moringa oleifera acquired from Egyptian Scientific Society for Moringa at National Research Centre, Dokki, Giza. plant material was documented and stored in Department of Horticulture and Crops Technology, under assigned voucher specimen number MO19.

Moringa oleifera Extract preparation (Aqueous Extraction):

Moringa oleifera leaves were arranged in a thin layer and dried in air under shaded conditions, ensuring that direct sunlight was avoided. To maintain their green color and prevent mold formation, leaves were regularly turned until they became crisp to touch. Leaves were carefully processed into a powder utilizing a blender once they had been entirely dried. A portion of 20g of this powdered Moringa oleifera was then mixed with 100 ml of distilled water and allowed to sit undisturbed for 24h. Afterward, mixture was filtered through a white muslin cloth. Remaining material was dried to determine its moisture-free weight, which was subtracted from original 20g to calculate Moringa oleifera quantity that had successfully dissolved in 100 milliliters of distilled water[11].

Animals:

A total of fifty adult male albino rats (Sprague Dawley), aged between 5 to 7 months and weighing between 200 to 250 grams, were obtained from Breeding Animal House at Faculty of Medicine, Zagazig University, Zagazig, Egypt. Rats were reserved in stainless steel cages in ordinary laboratory conditions at room temperature for an acclimation period This experiment was conducted in Anatomy Department ,Benha Faculty of Medicine. Throughout experiment, animals were granted unlimited access to conventional laboratory feed and water. Every experimental procedure followed Institutional Animal Care and Use Committee's recommendations and received approval from Faculty of Medicine, Benha University, Benha, Egypt, number (RC: 25-1-2024).

Experimental Design:

Randomly, rats were separated into 5 groups, with each comprising of 10 animals. assigned drugs were administered once daily over a four-week period.

Group I (Control Group): This group involved 10 rats, further divided into three subgroups:

- Subgroup Ia : Comprised of four animals that was not received any drug.
- Subgroup Ib: Consisted of three animals administered normal saline (0.9%) via intraperitoneal injection.
- Subgroup Ic: comprised of three rats that received distilled water via a gastric tube.

Group II (Non- treated Diabetic Group): This group consisted of 10 rats in which diabetes was induced through a single IP alloxan injection (140mg/kg/BW) in normal saline and rats were maintained on a regular rat chow diet[12].

Group III (Diabetic treated with Dapagliflozin): Ten rats were exposed to type 2 diabetes induction and subsequently treated with dapagliflozin (1mg/kg/day), melted in distilled water, and administered via oral gavage every day for 4 weeks[13].

Group IV (Diabetic treated with Moringa oleifera extract): comprised 10 rats in which T2DM was induced, followed by treatment with Moringa oleifera extract. Rats received Moringa oleifera extract via oral gavage at a dosage of 500mg/kg B.W every day for 4 weeks[14].

Group V (Diabetic treated with Dapagliflozin Plus Moringa oleifera extract): This group also consisted of 10 rats subjected to type 2 diabetes induction. Rats were treated with both dapagliflozin and Moringa oleifera extract at same dosages, time, and period as administered in Groups III and IV.

Experimental diabetes was prompted through a single IP injection of alloxan (140mg/kg B.W), with injection performed slowly. To mitigate initial hypoglycemic effect of Alloxan, a 2ml dose of a 5% glucose solution was administered orally just prior to Alloxan injection. Progress of hyperglycemia in animals was established by measuring fasting blood glucose levels two days' post-injection via an Accu-Chek glucometer (Roche, Germany). Rats demonstrating fasting blood glucose levels of 200mg/dL or greater were indicative of hyperglycemia and involved in this research. Serum glucose levels were recorded[15].

Sample blood & tissue collection:

Animals were required to fast overnight at the end of 4th week. In morning, rats were administered thiopental intraperitoneally 50mg/kg[16]. Abdominal cavity was exposed through a longitudinal incision. Blood samples were obtained through cardiac puncture using a needle and subsequently centrifuged at 600g for 10min to separate components. Liver tissues were dissected carefully for histopathological and biochemical studies.

Molecular markers detection:

RNA Extraction, Reverse Transcription, and Quantitative Analysis of Transcripts Utilizing qRT-PCR:

miRNeasy Serum/Plasma Kit was employed to purify total RNA, including miRNA, from serum samples (Qiagen, USA). Quality and quantity of RNA were determined utilizing a Nanodrop spectrophotometer (USA), with 260/280 nm absorbance ratio (A260/280) indicating purity of RNA. Mature miRNAs were reverse transcribed utilizing miRCURY LNA RT Kit (Qiagen, USA). cDNA generated from this reverse transcription process was promptly utilized for subsequent analysis on same day. For this purpose, miScript SYBR Green PCR Kit (Qiagen, USA) was utilized. complementary DNA (cDNA) was stored at -70°C.

Real-time PCR amplification was conducted utilizing after cycling parameters:

	Temperature	Time	Cycles
PCR initial heat activation	95 °C	2 min	1 cycle
Denaturation	95 °C	10 s	40 cycles
Combined annealing/extension	56 °C	60 s	
Melting curve analysis		60–95 °C	

primer sequences were the following:

MiR-34a, forward, 5′CTTGAACTCCTGGGGCCTGAAG3′ and reverse, 5′GCCAAAGAAACACTCACAGCT3′; and SIRT1, forward, 5′TAGCCTTGTCAGATAAGGAAGGA3′ and reverse, 5′ACAGCTTCACAGTCAACTTTGT3′. SCGN using following primers: Forward primer: TGGACCTGATGACAGCTCCCGGGAACCGAReverse primer: ACTGTGCTGGATAGAGCAAAAGGCAAAGCAGTC

GAPDH forward5-ACCACAGTCCATGCCATCAC-3 and reverse 5-CACCACCCTGTTGCTGTAGCC-30

The relative quantification of miR-34a, SCGN, and SIRT1 expression levels was determined using 2−ΔΔCt method and analysed with ABI Prism 7500 system. data were further processed using Boxplot software, with values normalized against GAPDH as reference gene.

Biochemical parameters evaluation:

Liver enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT)[17].

The methods of Ohkawa *et al*., (1979)[18] and Aebi (1984)[19] were used to measure the levels of serum gluthathione (GSH), along with malondialdehyde (MDA) and (TNF) tumor necrosis factor were quantified following protocols outlined by manufacturer using kits acquired from Spectrum Company, Cairo, Egypt.

Blood glucose levels were evaluated using glucose oxidase-peroxidase technique, utilizing kits (Cat No: 1001191, Spinreact, Spain). Serum insulin levels were measured by INS-ELISA kits (MBS 2024649, Biosource, Belgium)[20].

Calculation of insulin resistance index: A HOMA-IR score more than 2 indicates existence of insulin resistance, while a number below 2 indicates an insulin-sensitive state[21].

Free fatty acids (FFA) & Triglycerides (TGs):

The enzymatic colorimetric assays with commercially accessible Diamond Diagnostics Company kits (Cairo, Egypt) were utilized to determine amounts of Serum FFA and TGs. protocols described by manufacturers were followed[22].

Light microscopic study:

Histological study:

Liver samples were installed in 10% buffered formalin, handled, and inserted in paraffin blocks. These were then cutted up at 5µm thickness thereafter stained with Hematoxylin and Eosin (Hx. & E.) for a standard histology examination. Additionally, Masson Trichrome stain was employed for collagen fibers assessment[23].

