Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Comparative Study of The antidiabetic and Hepatoprotective Effects of Coriander Seed Extract and Garlic Extract in An experimental Diabetic Rat Model

Rania M. Elbatawy, Abdel-Baset I. El-Mashad, Aziza A. Amin, Shawky A. Mostafa and Ahmed A. Tantawy
Department of Pathology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.

Abstract

THE present study evaluates the potential antidiabetic and hepatoprotective effects of coriander seed extract (CSE) and garlic extract (GE) in streptozotocin (STZ)-induced diabetes. Thirty rats were separated into five groups Group 1; rats received citrate buffer orally (0.5 ml/kg). Rats in groups 2,3,4,5 were I.P injected with single dose of streptozotocin (50mg/kg). After 72 hours, rats in groups 3, 4 and 5 were received orally with CSE (250 mg/kg), GE (250 mg/kg) and glibenclamide (0.5 mg/kg). These extracts and glibenclamide were given daily for 28 successive days. Compared to control, diabetic rats showed significant increases in glucose, AST, and ALT values and most of their pancreatic sections revealed hypocellularity, degeneration, and necrosis of islets cells with acinar necrosis. Furthermore, hepatocellular degeneration, necrosis, and apoptosis, focal inflammatory cellular infiltration with portal fibrosis were predominant in the diabetic rats. Compared to control, there was significant decrease in area percentage of antinsulin antibodies in the pancreas with significant increase in caspase-3 positive cells and fibrotic area% in the liver of diabetic rats. CSE and GE therapy attenuated both pancreatic and hepatocellular damages induced by STZ with restoration of glucose and liver enzyme values. Furthermore, these extracts significantly increase the number of immunopositive β-cells and significantly decrease area percentage of caspase-3 positive immunoreaction and Masson's trichrome staining collagen fibers. Both CSE and GE had excellent antidiabetic and hepatoprotective impacts against STZ-induced diabetes probably due to their antiapoptotic and antifibrotic activities; however CSE had more beneficial effects.

Keywords: Diabetes; CSE; GE; Histopathology; Liver.

Introduction

Diabetes mellitus (DM) is a chronic condition that disrupts glucose metabolism, characterized by hyperglycemia due to abnormalities in insulin secretion, action, or both. It can cause a variety of problems, resulting in damage in numerous tissues and a poor quality of life [1].

The liver is integral to primary metabolic processes. It regulates blood glucose levels by balancing glucose intake and storage by glycogenesis with its release via glycogenolysis and gluconeogenesis [2]. The underlying mechanism of hyperglycemia-mediated liver injury consists of increased oxidative damage and an abnormal inflammatory response [3]. Defects in glucose metabolism in addition to oxidative stress in DM result in varying degrees of hepatic cell damage attributed by increased hepatocyte apoptosis and activation of the inflammatory process, which is furthered by necrosis, fibrosis, cirrhosis, and liver failure [4].

Insulin is the most effective treatment for Type I diabetes, effectively lowering blood glucose levels; nevertheless, it has significant side effects and frequently does not mitigate the problems associated with diabetes [5]. Natural antioxidants produced from plants are strong options for inhibiting free radical production and hence avoiding oxidative damage [6]. There were many methods via which natural substances demonstrated their efficacy in lowering blood glucose levels. Among these strategies, raising insulin activity, inhibiting insulinase activity, improving cell mass, and promoting cell regeneration are some of the promising ones [7].

*Corresponding authors: Rania M. Elbatawy, E-mail: Rania.Elbatawy@fvtm.bu.edu.eg Tel.: +20 12 86895668
(Received 05 March 2025, accepted 04 May 2025)
DOI: 10.21608/ejvs.2025.365881.2679

Coriander sativum is a naturally occurring polyphenolic compound with potent antioxidant properties and a potential reservoir of various bioactive constituents, such as flavonoids, polyphenols, vitamin C, and tocopherols, which may contribute to its therapeutic effects [8]. Furthermore, coriander sativum demonstrated antioxidant, antidiabetic, anticonvulsant, anti-inflammatory, antimutagenic, antibacterial, and hepatoprotective activity, among others, in addition to hormone balancing and analgesic characteristics [9].

Garlic (Allium sativum) is a well-known plant with therapeutic potential, having been utilized for both nutritional and medical purposes since ancient times [10]. Several research have proven garlic's hypolipidemic, antiatherosclerotic, anticoagulant, antidiabetic, antihypertensive, antibacterial, anticancer, hepatoprotective, and immunomodulatory effects [11]. Garlic's beneficial impact may be attributed to the presence of various bioactive organosulfur compounds such as diallyl thiosulfonate (allicin), diallyl sulfide (DAS), E/Z-ajoene and S-allyl-cysteine sulfoxide (alliin) [7,12].The positive benefits of garlic on type I and II diabetes have also been observed [13]. Along of its antidiabetic effects, garlic might also be effective in decreasing numerous diabetic complications due to its antioxidant impact via scavenging ROS [14]. Therefore, this investigation was aimed to compare both antidiabetic and hepatoprotective effects of CSE and GE against STZ-induced diabetes through serum biochemical analysis, histopathological,and immunohistochemical examination.

Material and Methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich Company, USA. Glibenclamide (DaoniL)^{R} 5mg was purchased from Sanofi Specialized Pharmaceuticals Company (Cairo, Egypt). All other chemicals were purchased as high analytic grade from commercial suppliers.

Preparation of coriander seed and GEs:

Ethanolic CSE and GE were prepared according to [15].The prepared extracts were kept in refrigerator at -4°C until use.

Induction of diabetes mellitus

Streptozotocin (STZ) was dissolved in 0.1 M citrate buffer at pH 4.5. Induction of diabetes was done by a single intraperitoneal injection of STZ at a dose of 50 mg/kg bw. After 72 hrs from injection, rats with fasting blood glucose higher than 250 mg/dl were declared diabetic [16].

Experimental animals

A total of 30 adult male albino rats weighing (200 ± 20 g) were acquired from the Egyptian Company for Production of Serums, Vaccines, and Drugs, Helwan, through the Experimental Animal House. These rats were kept in stainless steel wire cages, fed free clean water and a balanced commercial ration at all times, and kept in a clean environment with an ambient temperature of 23 ± 3 °C. They were also exposed to a natural 12-hour light/dark cycle every day.

Experimental design

Rats were split at random into five equal groups (N=6 in each). Group 1; control group, rats received citrate buffer orally (0.5 mL/kg) as a vehicle. Rats in groups 2,3,4,5 were I.P injected with STZ at a single dose of 50mg/kg bw [16]. After 72 hours, diabetic rats in the group 3 were received oral CSE (250 mg/kg body weight) [17]. Meanwhile, rats in group 4 were received orally GE (250 mg/kg) [7,18]. Additionally, rats in group 5 were orally given glibenclamide (0.5 mg/kg) [19]. These extracts and glibenclamide were administrated daily for 28 successive days.

In this study, animal care and all the experimental procedures were approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Benha University (Approval Ethical number: BUFVTM 10-11-23).

Biochemical analysis

Following an overnight fast at the end of the experiment, blood samples were drawn from each orbital sinus of each rat into sodium fluoride tubes for glucose determination. Meanwhile, gel and clot activator tubes were used for evaluation of liver function biomarkers. The blood samples were centrifuged for 15 minutes at 3,000 rpm after being allowed to clot at room temperature in order to separate the serum. Until they were utilized, the serum samples were kept at -20 °C. The serum glucose level, AST and ALT activities were assessed by using diagnostic kits purchased from Spectrum Company according to Ghanbari et al.[20] and Reitman and Frankel [21] , respectively. Measurement was performed by using a semi-auto chemistry analyzer.

Histopathological study

During necropsy, small tissue specimens were collected from the pancreas and liver of rats in all groups and immediately fixed in 10% neutral buffered formalin. Then five µm thick paraffin sections were routinely prepared and stained with hematoxylin and eosin and Masson's trichrome stain for assessment of overall liver lesions and the hepatic fibrosis, respectively [22, 23].

