EFFECTS OF *CHILIADENUS MONTANUS* EXTRACT ON STREPTOZOTOCIN INDUCED DIABETES AND ITS LIVER COMPLICATION IN RATS

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Abstract

The present work was designed to evaluate the effect of *Chiliadenus montanus* aerial parts extract on streptozotocin (STZ)-induced diabetes and liver complication with respect to its effect on α-amylase and total antioxidant capacity in rats. Forty male albino rats were assigned into four groups: normal control group, diabetic group STZ (50 mg/kg intraperitoneally), Glimepiride group (0.5 mg/kg; orally) and *Chiliadenus montanus* aerial parts group (50 mg/kg; orally) for 10 days. In diabetic rats, there was a marked elevation in fasting blood glucose, α-amylase, alanine transferase (ALT), aspartate transferase (AST) and total cholesterol levels accompanied with a marked decline in levels of serum high density lipoproteins (HDL) and total antioxidant capacity as compared to the normal rats, in addition reduction in islet cellularity and focal necrosis in pancreatic tissue and fibrosis in liver tissue, while administration of *Chiliadenus montanus* to STZ diabetic rats alleviated the disturbed biochemical changes and restored islet architecture and hepatocytes. These results revealed that *Chiliadenus montanus* has a significant hypoglycemic, hypolipidemic and antioxidant effects.

Key words: Diabetes mellitus; STZ; *Chiliadenus montanus;* α-amylase; Total antioxidant capacity; rats.

Introduction

Diabetes mellitus (DM) is highly linked to metabolic and endocrine disorder. diabetes global prevalence is estimated 415 million, in next 25 years, it is predicted to be elevated to 642 million. It has reached 1 in every 11 people in 21st century worldwide (Ogurtsova *et al.,* 2017). This growing pandemic prevalence is strongly associated with changes in lifestyle such as physical inactivity and unhealthy diet (Dos Santos *et al.,* 2015).

Chiliadenus montanus (Vahl.) Brullo [=*Jasonia montana*, *Varthemia montana*], a herb indigenous to the North Sinai region of Egypt, is a member of the Asteraceae (Hegazy *et al.,* 2014). Previous investigations showed the presence of monoterpenes (Ahmed and Jakupovic 1990), sesquiterpenes (Ahmed *et al.,* 2004), diterpenes (Al-Howiriny *et al.,* 2005), triterpenes, sterols and flavonoids in *Chiliadenus montanus* (Eid *et al.,* 1987).

Locally, it was known as Haneida (Täckholm 1974). This plant is used for the treatment of renal complications as a herbal tea and its chemical components exhibit anti-obesity, anti-diabetic, antiatherogenic, antimicrobial, antioxidant activities (Hussein 2011), antibacterial and antifungal activities (Hegazy *et al.,* 2014).

In Egypt, Sinai deserts have alot of medicinal plants that are rich in phenolic compounds, coumarins, flavonoids, terpenoids, and other constituents that may decrease diabetes incidence rates. So, the current study aimed to investigate the capacity of *Chiliadenus montanus* that is used in Egypt in folk medicine to treat hyperglycemia and dyslipidemia and liver complication in STZ-induced diabetic rats.

Materials and Methods

Instrumentation

Instrumentation included a Horiba SEPA-300 digital

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polarimeter (l = 5 cm) for specific rotation; a Shimadzu FTIR-8100 spectrometer for infra-red (IR) analysis; JEOL JMS-GCMATE mass spectrometer with tetramethylsilane as an internal standard for proton1 (H) (600 MHz) and Carbon (C) 13 (150 MHz) nuclear magnetic resonance (NMR) spectra; a Shimadzu refractive index detector (RID)-10A for High pressure liquid chromatography (HPLC) analysis and a COSMOSIL-Pack type (C18-MSII) (250 - 4.6 mm i.d.) and (250 -20 mm i.d.) columns for analytical and preparative separation, respectively. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography (cc), silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reverse-phase silica gel cc, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100– 200 mesh); thin layer chromatography (TLC), pre-coated TLC plates with silica gel 60F254 (Merck, 0.25 mm) (ordinary phase) and silica gel reverse-phase (RP)-18 F254S (Merck, 0.25 mm); RP-HPTLC, pre-coated TLC plates with silica gel RP-18 WF254S (Merck, 0.25 mm); and detection was achieved by spraying with (1:9) sulfuric acid-methanol (H_2SO_4-MeOH) followed by heating.

Plant material

Air-dried aerial parts of *Chiliadenus montanus* (Vahl.) Brullo. were collected in 2015, from Wadi Gebal, North Sinai, Egypt. Plant material was identified by Dr. El-Bialy E. Hatab, Egyptian Environmental Affairs Agency, Nature Conservation Sector, Siwa Protected Area, Siwa, Egypt. A voucher specimen SK-1001 has been deposited in the Herbarium of St. Katherine Protectorate, Egypt.