Immunohistochemical Analysis: sections were cleaned and deparaffinized by phosphate-buffered saline (PBS). To stop non-specific binding, sections were raised via 10% normal goat serum in PBS. After that, treated with primary antibodies, including Anti-activated caspase-3 polyclonal antibody from rabbits (dilution 1:100) (ab2302; Abcam, Cambridge, Massachusetts, USA) and rabbit polyclonal anti-proliferating cell nuclear antigen (PCNA) antibody (dilution 1:500) (ab152112, Abcam, Cambridge, Massachusetts, USA). Following this, they were raised with biotinylated IgG for 1 h at room temperature, after which a streptavidin-biotin-peroxidase complex was

applied for another hour. Diaminobenzidine (DAB) was used as chromogen, and Mayer's hematoxylin served as counterstain. The identical procedure was followed while processing negative controls, with the exception of using the primary antibody. Positive caspase-3 cells were identified by brown staining in cytoplasm, whereas cells positive for PCNA were characterized by brown staining localized specifically to nucleus[20].

Morphometric Study:

Morphometric analysis was performed by capturing images using a Leica light microscope (DM500, Switzerland) and subsequently analyzing them with "ImageJ" software (version 1.48v, National Institutes of Health, Bethesda, Maryland, USA). For each slide, 10 picked up at random, non-overlapping fields were studied at 400x magnification to estimate average area percentage occupied by collagen fibers, average area percentage showing positive immunohistochemical staining for both active caspase-3, and mean number of PCNA-immunopositive hepatocytes.

Statistical analysis:

All data were recorded and examined utilizing SPSS program, version 25. Qualitative data is displayed as frequency and distribution, whereas quantitative data is displayed as mean±standard deviation (SD). For analytical statistics, different tests were used based on non-normality of data established by Shapiro-Wilk test. Kruskal-Wallis test was utilized to ascertain the statistical significance of ordinal variables comparing more than two research groups. Correlation analysis, using either Spearman's or Pearson's method, determined degree of correlation between two quantitative variables, with correlation coefficient "r" signifying the direction and intensity of connection. Statistical significance was ascertained by a *P*-value of < 0.05., while a *P*-value >0.05 was insignificant[24].

RESULTS

Biochemical findings:

As shown in (Table 1), non- treated Diabetic rats and therapy of diabetic rats with Dapagliflozin (group III) and Moringa (group IV), had significantly elevated mirRNA34a as well as Secretagogin expression and decreased Sirtulin expression in comparison with control. But, administration of Dapagliflozin for diabetic rats in (group III) and Dapagliflozin +Moringa (group V), mirRNA34a and secretagogin expression levels reduced significantly and sirtulin elevated compared to non- treated diabetic rats. mirRNA34a, sirtulin and secretagogin expression were insignificantly different between Moringa -treated diabetic and non-treated diabetic rats as illustrated in (Table 1) .

Marked elevations in serum AST and ALT levels in the non-treated diabetic group compared with controls(P<0.05). However treatment with dapagliflozin or moringa alone significantly reduced AST and ALT levels relative to the diabetic control, however, enzyme levels remained higher than those of the non-diabetic group. Furthermore, the combined dapagliflozin and moringa treatment, significantly lowering both AST and ALT compared with untreated diabetic animals(P<0.05) and achieving values closer to normal controls. However, no significant variations in liver enzyme levels were detected between Treatment of diabetic rats with Moringa and those that remained untreated, as detailed in (Table 2).

Non-treated diabetic animals showed a significant reduction in GSH levels alongside a pronounced increase in MDA and TNF-α. Treatment with dapagliflozin or moringa alone significantly improved these parameters relative to the diabetic control, as evidenced by increased GSH and decreased MDA and TNF-α levels; however, values did not fully return to normal control levels. Notably, the combined treatment with dapagliflozin and moringa produced the greatest protective effect, significantly restoring GSH and markedly reducing MDA and TNF-α compared with the diabetic group and the single-treatment groups, approaching values observed in control, as shown in (Table 3).

Serum glucose levels and HOMA-IR were significantly elevated (p<0.05) in non-treated diabetic rats, as well as in diabetic treated with either Dapagliflozin (group III) or Moringa oleifera (group IV) as opposed to control. But, diabetes treatment with Dapagliflozin only group III or in combination with Moringa oleifera (group V) caused a significant diminish (p<0.05) in serum glucose level in contrast to non-treated diabetic rats. Additionally, combination of Dapagliflozin and Moringa in group V exhibited a marked diminish in HOMA-IR in contrast to untreated diabetic group.

Serum insulin levels were significantly raised (p<0.05) in untreated diabetic rats, as well as in diabetic treated with Dapagliflozin (group III), Moringa oleifera (group IV), or combination of Dapagliflozin and Moringa (group V), compared to controls. Notably, combination treatment with Dapagliflozin and Moringa (group 5) resulted in a significant diminish (p<0.05) in serum insulin levels relative to untreated diabetic rats.

Similarly, free fatty acid (FFA) concentrations were notably elevated (p<0.05) in untreated diabetic rats and the diabetic treated with Dapagliflozin (group III), Moringa (group IV), or Dapagliflozin plus Moringa (group V) in contrast to control. However, treatment of diabetic with combination of Dapagliflozin and Moringa (group V) brought about significant diminish (p<0.05) in FFA concentrations in contrast to untreated diabetic group. Serum TG was significantly higher (p<0.05) in non-treated diabetic group and the diabetic treated with Moringa (group IV) as opposed to control. Although, diabetic rats' therapy by Dapagliflozin (group III), and combination of Dapagliflozin and Moringa in group V led to a significant decline in TG level in contrast to non-treated group as shown in (Table 4).

As demonstrated in (Table 5), mirRNA34a exhibited significant positive correlation with secretagogin, AST, ALT, TNF, MDA, HOMA-IR, serum glucose concentrations and TG while there was significant negative correlation with sirtulin, and GSH. significant negative correlation between sirtulin with secretagogin, AST, ALT, TNF, MDA, HOMA-IR, serum glucose concentrations and TG while it had a significant positive correlation with GSH. Also, secretagogin showed significant positive correlation with AST, ALT, TNF, MDA, HOMA-IR, serum glucose concentrations and TG while it had significant negative correlation with GSH.

Histological examination:

The histological findings of liver sections stained by H&E from control subgroups (Ia, Ib, and Ic) were identical and showed normal hepatic lobule architecture. Central vein appeared normal, with hepatocytes arranged radiating from it. Hepatocytes exhibited acidophilic cytoplasm with single vesicular nuclei. Kupffer cells and normal blood sinusoids were also observed (Figure 1). The portal area seemed normal, containing bile duct, portal vein as well as hepatic artery. The hepatocytes radiating from portal triad, and Kupffer cells as well as blood sinusoids are also seen normal (Figure 2).

In diabetic group, hepatic lobule architecture was disturbed. Central vein appeared congested, and the blood sinusoids are dilated. Hepatocytes exhibited vacuolated cytoplasm, some with dark pyknotic nuclei, and there was infiltration of inflammatory cells (Figure 3). Additionally, hepatocytes displayed vacuolated cytoplasm (Figure 4). The portal area showed a disturbed structure, with portal vein appearing congested and draining dilated blood sinusoids. Hepatocytes exhibited vacuolated cytoplasm and focal areas of inflammatory cell infiltration (Figure 5).

The hepatic sections from diabetic group treated with dapagliflozin showed congested central vein, dilated blood sinusoids, and the hepatocytes displaying Small vacuoles still present and vesicular nuclei. There were few inflammatory cell infiltrations (Figure 6).The portal area seemed normal, containing a normal bile duct, portal vein, and normal hepatocytes. Binucleated hepatocytes, normal blood sinusoids with few inflammatory cell infiltrations were observed (Figure 7).

In diabetic group treated with moringa, central vein had a typical appearance, whereas blood sinusoids were seen to be dilated. Hepatocytes appeared more or less as in control group but few cells still had nuclei that were dark pyknotic (Figure 8). Portal area with congested portal vein, normal bile duct and hepatocytes, some binucleated cell and dilated, congested blood sinusoids with few inflammatory cell infiltrations are seen (Figure 9).