Immunohistochemical Examination

The remaining tissue paraffin sections were mounted on positively charged slides for immunohistochemistry detection of insulin and

caspase-3. The slices were deparaffinized in xylene, rehydrated, and treated with a polyclonal guinea pig anti-insulin antibody (dilution (1:100), N1542, Dako, Carpinteria, CA, USA). Caspase-3 activity was evaluated using the Caspase-3/CPP32 Fluorometric Assay Kit (K105), which was bought from Biovision, Inc. (Mountain View, Calif., USA). The reaction in each test was observed using 3,3-diaminobenzidine tetrahydrochloride (DAB) Substrate Kit, Thermo Fischer Scientific, Rockford, IL, USA). The sections were counterstained with Harris hematoxylin [24, 25].

These immunohistochemical and histopathological alterations were evaluated using a light microscope (Eclipse E800, Nikon) and photographed using Olympus camera. For morphometric analysis, the mean area % of Masson's trichrome stained collagen fibers, immunopositive expressions of anti-insulin antibodies, and caspase-3 activation were calculated in five non-overlapping randomly selected fields in five tissue paraffin sections among five rats in each group. The immunoexpression and Masson's trichrome fibrotic area % were analyzed with ImageJ software (ImageJ 1.54g, National Institutes of Health, and USA. Furthermore, the immunopositive β-cells % was counted by counting the number of cell nuclei as reference using the following equation: (number of beta-cells/ total number of islet cell nuclei) × 100[26].

Statistical analysis:

GraphPad Prism software version 9 (La Jolla, CA, USA) was used for statistical analyses. Tukey's multiple comparisons test was used after a one-way ANOVA. The means ± SD were used to express all of the data. Statistical significance was defined as a P value of less than 0.05.

Results

CSE and GE improved serum glucose levels and restored serum liver enzymes in STZ-induced diabetic rats

Serum biochemical analysis of serum glucose, AST, and ALT are illustrated in (Fig. 1). These results indicated that, STZ diabetic rats had significantly higher serum glucose levels than control. Meanwhile, oral administration of CSE, GE, and glibenclamide significantly reduced serum glucose levels compared to STZ group. However, diabetic rats treated with CSE and GE had low serum glucose levels than the glibenclamide-treated diabetic rats as their values closely comparable to those of the control. At the same time, there was indistinguishable difference in the antidiabetic effects of both extract. Furthermore, as compared to the control group, AST and ALT levels were significantly higher in STZ group. Interestingly, serum AST and ALT levels in glibenclamide-treated diabetic rats were nearly similar to those recorded in the diabetic group. Meanwhile, AST and ALT levels were much lower in diabetic rats received CSE and GE than those of diabetic non-treated rats and diabetic rats given glibenclamide. However, the effect of both extract treatments on serum liver enzymes were comparable.

CSE and GE ameliorated the STZ-induced β cell and acinar damage:

The pancreatic sections of rats in the control group exhibited normal pancreatic histoarchitecture, including pancreatic acini, ducts, and islets of Langerhans. The islet of Langerhans appeared as oval or round masses of cells interspersed between the acinar cells. The islets were formed from an aggregation of large spherical eosinophilic cells separated by blood capillaries (Fig.2A). In contrast, the majority of examined pancreatic sections of STZ diabetic rats revealed marked distortion of both the endocrine and exocrine pancreas. In most of these sections, the islets of Langerhans were hypocellular and small in size. In addition, most of these cells were degenerated, and even necrosed (Fig.2B).

Glibenclamide therapy showed moderate improvement in both exocrine and endocrine pancreatic tissue with an increase in islet sizes and number of cells compared to diabetic group but size and number of islets were still less than the control group (Fig. 2C). Meanwhile, almost all examined pancreatic sections of diabetic rats treated with CSE had no evidence of degeneration, or necrosis of islet cells and the majority of these cells were well-preserved in most examined pancreatic sections (Fig. 2D). Similarly, GE treatment attenuated the islet damage, although its impact was less noticeable than that of CSE, where most of the examined sections revealed nearly identical islets with a smaller number of cell than those recorded in CSE treated group (Fig. 2E). Moreover, more degenerated islets cell was detected in some examined sections in this group.

Fig.2F-J illustrates the findings of IHC evaluation of anti-insulin antibodies immunoexpressions in the pancreatic sections. The examined pancreatic sections of rats in control revealed a large number of insulin-positive cells which formed the majority of islet cell population (Fig.2F). Contrary, the diabetic group demonstrated a very low immunoreactive response to anti-insulin with a marked reduction in positive immunostained cell islets compared to the control group (Fig.2G). Treatment with glibenclamide (Fig.2H), CSE (Fig.2I), and GE (Fig.2J) increased the intensity of the brown color and number of immunopositive β-cells in islets compared to diabetic rats.

Semiquantitative analysis of area % of anti-insulin antibody and number of immunopositive β cells were shown in (Fig3. A&B). In pancreas of the STZ group, the area % of anti-insulin antibody

immunoreactivity and number of immunopositive β cells were significantly lower than control. While in glibenclamide treated group there were non-significant increases in the percentage area of anti-insulin antibody immunoreactivity or the number of immunopositive β cells compared to the STZ diabetic group. In comparison to diabetic rats, CSE and GE-treated rats showed significant increases in the area % of anti-insulin antibody immunoexpression and number of immunopositive β cells examined pancreatic sections. Furthermore, the CSE-treated group showed a much higher percentage area of anti-insulin antibody immunoreactivity and the number of positive immunoreactive β cells compared to the GE-treated group.

Regarding the effect of STZ and beneficial impacts of glibenclamide, CSE, and GE on the exocrine portion, it was cleared that, STZ induced severe pancreatic damage in the form of degeneration, necrosis, and dissociation of pancreatic acini (Fig4.A). Furthermore, severe periductal leukocytic cellular infiltration and fibrosis together with dilatation of some ducts with eosinophilic secretions inside these dilated ducts were commonly seen (Fig.4B). Additionally, the pancreatic blood vessels showed hypertrophy and vacuolation of tunica media with perivascular edema (Fig.4C). Thrombosis of some pancreatic vessels was occasionally observed. Glibenclamide therapy reduced the STZ-induced pancreatic acinar damage with minimal acinar degeneration and necrosis. In addition, congestion, ductal dilatation, periductal leukocytic cellular infiltration, and fibrosis were frequently observed in a few examined pancreatic sections (Fig.4D). Noticeably, both CSE and GE markedly reduced the STZ-induced acinar damage where almost all the pancreatic acini were well preserved and mild dilatation of pancreatic ducts with eosinophilic secretion was infrequently observed (Fig.4E, F).

CSE and GE alleviated the hepatic injury associated with STZ-induced diabetes

The examined liver of rats in the control group had normal liver parenchymal histoarchitecture. Typically, polyhedral hepatocytes were organized in cords separated by sinusoids and encircling central veins (Fig.5A). These hepatocytes had eosinophilic cytoplasm and centrally located vesicular nuclei. In contrast, most of the examined hepatic sections from the STZ group revealed severe hepatic disruption evidenced by hepatocellular dissociation and apoptosis (Fig.5B). Multifocal hepatic cell degeneration and necrosis were also prominent (Fig.5C). Furthermore, there was widespread mononuclear cellular infiltration of the hepatic parenchyma, particularly in-between degenerated and necrotic liver cells, portal areas, as well as inside the hepatic sinusoids (Fig.5D&E). Additionally, the examined portal areas showed portal fibrosis, mononuclear inflammatory cellular infiltration, and bile ductal hyperplasia, occasionally with severe congestion of portal veins and formation of new bile ductules (Fig.5F). The examined liver sections of the glibenclamide-treated group revealed minor attenuation of STZ induced hepatic cell damage. In this group, severe congestion with mild multifocal degeneration and necrosis of some hepatic cells were commonly observed (Fig. 6A). On the other hand, glibenclamide therapy considerably minimized portal inflammation, where a few inflammatory cellular infiltrations with mild periductal fibrosis along with hyperplasia and cystic dilatation of some bile ducts were occasionally observed (Fig.6B). Meanwhile, the examined liver sections of diabetic rats treated with CSE had no evidence of inflammatory response, hepatic degeneration, or apoptosis, and the majority of hepatic cells were well-preserved. However, minimal hepatic cell degeneration and necrosis surrounding the central veins were recorded in a few examined livers (Fig.6C). Additionally, CSE markedly ameliorated the STZ-induced portal fibrosis and inflammation where the majority of the portal tracts were intact and exhibited normal bile ducts and portal vessels, with a few inflammatory cells aggregating in some examined sections (Fig.6D). Similarly, GE treatment attenuated STZ induced liver cell damage. However, its effect was less pronounced than that of CSE, where minor hepatic cellular degeneration and mononuclear inflammatory cells were infrequently detected (Fig.6E). Furthermore, the examined portal tracts showed less protective efficacy of GE than CSE, where occasional portal congestion with moderate inflammatory cellular infiltration, bile ductal hyperplasia, and periductal fibrosis were seen (Fig.6F).