Extraction and isolation

Aerial parts (2.0 kg) of *Chiliadenus montana* were powdered and extracted with dichloromethane: methanol (CH_2Cl_2: CH_3OH) (1:1) (10L, 3 days) at room temperature. The combined extract was concentrated in vacuo to obtain a residue (175 g), which was fractionated on a silica gel column (6 × 120 cm) eluting with n-hexane (3 L) followed by a gradient of n-hexane-chloroform ($CHCl_3$) up to 100% $CHCl_3$ and then a $CHCl_3$– CH_3OH studies at up to 15% CH_3OH (3 L each of the solvent mixture).

The n-hexane:$CHCl_3$ (3:1) fraction was applied to a Sephadex LH-20 column (3 × 90 cm) eluted with n-hexane:CH_2Cl_2: CH_3OH (7:4:0.25), to give 10 (12 mg). The n-hexane: $CHCl_3$ (1:1) fraction was subjected to silica gel cc (4 × 120 cm) eluted with n-hexane: ethylacetate (EtOAc) (4:1). Fractions were obtained and combined into three parts A, B and C on the basis of their TLC

profiles. Sub-fraction A, was re-purified using silica gel cc (6 × 90 cm) eluted with n-hexane: EtOAc (6:1) to afford 3-oxo-g-costic acid (1.0 g) as a major compound (Tchamadeu *et al.,* 2017).

Animals

Adult male albino Wistar rats, weighing 120 – 140g were used in the current study. They were purchased from the National Research Centre (NRC; Cairo, Egypt) animal house. Animals received human care in compliance with the guidelines of the animal care and use committee of the NRC. The animals were kept in a quiet place and were allowed free access to water and standard food pellets throughout the period of experiment. Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee (IAEC). The animals were treated according to the national and international ethics guidelines stated by the ethics committee of NRC, Egypt and all procedures and experiments were performed according to the protocol approved (No 10010306).

Drugs

Glimepiride was obtained from Sanofi-Aventis, *Egypt*

Chemicals

STZ (98%), diethyl-ether, sodium citrate, citric acid and formaldehyde were obtained from Sigma Aldrich Chemical Co., USA, sterile saline was obtained from ADWIC, Egypt.

Experimental Design

Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg) dissolved in 0.1M citrate buffer (pH 4.5) (Khalaf *et al.,* 2012; Salama *et al.,* 2017). The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycemia (Kamel *et al.,* 2019). While, group 1: Negative control group rats were treated with the same volume citrate buffer only without STZ for 10 days. Diabetes was confirmed 48 h after STZ injection by measuring the glucose concentrations in blood samples obtained from the tail vein (One Touch SureStep Meter, LifeScan, Calif, USA). After diabetes was confirmed (>300mg/ml) (Kassem *et al.,* 2017), rats were assigned randomly into: Group 2: Diabetic control rats (STZ). Group 3: Diabetic rats received, the reference drug, Glimepiride (0.5 mg/kg; p.o.), (Mohamed *et al.,* 2013), for 10 days. Group 4: Diabetic rats received *Chiliadenus montanus* (50 mg/kg; p.o.), (Helal *et al.,* 2015), for 10 days.

Biochemical parameters

At the end of the experiment period (10 days) of treatment, the rats were anaesthetized with diethyl ether

and blood samples were collected for biochemical analyses. The blood was withdrawn from the retro-orbital plexus vein of each rat, then they were left to clot at room temperature and centrifuged at 3000 rpm for 10 min for serum separation. Serum samples were stored at -20ºC for biochemical assays(Elmotasem *et al.,* 2018).

Determination of blood glucose, serum levels of α-amylase, alanine transferase (ALT), aspartate transferase (AST), total cholesterol, high density lipoprotein (HDL) and total antioxidant capacity were performed using Biodiagnostic commercial kits.

Histopathology

At the end of the experiment; animals were sacrificed by decapitation (Salama *et al.,* 2016). Pancreas (splenic part) and liver were dissected and extracted from sacrificed animals. Organ tissues were fixed in 10% buffered formalin, processed through ascending grades of alcohol, cleared in xylene and prepared into paraffin blocks. Serial sections 5 microns thick were prepared from each block and stained with haematoxylin and eosin for routine histopathologic study (Drury *et al.,* 1976). The sections were examined under an Olympus CX41 research microscope at the Pathology Department, National Research Centre. Slide tissue microphotography was done using CCD digital camera Olympus DP-12 attached to the Olympus CX41 research microscope. Digital photomicrographic sections were taken at various magnifications

Statistical analysis

Results are expressed as mean ± standard error (S.E.) of at least six rats. The statistical evaluation was determined by one-way analysis of variance (ANOVA) followed by least significant difference (LSD). Graphpad Prism software, version 5 (Inc., USA) was used to carry out these statistical tests. The difference was considered significant when p < 0.05.