Meanwhile, hepatic sections from diabetic group treated with dapagliflozin plus moringa appeared to be returning to normal hepatic lobule architecture. Central vein appeared normal, with hepatocytes exhibiting acidophilic cytoplasm, single vesicular nuclei. some cells were binucleated, with Kupffer cells and normal blood sinusoids present (Figure 10). The portal area has a normal portal vein, bile duct, hepatic artery, and hepatocytes radiating from portal triad, with binucleated cells and normal blood sinusoids observed (Figure 11).

The histological findings in Masson's trichrome-stained sections from control group showed fine collagen fiber deposition in portal area (Figure 12). Diabetic group showed highly coarse collagen deposition in periportal area (Figure 13). In dapagliflozin-treated and moringa-treated groups, moderate collagen fiber deposition was observed in portal area (Figures 14, 15). Distribution of collagen fibers in dapagliflozin plus moringa-treated group resembled normal distribution of fine collagen fibers seen in control group (Figure 16).

Immunohistochemical examination:

Anti-caspase 3 immunohistochemical staining of hepatic sections from control group presented negative caspase 3 cytoplasmic immunoreactivity (Figure 17). In contrast, diabetic group exhibited a strong and diffuse positive caspase 3 cytoplasmic immunoreaction (Figure 18). Groups III (Figure 19) and IV (Figure 20) showed hepatocytes with moderate diffuse positive caspase 3 cytoplasmic immunoreactivity. However, dapagliflozin plus moringa-treated group showed only a few hepatocytes with positive caspase 3 cytoplasmic immunoreactivity (Figure 21).

The PCNA immune-histochemical stain of liver sections from control group presented few PCNA-positive nuclear immunoreactions (Figure 22). In contrast, diabetic group exhibited strong and diffuse positive PCNA nuclear immunoreactivity in almost all hepatocytes (Figure 23). Groups III (Figure 24) and IV (Figure 25) showed moderate positive PCNA nuclear immunoreactivity in some hepatocytes. However, dapagliflozin plus moringa-treated group showed only a few PCNA-positive nuclear immunoreactions (Figure 26).

Morphometric result:

The morphometric evaluation of area % related to collagen fiber distribution, as well as caspase-3 and PCNA immunostaining (Table 6), demonstrated the following findings:

In contrast to control, diabetic group demonstrated a significant rise ($p<0.05$) in area % of collagen distribution, as well as in caspase 3 and PCNA immunoreactivity.

In both diabetic treated with dapagliflozin group and diabetic treated with moringa oleifera extract group, a significant diminish ($p<0.05$) existed in main area % of collagen distribution, caspase 3 & PCNA immunoreactivity compared to diabetic group. Nevertheless, a noteworthy

rise (p<0.05) existed in average proportion of collagen fiber deposition, as well as increased caspase-3 and PCNA immunoreactivity, in comparison to group that received both dapagliflozin and Moringa oleifera extract. There was no noticeable disparity in average area percentage of collagen distribution, caspase 3, and PCNA immunoreactivity between control group and group treated with dapagliflozin + moringa oleifera extract.

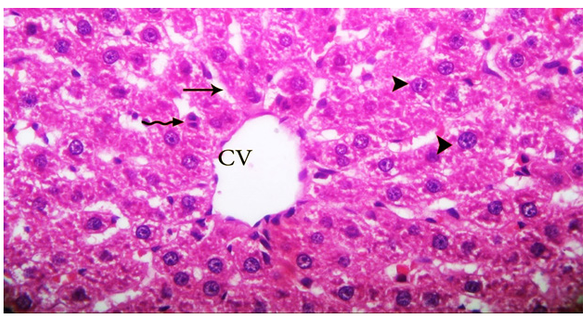

Fig. 1: Photomicrograph of liver section from the control group (I) exhibiting normal hepatic lobule architectures, central vein (CV) appears normal and hepatocytes (arrow heads) have acidophilic cytoplasm and single vesicular nucleus and Kupffer cells (wavy arrow) and normal blood sinusoids (arrow) (H & E stain x400)

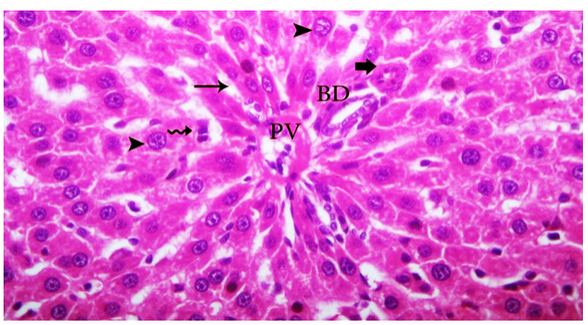

Fig. 2: Photomicrograph of liver section from the control group (I) exhibiting normal portal area that contains normal portal vein (PV), bile duct (BD), hepatic artery (thick arrow) and hepatocytes (arrow heads) radiating from the portal triad, Kupffer cells (wavy arrow) and normal blood sinusoids (arrow) (H & E stain x400)

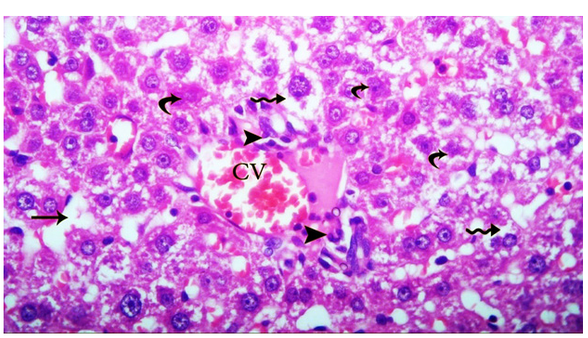

Fig. 3: Photomicrograph of liver section from diabetic group (II) displaying disturbed hepatic lobule architectures, the central vein (CV) appears congested and dilated blood sinusoids (arrow) hepatocytes have vacuolated cytoplasm (wavy arrow) and some have pyknotic nuclei (curved arrow) and inflammatory cell infiltration (head arrow) (H & E stain x400)

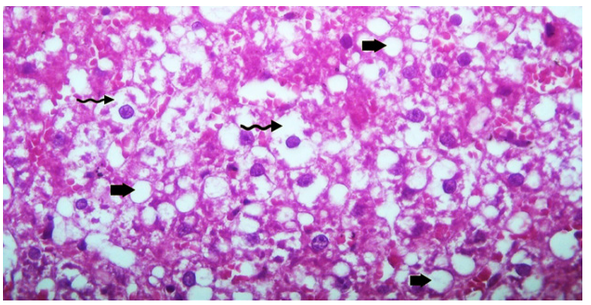

Fig. 4: Photomicrograph of liver section from diabetic group (II) exhibiting vacuolated cytoplasm (wavy arrow) and steatosis (thick arrow) (H & E stain x400)

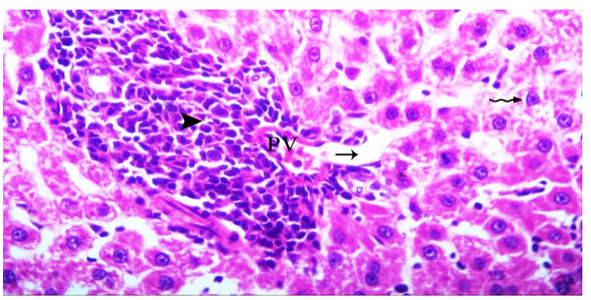

Fig. 5: Photomicrograph of liver section from diabetic group (II) exhibiting portal area with disturbed structure, the portal vein (PV) appears congested and drains dilated blood sinusoids (arrow) hepatocytes have vacuolated cytoplasm (wavy arrow) and focal area of inflammatory cell infiltration (head arrow) (H & E stain x400)