CSE and GE attenuated hepatic apoptosis in STZ-diabetic rats

Figure 7 demonstrates the results of IHC analysis of caspase-3 immunoexpression in hepatic sections. In the control group the examined liver sections exhibited a minimum immunoexpression localized to the walls of the hepatic sinusoids, as well as a few number of hepatic cells (Fig.7A). Compared to control, the diabetic group had a very high immunoreactive response to caspase3 with a marked rise in positive immunostained cells (Fig. 7B). Treatment with glibenclamide (Fig.7C), CSE (Fig.7D), and GE (Fig.7E) decrease the intensity of the brown color and the number of caspase-3 immuno-positive hepatocytes in comparison to diabetic rats. However, the examined sections of the liver in CSE and GE groups indicated weaker caspase-3 immunoreaction than in the glibenclamide-treated group.

Furthermore, semiquantitative analysis (Fig.7F) revealed that, area % of caspase-3 immunoreactivity in liver of STZ group was significantly higher than

control. Compared to diabetic group, CSE, glibenclamide, and GE-treated groups showed significant decreases in area% of caspase-3 immunoexpression. However, the CSE-treated group had a much lower percentage area of positive caspase-3 immunoreactive hepatic cells than the GE and glibenclamide-treated group. At the same time, the GE-treated group had a much lower area% immunoexpression than the glibenclamide-treated group.

CSE and GE alleviated hepatic fibrosis in STZ-diabetic rats.

The anti-fibrotic effects of CSE and GE on STZ-induced hepatic fibrosis in diabetic rats were assessed using Masson's trichrome stain. In control, only thin strands of bluish-stained collagen fibers were seen surrounding central veins and within portal areas surrounding the bile ducts (Fig.8A). In contrast, STZ-diabetic rats displayed multifocal densely packed bluish-stained collagen fibers in the portal areas, primarily surrounding congested portal veins and bile ducts (Fig.8B). Compared to diabetic group, the glibenclamide, CSE, and GE-treated groups revealed a decrease in bluish-stained collagen fibers (Fig.8C, D, and E). However, the proliferation of fibrotic tissue induced by STZ was markedly attenuated by treating rats with CSE, and GE compared to the glibenclamide-treated group.

Semi-quantitative evaluation of hepatic fibrosis in STZ diabetic non-treated rats as well as diabetic rats treated with glibenclamide, CSE, and GE were illustrated in (Fig.8F). The percentage areas of fibrotic tissue induced by STZ in the liver of the diabetic rats were significantly higher than those in liver of control. Compared to STZ diabetic rats, diabetic rats treated with glibenclamide showed a significant decrease in the area % of collagen fibers stained with Masson's trichrome in the liver. Furthermore, there was a significant decrease in area percentage of liver fibrosis in CSE and GE compared to diabetic group. CSE-treated group had a significantly decreased percentage area of Masson's trichrome staining of liver fibrosis than the GE and glibenclamide-treated group. At the same time, the area percentage of liver fibrosis in the GE-treated group was significantly decreased than glibenclamide-treated group.

Discussion

The overall number of diabetic patients worldwide is predicted to increase from 171 million in 2000 to 366 million by 2030, making it a severe health issue [27]. The pancreas and liver are important organs for maintaining blood glucose levels. Diabetes causes considerable alterations in both pancreas and liver, resulting in impaired glucose homeostasis and metabolism [28].

Diabetes caused chronic and persistent hyperglycemia in experimental animal models, which led to severe oxidative stress, impairing the antioxidative defense system's performance and encouraging the production of new free radicals [14]. STZ exerts powerful toxic effects on islet β cells as a result of oxidative stress resulting in dysfunction of the pancreatic β cell [29,30]. In this work, STZ-induced hyperglycemia with substantial pathological changes in the islets of Langerhans, including hypocellularity, degeneration, and necrosis. These alterations were verified by immunohistochemistry results, which revealed a very low immunoreactive response to anti-insulin, as well as a significant reduction in area % of the positive immunostaining β cells, indicating insufficient insulin synthesis. These findings were agreed with Metawea et al.[31]. In the current investigation, daily glibenclamide, CSE and GE therapy significantly lowered glucose levels and reduced pancreatic damage induced by STZ. However, the CSE and GE therapy lead to significant decreases in blood glucose levels than the glibenclamide-treated group. These findings were consistent with Moharib and Adly[32] and Eidi et al. [15]. Anti-hyperglycemic and protective effects of CSE against STZ islet damage could be related to its polyphenol and flavonoid antioxidant substances [33]. Furthermore, CSE inhibits lipid peroxidation and activates antioxidant enzymes, which minimize islet damage associated with diabetes [32]. Similarly, the antidiabetic benefits of GE were linked to sulfur compounds or allicin-type compounds [34]. The pancreatic protective effect of CSE, GE, and glibenclamide were validated by histopathological and immunohistochemical examination, with almost all islets of Langerhans and pancreatic acini appearing nearly identical to the control group, with only mild acinar degeneration and necrosis in GE treated group. The immunohistochemical study of anti-insulin antibodies supported these findings, where there was a significant increase in anti-insulin antibody area percentage and immunopositive β-cells in these groups compared to diabetic group. These finding was supported by Xie et al. [12]. However, the examined sections of the pancreas in the CSE and GE groups indicated stronger anti-insulin expression than in the glibenclamide-treated group. Diabetes affects protein, carbohydrate, and lipid metabolism and causes liver lesions such as fatty liver disease, steato-hepatitis, fibrosis, and cirrhosis [35]. In the current investigation, hyperglycemia induced by STZ was linked with hepatocellular degeneration, necrosis, and apoptosis. The apoptotic effect was validated by caspase-3 immunostaining, which revealed that the liver sections in the diabetic group had a very high immunoreactive response to caspase-3, with a significant rise in area % of positive immunostaining cells, indicating an increase in

apoptotic hepatic cell numbers. This diabetic hepatopathy was supported byAlqahtani et al.[35]who mentioned that hyperglycemia triggered apoptosis through a considerable increase in hydroxyl radical generation, which was connected with lipid peroxidation (LPO) levels. Furthermore, chronic persistent hyperglycemia lowers antioxidant defense system function, resulting in the generation of free radicals. These increased free radical levels, with natural antioxidant system failure, often result in hepatocellular death [35].These recorded histopathological and immunohistochemical hepatic alterations in the present work were supported by serum biochemical examination, which revealed that diabetic rats had significantly higher levels of liver enzymes (AST, ALT) than the control. These increases result from the release of these enzymes from injured liver cells [36]. In addition, the examined liver of diabetic rats exhibited multiple areas of portal fibrosis confirmed by the Masson's trichrome stain, which revealed abundant bluish collagen fibers in the portal tracts, with an increase in the fibrotic area%. This hepatic fibrosis has been linked to hyperglycemia [37].

In the present work, CSE and GE treatment demonstrated outstanding hepatoprotective effect with antiapoptotic and antifibrotic activitie. Almost all examined liver of diabetic rats received CSE and GE revealed no evidence of inflammatory response, infrequent hepatic necrosis with minimal hepatic cell degeneration and apoptosis particularly around the central veins in a few of the examined livers. The anti-apoptotic impact of CSE and GE was validated by immunohistochemistry results, which showed a substantial decrease in the area % of caspase-3 immuno-positive hepatocytes compared to diabetic rats. However, CSE-treated group had a much lower percentage area of positive immunoreactive hepatic cells than the GE. These result was recorded by Naveen and Khanum[38]. This improvement in the microscopic hepatic picture was reflected in the results of serum biochemical analysis, where ALT and AST values in CSE and GE-treated groups were nearly identical to those recorded in the control. This protective effect of CSE could be explained by linalool, the principal component of CSE, which possesses antioxidant characteristics that are commonly used to treat complications related to oxidative damage in DM [39]. Similarly, the protective effect of GE could be attributed to numerous active phenolic compounds that play a significant role in induced oxidative stress, inflammatory responses, and apoptotic effects of STZ [33]. In addition, CSE and GE therapy successfully reduced liver fibrosis progression, as evidenced by microscopic examination of the liver sections which showed mild periductal fibrosis and a significant reduction in the percentage of positive Masson's trichrome stained fibrotic areas. However, the CSE-treated group had a significantly decreased percentage area of Masson's trichrome staining of liver fibrosis than the GE and glibenclamide-treated group. At the same time, the area percentage of liver fibrosis in the GE-treated group significantly decreased more than the glibenclamide-treated group. This regression of liver fibrosis were described by D'Argenio et al. [40] who assess the potential effect of GE in regression of liver fibrosis through inhibiting tissue transglutaminase (tTG) and reducing myofibroblasts.

Conclusion

Both CSE and GE demonstrated outstanding antidiabetic effects and hepatoprotective efficacy against hepatic damage linked to STZ-induced diabetes in a rat model. Nevertheless, the total protective benefits offered by CSE exceeded those of GE. These benefits might be explained by anti-hyperglycemic, antiapoptotic, and antifibrotic activities of these extracts as well as their antioxidant contents.

Acknowledgments

The authors are thankful to Faculty of Veterinary Medicine, Benha University for completion this work.

Funding statement

The authors did not get any funds for their work.

Declaration of Conflict of Interest

The authors state that there is no conflict of interests.

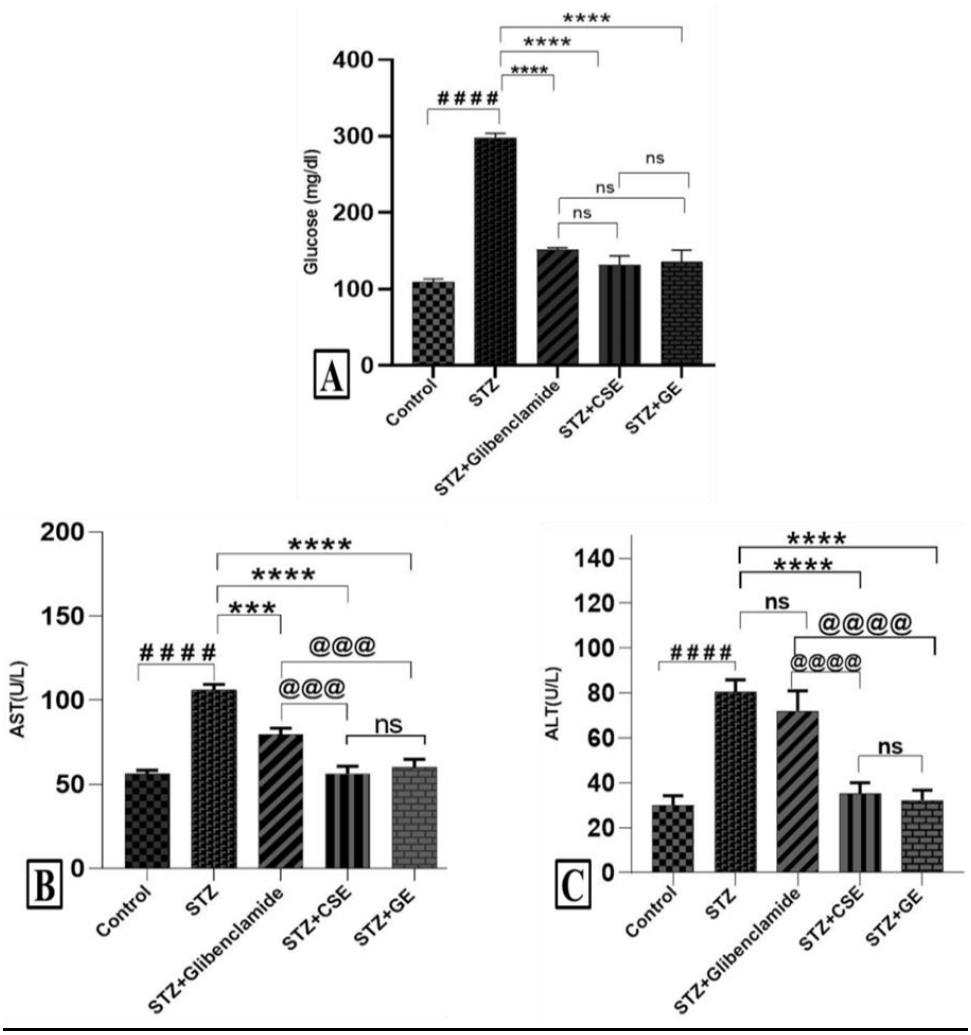

Fig.1. Effect of STZ, CSE, GE, and glibenclamide on glucose (A), AST(B), and ALT(C) activities. $^{\#\#\#\#}$p<0.0001 when compared to control. ****p<0.0001,***p <0.001when compared to STZ group. $^{@@@@}$p<0.0001, $^{@@@}$ p <0.001 when compared to glibenclamide-treated group. ns p non-significant. Data are expressed as means ± SD.