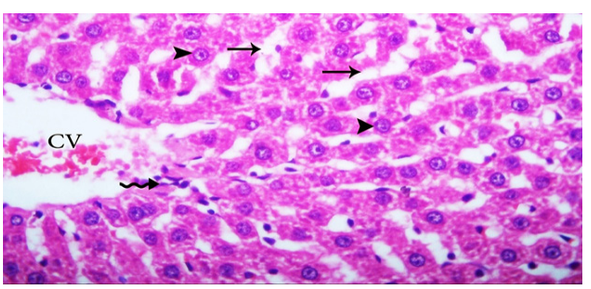

Fig. 6: Photomicrograph of liver section from diabetic group treated with dapagliflozin (III) exhibiting congested central vein (CV) and dilated blood sinusoids (arrow) normal hepatocytes with acidophilic cytoplasm and vesicular nucleus (arrow heads) and few inflammatory cell infiltration (wavy arrow) (H & E stain x400)

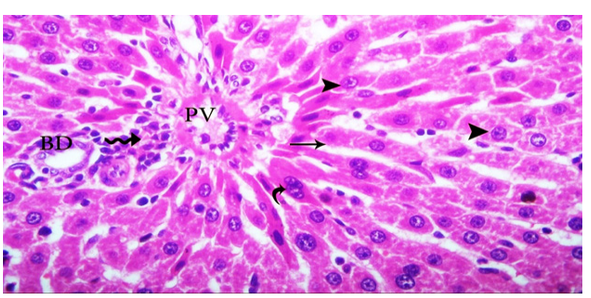

Fig. 7: Photomicrograph of liver section from diabetic group treated with dapagliflozin (III) exhibiting normal portal area with normal portal vein (PV), bile duct (BD) and hepatocytes (arrow heads) with acidophilic cytoplasm and vesicular nucleus, another binucleated cell (curved arrow) and normal blood sinusoids (arrow) with few inflammatory cell infiltration (wavy arrow) (H & E stain x400)

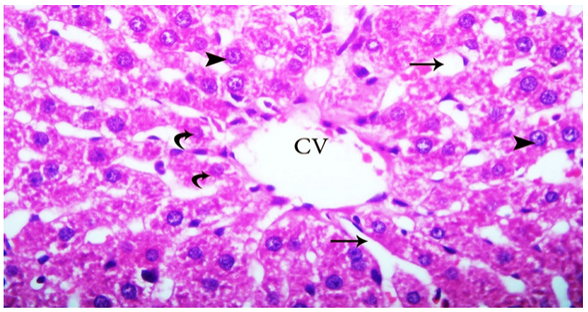

Fig. 8: Photomicrograph of liver section from diabetic group treated with moringa (IV) exhibiting normal central vein (CV) and dilated blood sinusoids (arrows) normal hepatocytes with acidophilic cytoplasm and vesicular nucleus (arrow heads) and few cells with pyknotic nucleus (curved arrow) (H & E stain x400)

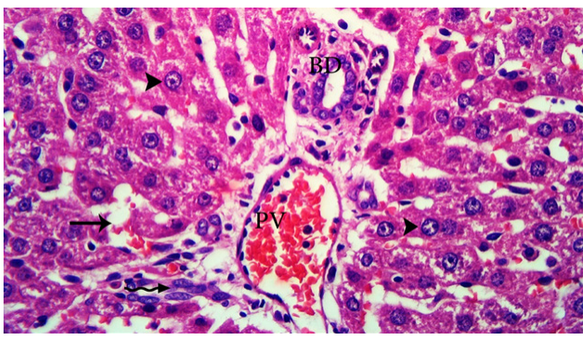

Fig. 9: Photomicrograph of liver section from diabetic group treated with moringa (IV) displaying normal portal area with congested portal vein (PV), normal bile duct (BD) and hepatocytes (arrow heads) with acidophilic cytoplasm and vesicular nucleus and dilated, congested blood sinusoids (arrow) with few inflammatory cell infiltration (wavy arrow) (H & E stain x400)

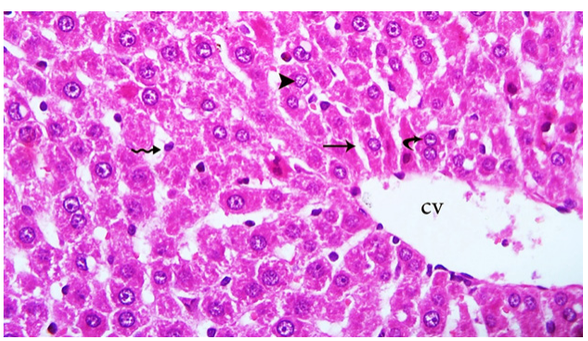

Fig. 10: Photomicrograph of liver section from diabetic group treated with dapagliflozin plus moringa (V) showing normal hepatic lobule architectures, central vein (CV) appears normal and hepatocytes (arrow heads) have acidophilic cytoplasm and single vesicular nucleus, another cell is binucleated (curved arrow) and Kupffer cells (wavy arrow) and normal blood sinusoids (arrow) (H & E stain x400)

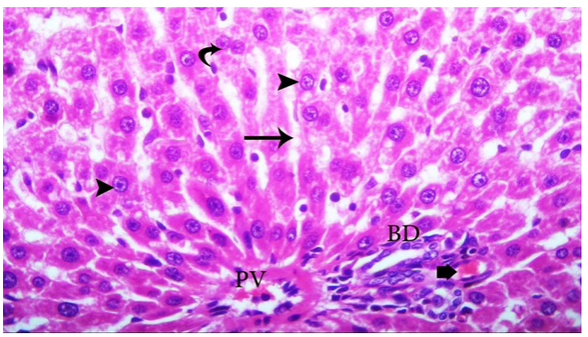

Fig. 11: Photomicrograph of liver section from diabetic group treated with dapagliflozin plus moringa (V) showing normal portal area that contains normal portal vein (PV), bile duct (BD), hepatic artery (thick arrow) and hepatocytes (arrow heads) radiating from the portal triad, binucleated cell (curved arrow) and normal blood sinusoids (arrow) (H & E stain x400)

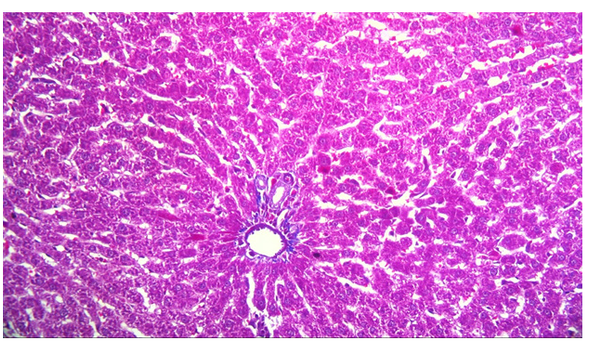

Fig. 12: Photomicrograph in the control group (I) exhibiting fine collagen fibers deposition in the portal area (Masson trichrome stain x 200)

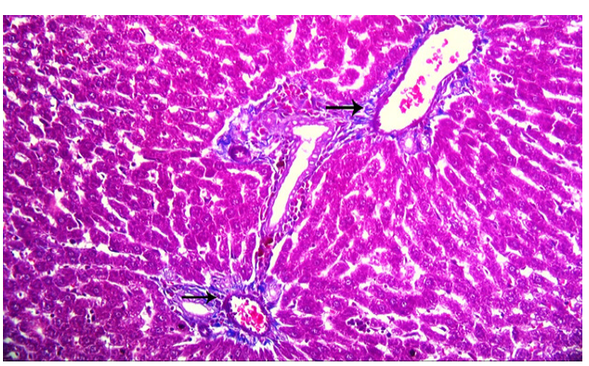

Fig. 13: Photomicrograph in the diabetic group (II) exhibiting highly coarse collagen deposition in the portal area (arrow) (Masson trichrome stain x 200)

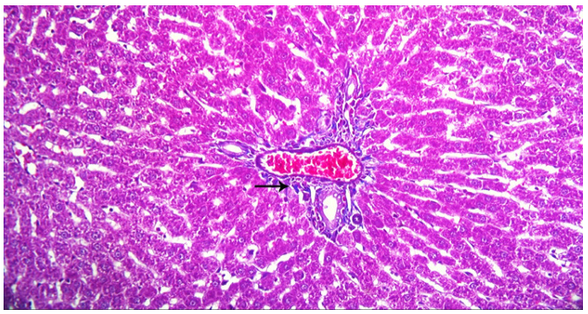

Fig. 14: Photomicrograph in the diabetic group treated with dapagliflozin (III) exhibiting moderate collagen fibers deposition in the portal area (arrow) (Masson trichrome stain x 200)

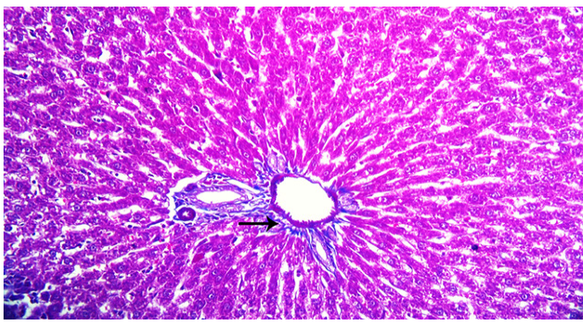

Fig. 15: Photomicrograph in the diabetic group treated with morenga (IV) exhibiting moderate collagen fibers deposition in the portal area (arrow) (Masson trichrome stain x 200)

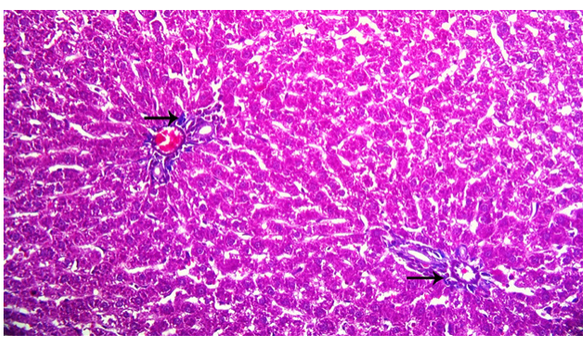

Fig. 16: Photomicrograph in the diabetic group treated with dapagliflozin plus moringa (V) showing fine collagen fibers deposition in the portal area (arrow) (Masson trichrome stain x 200)

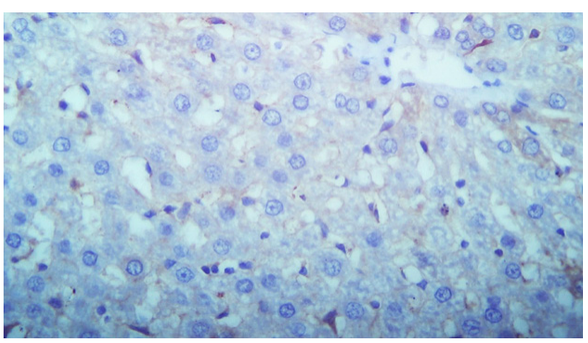

Fig. 17: Photomicrograph in the control group (I) exhibiting negative caspase 3 cytoplasmic immunoreaction. (Caspase 3 immunostain x 400)

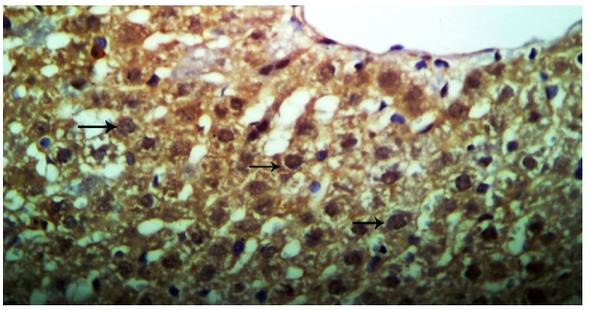

Fig. 18: Photomicrograph in diabetic group (II) showing highly diffuse strong positive (arrow) caspase 3 cytoplasmic immunoreactions. (Caspase 3 immunostain x 400)

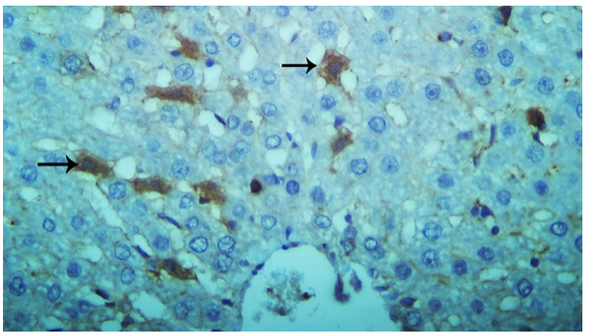

Fig. 19: Photomicrograph in the diabetic group treated with dapagliflozin (III) showing hepatocytes with moderate diffuse positive (arrow) caspase 3 cytoplasmic immunoreactions. (Caspase 3 immunostain x 400)

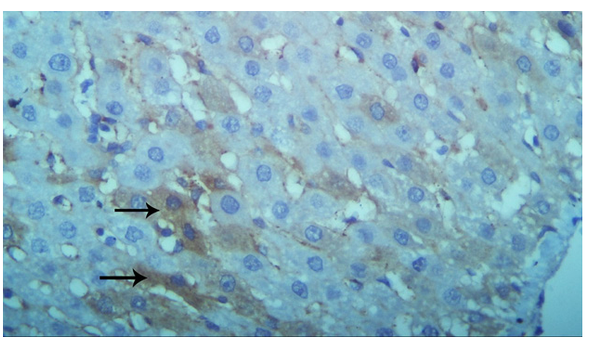

Fig. 20: Photomicrograph in the diabetic group treated with moringa (IV) showing hepatocytes with moderate diffuse positive (arrow) caspase 3 cytoplasmic immunoreactions. (Caspase 3 immunostain x 400)

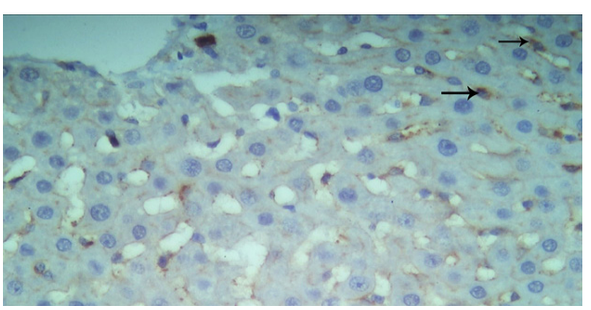

Fig. 21: Photomicrograph in the diabetic group treated with dapagliflozin plus moringa (V) exhibiting few hepatocytes with positive (arrow) caspase 3 cytoplasmic immunoreactions. (Caspase 3 immunostain x 400)

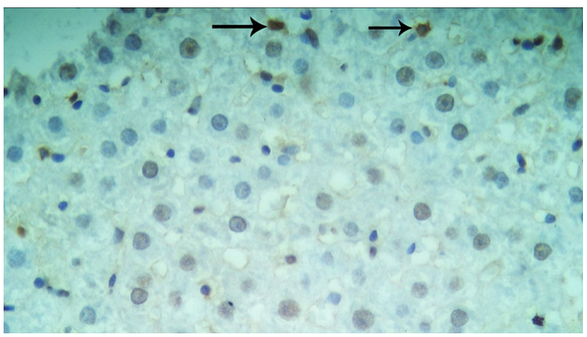

Fig. 22: Photomicrograph in control group (I) exhibiting few PCNA nuclear positive immunoreactions (arrow). (PCNA immunostain x 400)