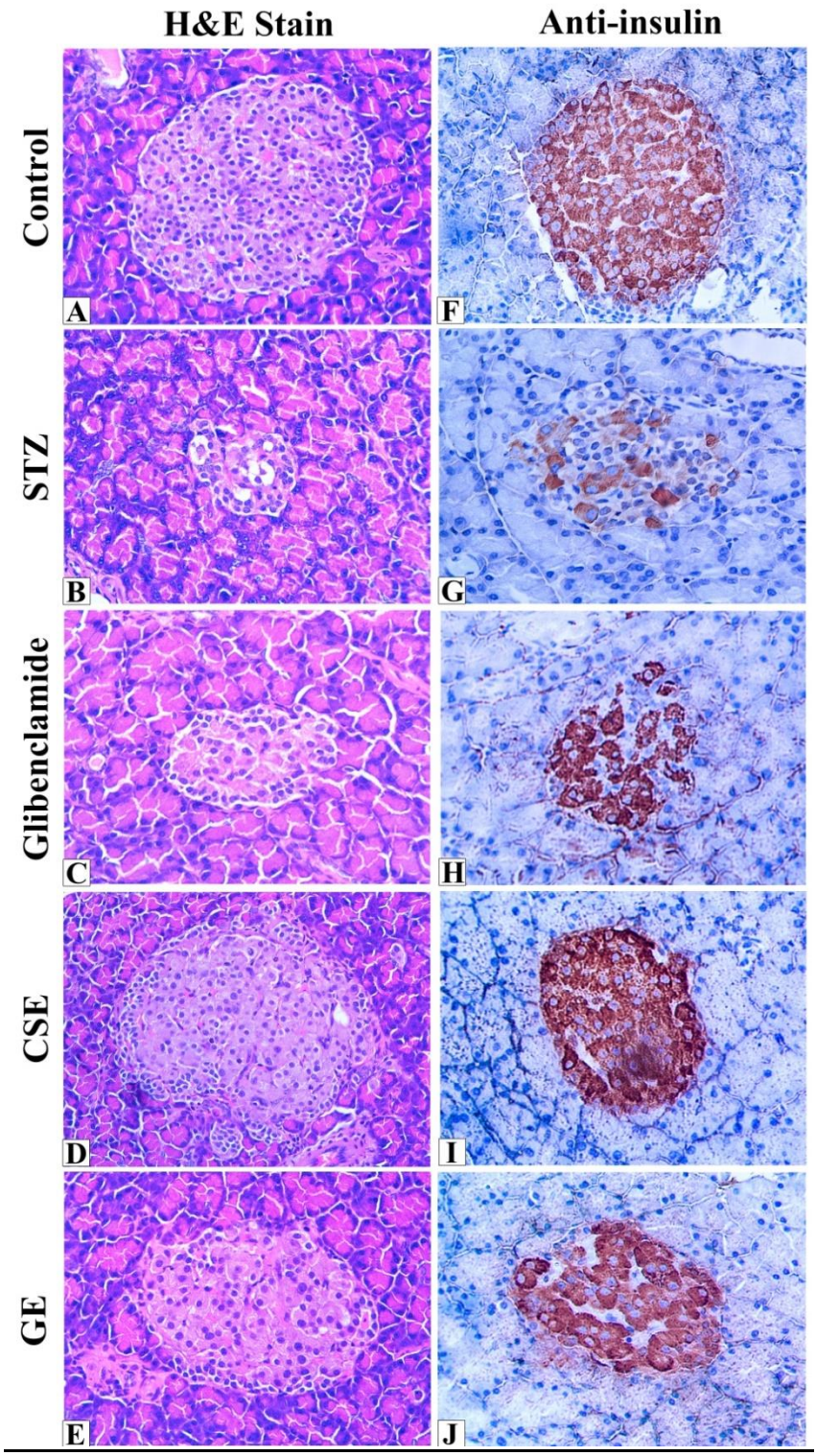

Fig.2. Representative photomicrographs of H&E (A-E) and immunohistochemical (F-J) stained pancreatic sections ; (A) Control group showing a regular, well-defined, pale, rounded mass of islets of Langerhans separated by capillaries in-between typical pancreatic acini, (B) Diabetic group revealing hypocellularity with degenerated and necrotic islets cells, (C) Glibenclamide-treated group showing small sized mass of islets of Langerhans, (D) CSE-treated group revealing a large cluster of typical islet cells similar to the control group surrounded by normal pancreatic acini, (E) GE-treated group revealing nearly identical islets, (F) Control group showing a very high immunoreactive response of β cells to anti-insulin, STZ diabetic group (G), revealing a very low immunoreactive response to anti-insulin within the islets of Langerhans, Glibenclamide-treated groups (H), displaying moderate immunopositive β cells. CSE-treated groups (I), showing marked immunopositive to anti-insulin antibodies in the majority of β cells, GE-treated groups (J) showing immunoreactivity similar to CSE but with less positive β cells. X 200

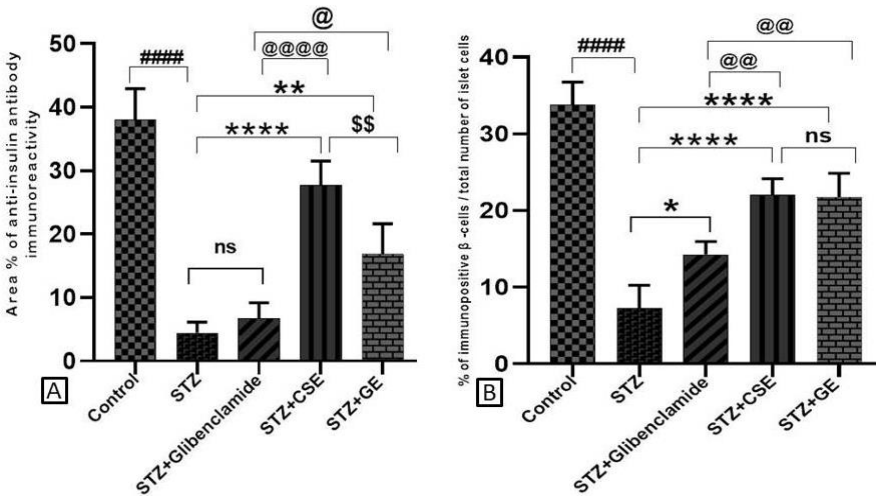

Fig. 3: Morphometric measurements in different experimental groups showing changes in the mean values of: A. Area % of anti-insulin immunoreactivity ; B. Percentage of β -cells per total number of islet cells, $^{####}$p<0.0001 when compared to control. ****p<0.0001,p <0.01,*p <0.05 when compared to STZ group. $^{@@@@}$p<0.0001, $^{@@}$p<0.01, $^{@}$p <0.05 when compared to glibenclamide-treated group. $^{\$\$}$p< 0.01 when compared to GE-treated group, nsP non-significant. Data are expressed as means ± SD.**

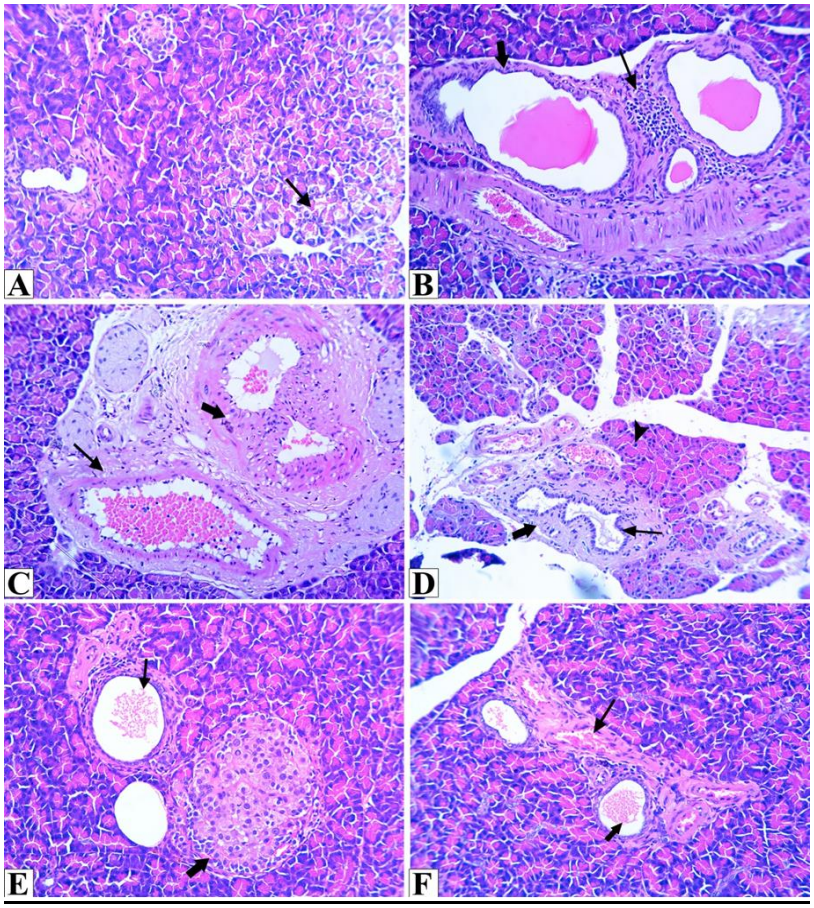

Fig.4. Representative photomicrographs of pancreatic sections in all experimental groups. STZ group (A-C) showing (A), severe degeneration and necrosis of acinar cells (arrow), (B) cystic dilatation of the pancreatic ducts (thick arrow) , focal hyperplasia in the lining epithelium of pancreatic duct with periductal fibrosis and inflammatory cellular infiltration (thin arrow), (C) congestion and perivascular edema (thin arrow) with hypertrophy and vacuolation of tunica media of pancreatic blood vessels (thick arrow) (D) Glibenclamide-treated group revealing pyknotic nuclei of the acinar cells (arrow head) with congestion and moderate pancreatic duct dilatation (thin arrow) with periductal fibrosis (thick arrow) (E) CSE-treated group displaying a large cluster of nearly identical islet cells in between intact pancreatic acini (thick arrow) with mild dilatation of pancreatic ducts containing little eosinophilic debris (thin arrow), (F) GE-treated group showing congestion of pancreatic blood vessels (thin arrow) with eosinophilic debris inside the lumen of dilated ducts (thick arrow). H&E stain X 200.

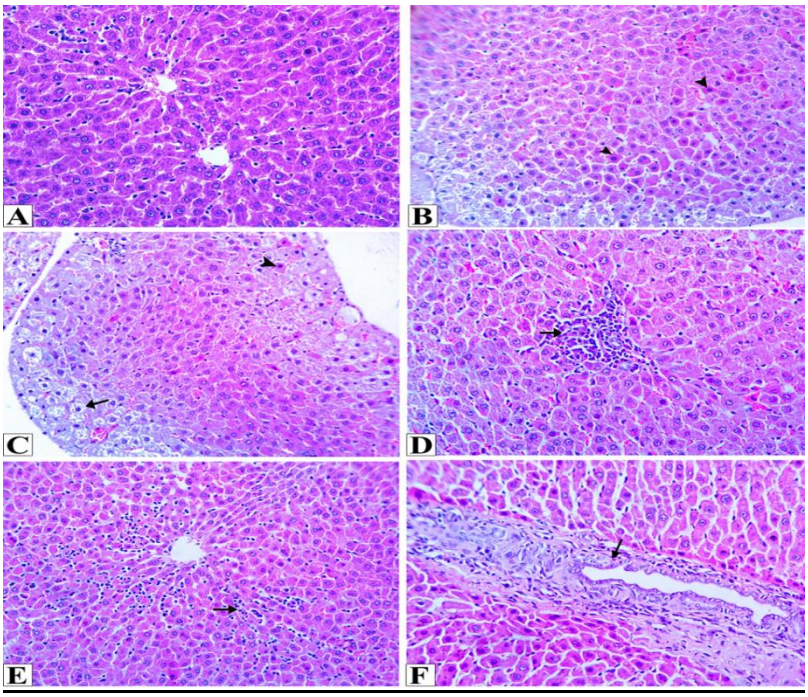

Fig. 5. Representative photomicrographs of liver sections in control and STZ groups. (A) The control group showing typically hexagonal hepatocytes organized in cords separated by sinusoids and encircling central veins. STZ group (B-F) showing (B) hepatocellular dissociation and apoptosis (arrow head), (C) severe degeneration (thin arrow) and necrosis of the hepatic cells (arrow head), (D) mononuclear cell infiltration in the portal area (thin arrow), and hepatic sinusoids (E, thin arrow), (F) portal fibrosis with mononuclear inflammatory cell aggregation, and marked bile ductal hyperplasia (thin arrow). H&E stain X200.

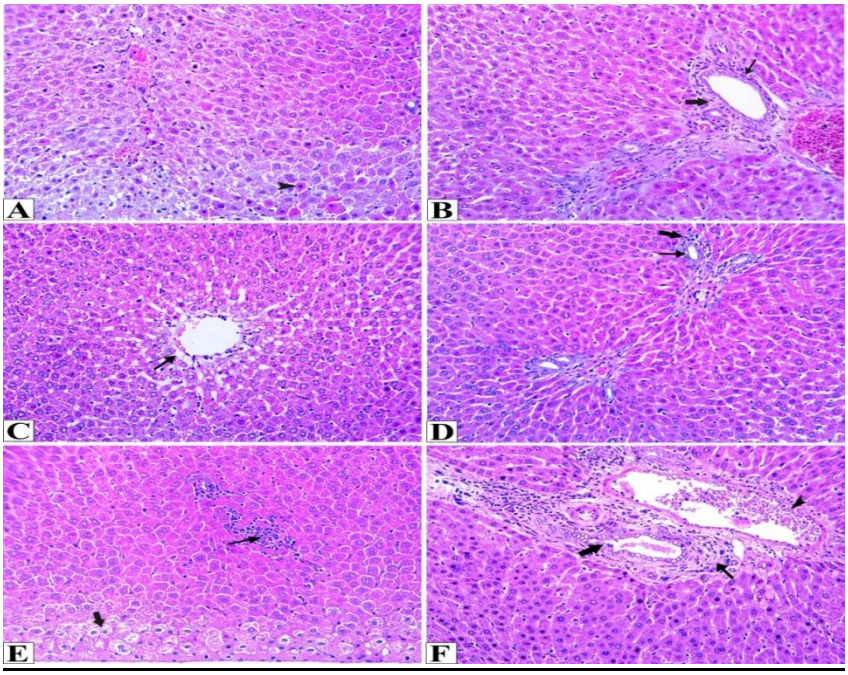

Fig.6. Representative photomicrographs of the liver sections of diabetic-treated rats (A-F). Glibenclamide-treated group (A&B) showing, (A) congestion of blood sinusoids with degeneration and apoptosis of hepatic cells (arrow head), (B) mild hyperplasia (thin arrow), and cystic dilatation of the bile ducts with a few inflammatory cellular infiltration and mild periductal fibrosis (thick arrow).The CSE-treated group (C&D) displaying, (C) well-preserved hepatic cells with minimal hepatic cell degeneration and necrosis around the central vein (arrow), (D) a very few inflammatory cell infiltration in the intact portal area (thick arrow) with typical bile ducts (thin arrow). GE-treated group (E&F) showing, (E) hepatic cellular degeneration (thick arrow) and mononuclear inflammatory cell aggregation (thin arrow), (F) congestion of portal vein (arrow head), inflammatory cellular infiltration (asterisk) with bile ductal hyperplasia (thick arrow) and periductal fibrosis (thin arrow), H&E stain X200.

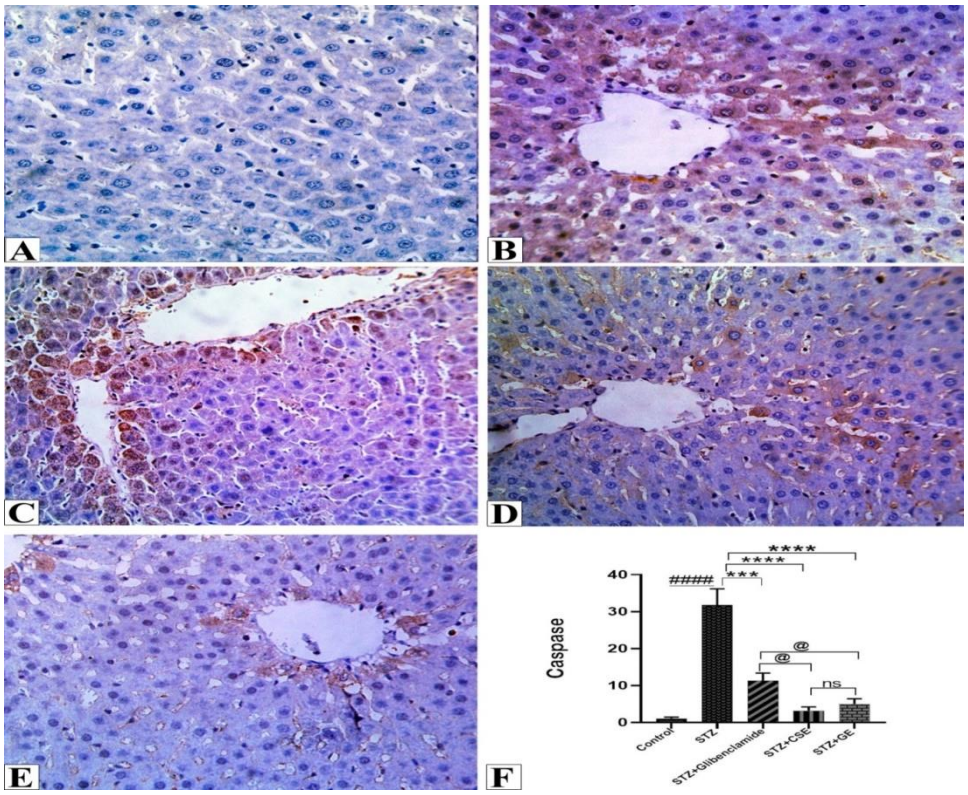

Fig.7. Representative photomicrographs showing the immunohistochemical expression of caspase-3 in the hepatic tissues of rats x200, (A) Control group exhibiting a minimum immunoexpression in the walls of hepatic sinusoids. (B) STZ diabetic rat revealing a very high immunoreactive response to caspase-3 with a marked increase in positive immunostained cells. Treatment with glibenclamide (C), CSE (D), and GE (E) displaying marked decreases in the intensity of the brown color and the number of immuno-positive hepatocytes. (F) Area % of caspase-3 immunoreactivity in different experimental groups, $^{\#\#\#\#}$p<0.0001 when compared to control. ****p<0.0001, ***p<0.001,$^{@}$p<0.05 compared to STZ group, ns P non-significant. Data are expressed as means ± SD.