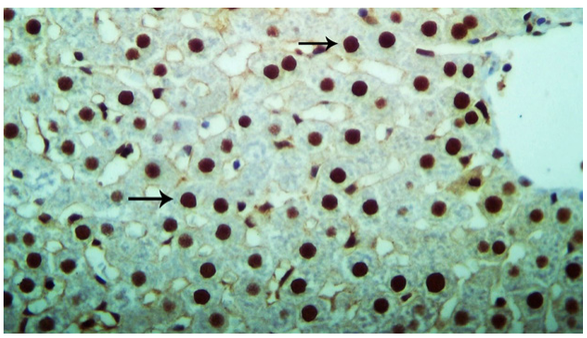

Fig. 23: Photomicrograph in diabetic group (II) exhibiting strong diffuse positive PCNA nuclear immunoreaction in almost all hepatocytes (arrow). (PCNA immunostain x 400)

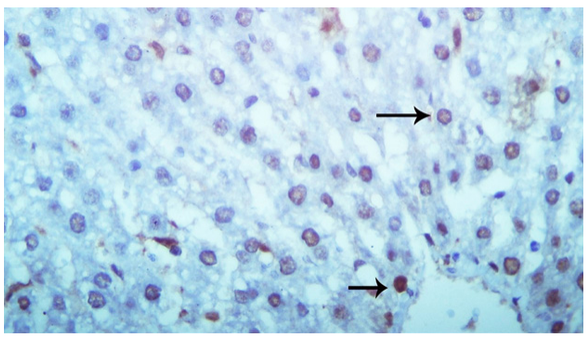

Fig. 24: Photomicrograph in diabetic group treated with dapagliflozin (III) showing moderate positive PCNA nuclear immunoreaction in some hepatocytes (arrow). (PCNA immunostain x 400)

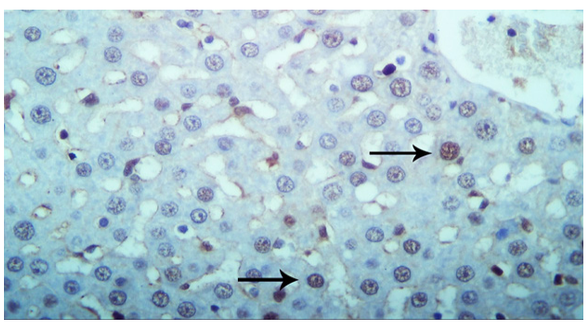

Fig. 25: Photomicrograph in diabetic group treated with moringa (IV) showing moderate positive PCNA nuclear immunoreaction in some hepatocytes (arrow). (PCNA immunostain x 400)

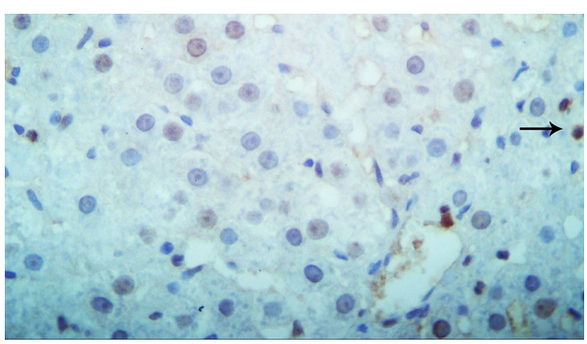

Fig. 26: Photomicrograph in diabetic group treated with dapagliflozin plus moringa (V) showing few PCNA nuclear positive immunoreactions (arrow). (PCNA immunostain x 400)

Table 1: Comparison of mirRNA34a, Sirtulin and Secretagogin expression among groups.

Studied groups	mirRNA34a expression	Sirtulin	Secretagogin
	Mean±SD	Mean±SD	Mean±SD
Group 1: Controls	1±0	1±0	1±0
Group 2: Non- treated Diabetic	9.072±0.622 [a, c]	0.557±0.275	8.32±0.275 [a, c]
Group 3: Diabetic treated with Dapagliflozin	4.483±0.734 [a, b]	2.739±1.095 [b]	4.06±1.095 [b]
Group 4: Diabetic treated with Moringa	6.021±0.898 [a, c]	1.189±0.763 [c]	5.26±0.763 [a, c]
Group 5: Diabetic treated with Dapagliflozin +Moringa	2.281±0.541 [b]	5.119±0.685 [a, b]	1.19±0.685 [b]

SD: standard deviation, [a]*P*<0.05 values are significant as opposed to non-diabetic control (group I); [b]*P*<0.05 values are significant in contrast to diabetic control (group II); [c] *P*<0.05 values are significant as opposed to diabetic treated with Dapagliflozin +Moringa (group V)

Table 2: Comparison of AST & ALT among groups.

Studied groups	AST (IU/l)	ALT (IU/l)
	Mean±SD	Mean±SD
Group 1: Controls	159.9±1.97	45.5±1.43
Group 2: Non- treated Diabetic	400.1±8.62 [a]	85.8±2.39 [a]
Group 3: Diabetic treated with Dapagliflozin	299.2±14.16 [a, b]	57.8±2.66 [a]
Group 4: Diabetic treated with Moringa	316.3±19.64 [a]	61.4±4.70 [a]
Group 5: Diabetic treated with Dapagliflozin +Moringa	271.1±32.05 [b]	52.4±2.46 [b]

SD: standard deviation; [a]*P*<0.05 values are significant as opposed to non-diabetic control (group 1); [b]*P*<0.05 values are significant in contrast to diabetic control (group 2); [c] *P*<0.05 values are significant as opposed to diabetic treated with Dapagliflozin +Moringa (group 5)

Table 3: Comparison of GSH, MDA and TNF among groups.

Studied groups	GSH(mg/g)	MDA(nmol)	TNF (pg/ml)
	Mean± SD	Mean± SD	Mean± SD
Group 1: Controls	53.13±1.71	36.69±1.46	3.1±0.66
Group 2: Non- treated Diabetic	15.66±1.12 [a, c]	100.7±3.47 [a, c]	165.6±8.55 [a]
Group 3: Diabetic treated with Dapagliflozin	34.12±1.63 [a, b]	59.40±2.41 [a, b]	56.4±6.11 [a]
Group 4: Diabetic treated with Moringa	29.47±1.66 [a, c]	65.90±2.85 [a, c]	62.9± 6.54 [a]
Group 5: Diabetic treated with Dapagliflozin +Moringa	42.43±1.66 [b]	46.70±3.89 [b]	27.0±4.29 [b]

SD: standard deviation; [a]*P*<0.05 values are significant as opposed to nondiabetic control (group 1); [b]*P*<0.05 values are significant as opposed to diabetic control (group 2); [c] *P*<0.05 values are significant as opposed to diabetic treated with Dapagliflozin +Moringa (group 5)

Table 4: Comparison of HOMA-IR, serum glucose and serum FFA & TG among groups.