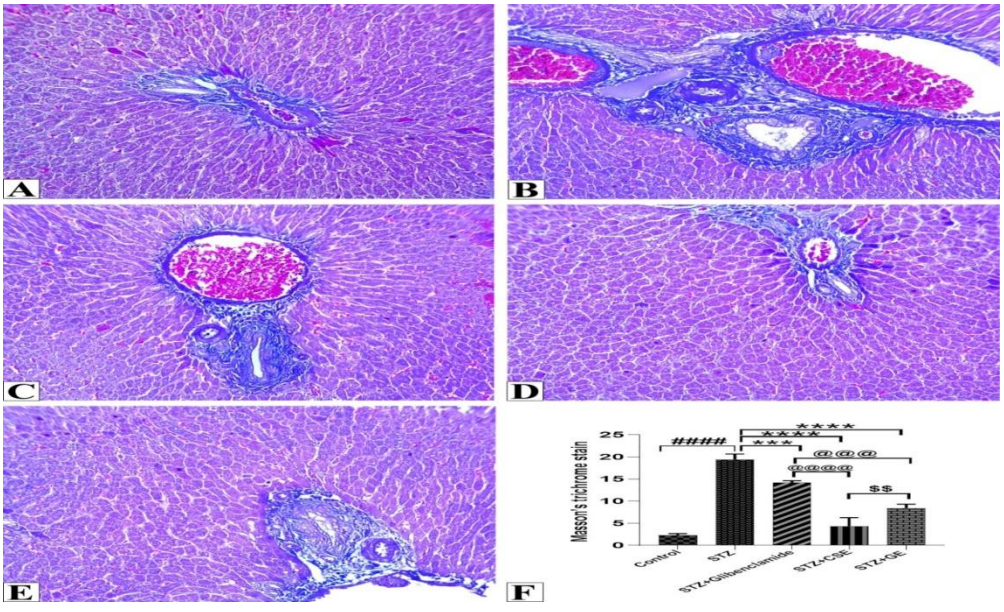

Fig.8. Representative photomicrographs of Masson's trichrome stained liver sections X200 (A) control group revealing thin bluish strands of collagen fibers in portal area. STZ diabetic rats showing (B), positive Masson's trichrome stained portal fibrosis, (C) Glibenclamide-treated group showing (moderate perivascular and periductal fibrosis, (E) CSE-treated group showing very mild periductal fibrosis. (D) GE- treated group showing mild fibrosis around the bile duct, (F) Area % of Masson's Trichrome staining in different experimental groups, $^{\#\#\#\#}$p<0.0001 when compared to control. ****p<0.0001, ***p<0.001when compared to STZ group. $^{@@@@}$p<0.0001, $^{@@@}$ p <0.001 when compared to glibenclamide-treated group. $^{\$\$}$p<0.01 when compared to GE-treated group. Data are expressed as means ± SD.

References

1. Kuang, Y., Chai, Y., Su, H., Lo, J.-Y., Qiao, X. and Ye, M. A network pharmacology-based strategy to explore the pharmacological mechanisms of Antrodia camphorata and antcin K for treating type II diabetes mellitus. *Phytomedicine,* 96153851(2022).

2.Sharabi, K., Tavares, C.D., Rines, A.K. and Puigserver, P. Molecular pathophysiology of hepatic glucose production. *Molecular Aspects of Medicine,* 4621-4633(2015).

3.Mohamed, J., Nafizah, A.N., Zariyantey, A. and Budin, S. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan qaboos university medical journal,* **16**(2),e132(2016).

4.Nowotny, K., Jung, T., Höhn, A., Weber, D. and Grune, T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules,* **5**(1),194-222(2015).

5.Jain, S., Pandhi, P., Singh, A. and Malhotra, S. Efficacy of standardised herbal extracts in type 1 diabetes-an experimental study. *African Journal of Traditional, Complementary and Alternative Medicines,* **3**(4),23-33(2006).

6. Ashour, M. N., Megahed, H. A., Morsy, S. M., Eltoukhy, S. I., Youness, E. R., Habib, D. F. and Wafai, H. A. Antioxidant and radical scavenging properties of garlic oil in streptozotocin induced diabetic rats. *Aust. J. Basic. Appl. Sci.,* **5**(10),280-286 (2011).

7. Kaur, G., Padiya, R., Adela, R., Putcha, U. K., Reddy, G., Reddy, B., Kumar, K., Chakravarty, S. and Banerjee, S. K. Garlic and resveratrol attenuate diabetic complications, loss of β-cells, pancreatic and hepatic oxidative stress in streptozotocin-induced diabetic rats. *Frontiers in Pharmacology,* 7360 (2016).

8.Attaallah, A., Elmrazeky, A.R., El-Beltagy, A.E-F.B., Abdelaziz, K.K., Soliman, M.F. Modulatory role of Coriandrum sativum (coriander) extract against diabetic complications on the gonads of female rats and their offspring. *Tissue and Cell,* 83102127(2023).

9.Priya, A.V. and Kumar, V.S. A Comprehensive Review OnCoriandrumSativum Linn. *NeuroQuantology,* **19**(6),148(2021).

10.Thomson, M., Al-Amin, Z.M., Al-Qattan, K.K., Shaban, L.H. and Ali, M. Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats. *International Journal of Diabetes and Metabolism,* **15**(3),108-115(2007).

11.Sadeghabadi, Z.A., Lotfi, F., Moheb, S.S., Abbasalipourkabir, R., Goodarzi, M.T and Ziamajidi, N. Effects of garlic extract on inflammatory cytokines in rats with type 1 and type 2 diabetes. *Gene Reports,* 26101474(2022).

12.Xie, C., Gao, W., Li, X., Luo, S., Wu, D. and Chye, F.Y. Garlic (Allium sativum L.) polysaccharide ameliorates type 2 diabetes mellitus (T2DM) via the regulation of hepatic glycogen metabolism. *NFS Journal,* 3119-3127(2023).

13.Padiya, R., Khatua, T.N., Bagul, P.K., Kuncha, M. and Banerjee, S.K. Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats. *Nutrition & Metabolism,* 81-88(2011).

14.Moharib, S. Antidiabetic and antioxidant effects of parsley (Petroselinum sativum) extract in streptozocin-induced diabetic rats. *International Atomic Energy Agency (IAEA)*, 43(2), 341-357(2016).

15.Eidi, A., Eidi, M. and Esmaeili, E. Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine,***13**(9-10), 624-629(2006).

16.El-Shaer, N.O., Hegazy, A.M. and Muhammad, M.H. Protective effect of quercetin on pulmonary dysfunction in streptozotocin-induced diabetic rats via inhibition of NLRP3 signaling pathway. *Environmental Science and Pollution Research,* **30**(14),42390-42398(2023).

17.Naquvi, K.J., Ali, M. and Ahmad, J. Antidiabetic activity of aqueous extract of Coriandrum sativum L. fruits in streptozotocin induced rats. *Indian J. Exp. Biol.,* **42**(9),909-12(2004).

18.Mittal, P. and Juyal, V. Drug-dietary interaction potential of garlic on glimepiride treated type 2 diabetic Wistar rats. *Journal of Diabetology,* **3**(3),3(2012).

19.Rabbani, S.I., Devi, K. and Khanam, S. Protective role of glibenclamide against nicotinamidestreptozotocin induced nuclear damage in diabetic Wistar rats. *Journal of Pharmacology and Pharmacotherapeutics,* 1(1),18-23(2010).

20.Ghanbari, E., Nejati, V. and Khazaei, M. Improvement in serum biochemical alterations and oxidative stress of liver and pancreas following use of royal jelly in streptozotocin-induced diabetic rats. *Cell Journal (Yakhteh),* **18**(3),362(2016).

21.Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology,* **28**(1),56-63(1957).

22.Bancroft, J.D. and Layton, C. Connective and other mesenchymal tissues with their stains. *Bancroft's Theory and Practice of Histological Techniques,*153-175(2018).

23.Suvarna, K.S., Layton, C. and Bancroft, J.D. Bancroft's theory and practice of histological techniques: Elsevier Health Sciences; 2018.

24.Abunasef, S.K., Amin, H.A. and Abdel-Hamid, G.A. A histological and immunohistochemical study of beta cells in streptozotocin diabetic rats treated with caffeine. *Folia Histochemica et Cytobiologica,* **52**(1),42-50(2014).

25.Nagayach, A., Patro, N. and Patro, I. Experimentally induced diabetes causes glial activation, glutamate toxicity and cellular damage leading to changes in motor function. *Frontiers in Cellular Neuroscience,* 8355(2014).