Studied groups	HOMA-IR	serum insulin (uIU/ml)	Serum glucose (mg/dl)	FFA mEQ/l	Serum TG (mg/dl)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Group 1: Controls	0.98±0.01	4.69±0.17	96.8±2.57	0.90±0.10	55.47±1.21
Group 2: Non- treated Diabetic	3.32±0.12 [a, c]	8.67±0.97 [a, c]	193.7±6.29 [a, c]	6.12±0.56 [a, c]	139.5±2.84 [a, c]
Group 3: Diabetic treated with Dopagliflozin	2.50±0.33 [a]	7.37±1.05 [a]	146.0±4.94 [a, b]	4.82±0.48 [a]	63.0±3.46 [b]
Group 4: Diabetic treated with Moringa	3.02±0.45 [a, c]	7.71±0.74 [a, c]	154.4±7.04 [a, c]	5.82±0.27 [a, c]	70.2±3.94 [a, c]
Group 5: Diabetic treated with Dopagliflozin +Moringa	1.24±0.13 [b]	5.11±0.51 [a, b]	125.2±7.38 [b]	2.42±0.56 [b]	56.1±2.28 [b]

SD: standard deviation; [a]*P*<0.05 values are significant as opposed to nondiabetic control (group 1); [b]*P*<0.05 values are significant in contrast to diabetic control (group 2); [c] *P*<0.05 values are significant in contrast to diabetic treated with Dopagliflozin +Moringa (group 5)

Table 5: Correlations of mirRNA34a , Sirtulin and secretagogin with AST, ALT, TNF, MDA, HOMA-IR, serum glucose concentrations and TG

	mirRNA34a	Sirtulin	secretagogin	AST IU/l	ALTIU/l	TNF PG/ml	GSH	MDA	HOMA-IR	serum glucose (mg/dl)	serum TG
mirRNA34a	-	-.479-**	.893**	.872**	.911**	.936**	-.964-**	.949**	.921**	.951**	.932**
Sirtulin		-	-.610-**	-.396-**	-.393-**	-.452-**	.457**	-.457-**	-.403-**	-.466-**	-.524-**
Secretagogin			-	.826**	.900**	.903**	-.894-**	.889**	.894**	.923**	.877**
AST IU/l				-	.859**	.908**	-.894-**	.899**	.877**	.867**	.831**
ALTIU/l					-	.924**	-.921-**	.929**	.898**	.924**	.870**
TNF pg/ml						-	-.959-**	.936**	.924**	.925**	.896**
GSH							-	-.956-**	-.935-**	-.935-**	-.937-**
MDA								-	.930**	.934**	.935**
HOMA-IR									-	.936**	.886**
serum glucose (mg/dl)										-	.909**
serum TG											-

**. Correlation is significant at 0.01 level (2-tailed)

Table 6: Comparison of area % of collagen fiber distribution, caspase 3, and PCNA between all studied groups.

area% of	Control Group	Group II	Group III	Group IV	Group V
Collagen fiber deposition	3.3 ± 1.1	43.3 ± 3.6 $^{a,c,d\ \&\ e}$	14.4 ± 0.8 $^{a,b\ \&\ e}$	16.3 ± 1.4 $^{a,\ b\ \&\ e}$	4.8 ± 2.1 $^{b,c\ \&\ d}$
Caspase 3 immunoreaction	5.31.3 ±	47.2 ± 4.6 $^{a,c,d\ \&\ e}$	17.4 ± 2 $^{a,b\ \&\ e}$	18.4 ± 1.7 $^{a,\ b\ \&\ e}$	7.3 ± 2.6 $^{b,c\ \&\ d}$
PCNA immunoreaction	2.60.5 ±	39.2± 8.6 $^{a,c,d\ \&\ e}$	14.5 ± 0.9 $^{a,b\ \&\ e}$	19.6 ± 0.7 $^{a,\ b\ \&\ e}$	3.6 ± 0.6 $^{b,c\ \&\ d}$

Data displayed as mean±SD, *: significance ≤ 0.05 a: Significance vs Control, b: Significance vs group II, c: Significance vs group III, d: Significance vs group IV, e: Significance vs group V.

DISCUSSION

Diabetes mellitus is a chronic metabolic disease typified by large glucose level in the blood, which can cause substantial destruction to several organs, like liver[2].

Dapagliflozin is an oral drug that specifically inhibits sodium-glucose cotransporter 2 (SGLT2i). It operates by inhibiting the reabsorption of glucose in proximal tubules, causing an excretion of glucose in urine[5].

Multiple research have emphasized strong effectiveness of Moringa oleifera, a plant known for its extensive diversity of pharmacological qualities, for example antioxidant, antidiabetic, antihyperlipidemic and anti-inflammatory impacts[6].

In our study, there was a significant upregulation of miRNA34a and Secretagogin and a downregulation of Sirtulin expression in untreated diabetic rats. Also untreated diabetic rats demonstrated markedly increased serum HOMA-IR, insulin level, glucose, ALT, AST, TNF, TG, FFA and MDA levels, alongside a significant reduction in GSH. These observations corroborate findings reported by Al-Attar *et al*.,[25].

In our research, miRNA34a exhibited a significant positive connection with secretagogin, AST, ALT, TNF, MDA, HOMA-IR, serum glucose concentrations, FFA and TG, while there was a significant negative correlation with Sirtulin and GSH. Additionally, Sirtulin demonstrated a significant negative connection with secretagogin, AST, ALT, TNF, MDA, HOMA-IR, serum glucose concentrations, and TG, but a significant positive connection with GSH. Numerous miRNAs have been characterized as key regulators of hepatic gluconeogenesis. According to Castro *et al*.,[26], a significant positive connection was existed among level of miR-34a expression and extent of hepatic damage. Cermelli *et al*.,[27] noted that while elevated miR-34a levels are not commonly detected in serum of individuals with a healthy liver, such elevations are prevalent in patients diagnosed with NAFLD. Furthermore, Liu *et al*.,[28] documented that increased serum concentrations of miR-34a are indicative of advancing stages of NAFLD and NASH, with these elevations correlating with liver enzyme levels, degree of fibrosis, and activity of inflammation. miR-34a is a liver-specific, lipid-sensitive microRNA that regulates oxidative stress & lipid metabolism.

GSH plays a significant function in regulating hepatocyte cell death. As in reduction of GSH there was a rise in reactive oxygen species (ROS). Additionally, MDA is a critical marker associated to oxidative damage & ROS and a rise in free radicals leads to elevated MDA secretion[29]. MicroRNAs act as vital intermediaries of oxidative stress. miRNAs function as negative controllers of gene expression, as well as modulation of redox signalling pathways[30].

In this study, light microscopic examination revealed alterations in liver of diabetic rats, including disturbed hepatic lobule architecture. central vein appeared congested with dilated blood sinusoids, and hepatocytes exhibited vacuolated cytoplasm, some with pyknotic nuclei, along with inflammatory cell infiltration. The same outcomes were documented by Aboonabi *et al*.,[31].

According to Hazlehurst *et al*.,, in context of DM, hepatic system struggles to effectively secrete triglycerides as VLDL. Consequently, this impairment results in triglyceride accumulation within liver, culminating in steatosis and hepatocellular inflammation. This inflammatory response is exacerbated by free radicals produced from mitochondrial and lysosomal oxidation of fatty acids[32]. Chen and Fang concluded that elevated blood glucose levels initiate a surge in generation of free radicals and ROS, which in turn precipitates oxidative stress & resultant tissue destruction[33].

In our study, diabetic group showed a significant coarse collagen deposition in periportal area in Masson's trichrome-stained sections. In addition, there were widespread, intense positive caspase 3 cytoplasmic immunoreaction, and high level intense PCNA nuclear immunoreactivity in nearly all liver cells.

Al-Attar *et al*., established that an elevated oxidative state facilitates progression of hepatic disorders by triggering apoptosis in hepatocytes, initiating an inflammatory response within liver, and driving fibrogenesis[25]. Yazdi *et al*., provided evidence that diabetes can cause hepatopathies like fat and glycogen buildup and liver fibrosis, and activation of hepatic enzymes[34].

In hepatic fibrosis models, rat liver tissues exhibit upregulation of miR-34a and acetyl-p53, accompanied by a reduction in SIRT1 levels. Notably, SIRT1 activation markedly diminishes miR-34a and acetyl-p53 levels and curtails fibrosis, implicating miR-34a/SIRT1/p53 signalling axis in fibrotic processes. Further in vitro analyses have corroborated role of miR-34a/SIRT1/p53 pathway.

miR-34a modulates cellular growth, differentiation, and metabolism through negative regulation of its target genes, interacting with multiple targets to influence fibrosis through various mechanisms[35].

Deepthi *et al*., elucidated that elevated blood glucose levels precipitate apoptosis, predominantly by enhancing caspase-3 activity within hepatic tissues of rats[36].