26.O'brien, T., Hayden, D., Johnson, K. and Fletcher, T. Immunohistochemical morphometry of pancreatic endocrine cells in diabetic, normoglycaemic glucose-intolerant and normal cats. *Journal of Comparative Pathology,* **96**(4),357-69(1986).

27.Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care,* **27**(5),1047-1053(2004).

28.George, N., Kumar, T.P., Antony, S, Jayanarayanan, S. and Paulose, C. Effect of vitamin D3 in reducing metabolic and oxidative stress in the liver of streptozotocin-induced diabetic rats. *British Journal of Nutrition,* **108**(8),1410-1418(2012).

29.Ardestani, A., Yazdanparast, R. and Jamshidi, S. Therapeutic effects of Teucrium polium extract on oxidative stress in pancreas of streptozotocin-induced diabetic rats. *Journal of Medicinal Food,* **11**(3),525-532 (2008).

30.Roosdiana, A., Permata, F.S., Fitriani, R.I., Umam, K. and Safitri, A. Ruellia tuberosa L. Extract Improves Histopathology and Lowers Malondialdehyde Levels and TNF Alpha Expression in the Kidney of Streptozotocin-Induced Diabetic Rats. *Veterinary Medicine International,* **2020** (1),8812758(2020).

31.Metawea, M.R., Abdelrazek, H.M., El-Hak, H.N.G., Moghazee, M.M. and Marie, O.M. Comparative effects of curcumin versus nano-curcumin on histological, immunohistochemical expression, histomorphometric, and biochemical changes to pancreatic beta cells and lipid profile of streptozocin induced diabetes in male Sprague–Dawley rats. *Environmental Science and Pollution Research,* **30**(22),62067-62079(2023).

32.Moharib, S.A. and Adly, R.S. Hypoglycemic and hepatoprotective activities of coriander (Coriandrum sativum) Extract in Streptozocin Induced Diabetic Rats. *Journal of Advances in Biology & Biotechnology,* **27**(2),15-38(2024).

33. Mousa, A. M., Soliman, K. E., Alhumaydhi, F., Almatroudi, A., Al Rugaie, O., Allemailem, K. S., Alrumaihi, F., Khan, A., Rezk, M. Y. and Aljasir, M. Garlic Extract Alleviates Trastuzumab-Induced Hepatotoxicity in Rats Through Its Antioxidant, Anti-Inflammatory, and Antihyperlipidemic Effects. *Journal of Inflammation Research,* 6305-16(2021).

34.Augusti, K. Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.). *Indian Journal of Experimental Biology,* **34**(7),634-640 (1996).

35. Alqahtani, Q. H., Alshehri, S., Alhusaini, A. M., Sarawi, W. S., Alqarni, S. S., Mohamed, R., Kumar, M. N., Al-Saab, J. and Hasan, I. H. Protective Effects of Sitagliptin on Streptozotocin-Induced Hepatic Injury in Diabetic Rats: A Possible Mechanisms. *Diseases,* **11**(4),184(2023).

36.Ouyang, G., Wang, N., Tong, J., Sun, W., Yang, J. and Wu, G. Alleviation of taurine on liver injury of type 2 diabetic rats by improving antioxidant and anti-inflammatory capacity. *Heliyon,* **10**(7),e28400(2024).

37.Basson, R. and Bayat, A. Fibrosis and diabetes: Chronic hyperglycemia triggers organ-specific fibrotic mechanisms. Wound Healing, Tissue Repair, and Regeneration in Diabetes: Elsevier; 2020. p. 121-47.

38.Naveen, S. and Khanum, F. Anti-diabetic, anti-oxidant, anti-dyslipidemic and hepatoprotective properties of coriander seed extract in streptozotocin induced diabetic rats. *Journal of Herbal Medicine and Toxicology,* **6**(2),61-67(2012).

39.Laribi, B., Kouki, K., M'Hamdi, M. and Bettaieb, T. Coriander (Coriandrum sativum L.) and its bioactive constituents. *Fitoterapia,* 1039-1026(2015).

40. D'argenio, G., Mazzone, G., Ribecco, M. T., Lembo, V., Vitaglione, P., Guarino, M., Morisco, F., Napolitano, M., Fogliano, V. and Caporaso, N. Garlic extract attenuating rat liver fibrosis by inhibiting TGF-β1. *Clinical Nutrition,* **32**(2),252-258(2013).

دراسة مقارنة للتأثيرات المضادة لمرض السكري والحماية للكبد لمستخلص بذور الكزبرة ومستخلص الثوم في نموذج تجريبي للفئران المصابة بمرض السكري.

رانيا محمد البطاوي، عبد الباسط إسماعيل المشد، عزيزة عبد الفتاح امين، شوقي أحمد مصطفى وأحمد عبد الحافظ طنطاوي

قسم علم الأمراض، كلية الطب البيطري، جامعة بنها، طوخ 13736، مصر.

الملخص:

تم خلال هذا البحث إجراء دراسة لمقارنة التأثيرات المُحتملة المضادة لمرض السكري وحماية الكبد لكل من مستخلص بذور الكزبرة (CSE) ومستخلص الثوم (GE) في نموذج تجريبي للفئران المصابة بمرض السكري المُستحث بالستربتوزوتوسين (STZ) . حيث ُقسِّمت ثلاثون جرذًان إلى خمس مجموعات متساوية: المجموعة الأولى: تم تجريع الجرذان بمحلول سترات عن طريق الفم (0.5 مل/كجم). وحُقنت الجرذان في المجموعات 2، 3، 4، و5 داخل الصفاق بجرعة واحدة من الستربتوزوتوسين (50 مل/كجم). بعد 72 ساعة، تم تجريع الجرذان في المجموعات 3، 4، و5 عن طريق الفم بمستخلص بذور الكزبرة (250 مل /كجم)، ومستخلص الثوم (250 مل /كجم)، وجليبنكلاميد (0.5 مل/كجم) على التوالي. وقد أُعطيت هذه المستخلصات وجليبنكلاميد يوميًا لمدة 28 يومًا متتالية. وأظهرت النتائج زيادات ملحوظة في قيم الجلوكوز، وAST، وALT بالفئران المصابة بداء السكري مقارنة مع المجموعة الضابطة. وكشف الفحص المجهري لمعظم مقاطع البنكرياس لهذه الفئران عن نقص خلوي، وتنكس، ونخر في خلايا جز لانجرهانز مع نخر أسيني. علاوة على ذلك، كان التنكس الخلوي الكبدي، والنخر، وموت الخلايا المبرمج، والتسلل الخلوي الالتهابي البؤري مع تليف البوابة هي التغيرات السائدة بأنسجة الكبد لدى الفئران المصابة بداء السكري. وبالمقارنة مع المجموعة الضابطة، أظهر فحص كيمياء الانسجة المناعية وجود انخفاض ملحوظ في النسب المئوية لمساحة التفاعل المناعي الإيجابي للأجسام المضادة للأنسولين وعدد خلايا بيتا الإيجابية للتفاعل المناعي في البنكرياس مع زيادة ملحوظة في النسب المئوية لمساحة التفاعل المناعي الإيجابي ل caspase-3 ونسبة المساحة الليفية في كبد الفئران المصابة بداء السكري. وقد خفف علاج مستخلص الكزبرة والثوم من هذه الأضرار البنكرياسية والكبدية الناجمة عن STZ مع استعادة قيم الجلوكوز وإنزيمات الكبد. علاوة على ذلك، فقد صاحب العلاج بهذه المستخلصات زيادة بشكل ملحوظ في النسب المئوية لمساحة الأجسام المضادة للأنسولين وعدد خلايا بيتا الإيجابية للتفاعل المناعي مع نقص بشكل ملحوظ في النسبة المئوية لمساحة التفاعل المناعي الإيجابي ل caspase-3 وألياف الكولاجين المصبوغة بصابغة ماسون. وقد خلصت الدراسة أن لهذه المستخلصات تأثيرات مضادة ممتازة لمرض السكري ولحماية الكبد ضد مرض السكري الناجم عن STZ ربما بسبب أنشطتهما المضادة للموت الخلوي المبرمج والمضادة للتليف؛ إلا أن كان لمستخلص الكزبرة تأثيرات أكثر فائدة.

الكلمات الداله: داء السكري، مستخلص بذور الكزبرة، مستخلص الثوم، الفحص النسيجي، الكبد.