Another study stated that increase the glucose level in diabetes resulted in apoptosis and elevating caspase-3 positive activity. Also it was established that hyperglycaemia augmented cytochrome C release, therefore induced apoptosis[37].

In our study after administration of Dapagliflozin and moringa oleifera extract in group III & IV there was non-significant downregulation of miRNA34a and Secretagogin but, there was up-regulation of Sirtulin expression. also decrease in blood glucose, insulin, HOMA-IR levels and FFA, TG and liver enzymes as ALT, AST& indicators of oxidative stress MDA, TNF. Simultaneously, there was rise in antioxidant indicator glutathione GSH level.

Additionally, histological examination of liver tissues from group III & IV revealed moderate improvement in hepatic sections as there were normal hepatocytes but, there was a small number of cells that had pyknotic nuclei, few inflammatory cells infiltrating and congested central vein with dilated blood sinusoids.

According to Tang *et al*.,'s research, dapagliflozin medication for four weeks notably decreased blood glucose levels and raised glucose excretion through urine. Although no alteration in body weight, dapagliflozin therapy was also related with a diminish in indicators of liver inflammation, oxidative stress, and fibrosis, as well as higher hepatic enzyme activity and fat content[38].

The improvement shown in liver function indicators is likely attributed to beneficial effect of Moringa oleifera on structure of hepatic tissue, maybe due to its antioxidant characteristics. This is supported by decreased levels of malondialdehyde (MDA) and nitric oxide (NO), as well as increased amounts of glutathione (GSH) within liver tissues[39].

The diabetic group receiving dapagliflozin and moringa oleifera extract in group III & IV in this research had moderate collagen fiber deposition in the portal region when stained with Masson's trichrome stain. Also, there was moderate diffuse positive caspase 3 cytoplasmic immunoreactivity and moderate positive PCNA nuclear immunoreactivity in some hepatocytes. This was in line with the outcomes of Tang *et al*.,, who exhibited that dapagliflozin treatment may stop the development of hepatic fibrosis by lowering liver inflammation[38].

Berkovich *et al*., stated that hepatoprotective influence of Moringa oleifera might be ascribed to its scavenging of free radicals and down regulation of NF-κB.[40]. Kim *et al*., clarified that Moringa oleifera leaf extract resulted in significant decrease of inflammatory cells infiltration, fibrosis, lipid accumulation, hepatic cells necrosis, hepatocellular deterioration, sinusoidal dilatation, and vesicular congestion[41].

In a related study, Abd Eldaim *et al*., elucidated that aqueous extract of Moringa oleifera effectively preserved structural integrity of hepatic tissues, countering alterations in their histoarchitecture. Furthermore, it modulated gene expression of caspase-3 within liver tissue[42].

Giving of Moringa oleifera to albino rats effectively mitigated detrimental effects of alloxan by reducing occurrence of high blood sugar levels, lipid peroxidation, and caspase-3 gene expression. Additionally, it enhanced activity of antioxidant defence system[43].

In our study administration of Dapagliflozin plus moringa oleifera extract in group V there was significant downregulation of miRNA34a and Secretagogin but, there was a significant upregulation of Sirtulin expression. similarly, there was significant diminish in blood glucose, insulin, HOMA-IR levels and numerous biochemical parameters, as FFA, TG & ALT, AST, indicators of oxidative stress MDA, TNF. Simultaneously, there was a significant elevation in antioxidant indicator glutathione GSH level.

In present study, histological examination of liver tissues from combination of dapagliflozin and Moringa oleifera in group V showed restored normal hepatic lobule architecture. Central vein appeared normal, and hepatocytes exhibited acidophilic cytoplasm with a single vesicular nucleus; some cells were binucleated. Kupffer cells, blood sinusoids, and portal area appeared normal, deposition of collagen fibres in group V resembled normal deposition of fine collagen fibres in control rats. Few hepatocytes showed positive caspase-3 cytoplasmic immunoreactions, and there were also few PCNA nuclear positive immunoreactions.

The protective impact of Moringa oleifera on the liver can be owing to free radical scavenging and the NF-κB down-regulation, which inhibits COX-2 expression, inducible nitric oxide synthase (iNOS), and NO generation[40]. Additionally, dapagliflozin inhibits cell death by activating PI3K/Akt signalling pathway[44].

CONCLUSIONS

Administration of Dapagliflozin with Moringa oleifera in diabetic rats improves liver structure function and inhibit progression of hepatic complications. MicroRNAs may serve as valuable indicators for disease prognosis and as targets for innovative therapeutic strategies.

CONFLICT OF INTERESTS

There are no conflicts of inetrest.

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الملخص العربى

التأثير الوقائي المحتمل للداباجليفلوزين والمورينجا منفردين أو مجتمعين على كبد الجرذان البيضاء المصابة بداء السكري من النوع الثاني: دراسة كيميائية حيوية ونسيجية ومناعية كيميائيه

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الخلفية: يسبب داء السكري من النوع الثاني تأثيرات ضارة على الكبد وذلك بسبب ارتفاع مستويات السكر في الدم والدهون والإجهاد التأكسدي وزيادة حدوث الالتهابات وبالتالي يمكن أن تؤدي هذه العوامل إلى أمراض الكبد المختلفة.
هدف الدراسة: تهدف هذه الدراسة الي تقييم تأثير داء السكري من النوع الثاني على التركيب النسيجي للكبد والخلل الجزيئي والتمثيل الغذائي والدور الوقائي المحتمل للداباجليفلوزين والمورينجا.
المواد والطرق: تم تقسيم خمسين من ذكور الجرذان البيضاء البالغة إلى خمس مجموعات: المجموعة الضابطة, مجموعة مرضى السكري غير المعالجين، مجموعة مرضى السكري الذين عولجوا بالداباجليفلوزين، مجموعة مرضى السكري الذين عولجوا بمستخلص المورينجا أوليفيرا و مجموعة مرضى السكري الذين عولجوا بالداباجليفلوزين بالإضافة إلى مستخلص المورينجا أوليفيرا معا . في نهاية التجربة، تم أخذ عينات من الدم مباشرة من القلب لتقييم المعايير البيوكيميائية و الجينات Sirtulin miRNA، Secretagogin، ٣٤a كما تمت فحص عينات الكبد النسيجية والكيميائية المناعية.
النتائج: داء السكري يسبب ضعف في وظائف الكبد مع اضطراب في البنية النسيجيه الكبد و لقد أظهر اعطاء الداباجليفلوزين والمورينجا كل علي حدا في المجموعة الثالثة والرابعة انخفاضًا غير ملحوظ في مستوى mirRNA٣٤a و سيكرتاجوجين مع تحسن معتدل في النتائج الكيميائية الحيوية والنسيجية . بخلاف ذلك، بعد علاج الجرذان المصابة بداء السكري بواسطة داباجليفلوزين بالإضافة إلى المورينجا في المجموعة الخامسة، انخفضت مستويات كل من mirRNA٣٤a وسكريتاجوجين بشكل ملحوظ وارتفعت مستويات السيرتولين مقارنة بالجرذان المصابة بداء السكري غير المعالجة. يتضح التحسن في مؤشرات وظائف الكبد، وعلامات الإجهاد التأكسدي ومستويات الدهون والجلوكوز، هناك أيضًا تحسن في نتائج التركيب النسيجي في المجموعة الخامسة وانخفاض كبير في نسبه توزيع كل من الياف الكولاجين، والنشاط المناعي السيتوبلازمي الإيجابي للكاسبيز ٣ والنشاط المناعي النووي الإيجابي PCNA بالمقارنة لمجموعة مرضى السكري غير المعالجة.
الخلاصة: ان تناول داباجليفلوزين بالإضافة إلى مستخلص المورينجا أوليفيرا يؤدي إلى تحسين ملحوظ في التغيرات الكبدية الناجمة عن داء السكري من النوع الثاني في الجرذان.