Results

Effect of *Chiliadenus montanus* on blood glucose and α-Amaylase levels

In the present study, injection with STZ (50 mg/kg, i.p) showed a significant increase in blood glucose and á-Amaylase levels after 10 days by 242% and 96% respectively, when compared to normal animals. While treatment with Glimepiride (0.5 mg/kg, orally) and *Chiliadenus montanus* (50 mg/kg, orally) caused a significant decrease in blood glucose levels after 10 days by 69.44% and 64.63%, respectively, as well as α-Amaylase levels by 60% and 55% respectively, when compared to STZ- induced animals Fig. 1.
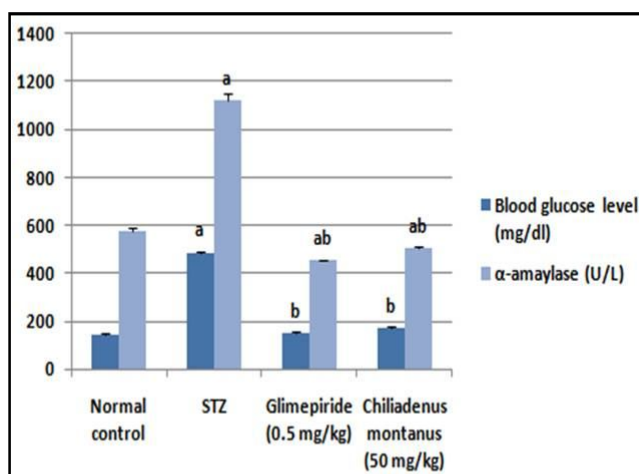

Fig. 1: Effect of *Chiliadenus montanus* on blood glucose and á-Amaylase levels in streptozotocin-induced diabetic rats.
Statistical analysis was carried out by one-way ANOVA followed by LSD test.
a. Significantly different from normal control (Saline) at P<0.05.
b. Significantly different from STZ control group at P<0.05.

Effect of *Chiliadenus montanus* on cholesterol and High density lipoprotein cholesterol (HDL)

In the present study, injection with STZ significantly increased serum cholesterol level by 23.38% and showed a significant decrease in serum HDL level by 51% after 10 days when compared to normal animals. But treatment with Glimepiride, *Chiliadenus montanus* decreased cholesterol serum levels by 10.39% and 12.71%, respectively, as well as increased serum HDL levels by 78% and 264%, respectively, after 10 days, when compared to STZ-induced animals table 1.

Effect of *Chiliadenus montanus* on ALT and AST serum levels

In the present study, injection with STZ showed a significant increase in ALT and AST serum levels after 10 days by 42.64% and 47.89% respectively, when

Table 1: Effect of *Chiliadenus montanus* on total cholesterol and HDL-cholesterol in streptozotocin-induced diabetic rats.

	Normal control	STZ	Glimepiride (0.5 mg/kg)	*Chiliadenus montanus* (50 mg/kg)
Total Cholesterol (mg/dl)	218.77 ±15.88	269.91 ±8.55[a]	241.87 ±2.51[b]	235.59 ±2.08[b]
HDL-cholesterol (mg/dl)	393.25 ±5.50	192.50 ±4.35[a]	342.10 ±0.51[ab]	398.75 ±47.08[b]

Statistical analysis was carried out by one-way ANOVA followed by LSD test. Results were expressed as means ± SE, N=6. *a Significantly different from normal control (Saline) at P<0.05. b Significantly different from STZ control group at P<0.05.*

Table 2: Effect of *Chiliadenus montanus* on ALT and AST in streptozotocin-induced diabetic rats.

	Normal control	STZ	Glimepiride (0.5 mg/kg)	*Chiliadenus montanus* (50 mg/kg)
ALT (U/mL)	50.65± 0.58	72.25± 0.88 [a]	57.98± 0.17 [b]	54.52± 0.51 [b]
AST (U/mL)	60.45± 2.59	89.41± 1.33 [a]	69.79± 0.85 [b]	66.47± 3.49 [b]

Statistical analysis was carried out by one-way ANOVA followed by LSD test. Results were expressed as means ± SE, N=6. *a Significantly different from normal control (Saline) at P<0.05. b Significantly different from STZ control group at P<0.05.*

compared to normal animals. While treatment with Glimepiride caused a significant decrease in ALT and AST serum levels after 10 days by 19.75% and 21.94%, respectively, also, *Chiliadenus montanus* (50 mg/kg, orally) caused a significant decrease in ALT and AST serum levels after 10 days by 24.54% and 25.65% respectively, when compared to STZ-induced animals table 2.

Effect of *Chiliadenus montanus* on total antioxidant capacity

In the present study, injection with STZ showed a significant decrease in serum total antioxidant capacity after 10 days by 19.30% when compared to normal animals. While treatment with Glimepiride and *Chiliadenus montanus* caused a significant increase in serum total antioxidant capacity after 10 days by 125.22% and 100.45% respectively, when compared to STZ-induced animals Fig. 2.
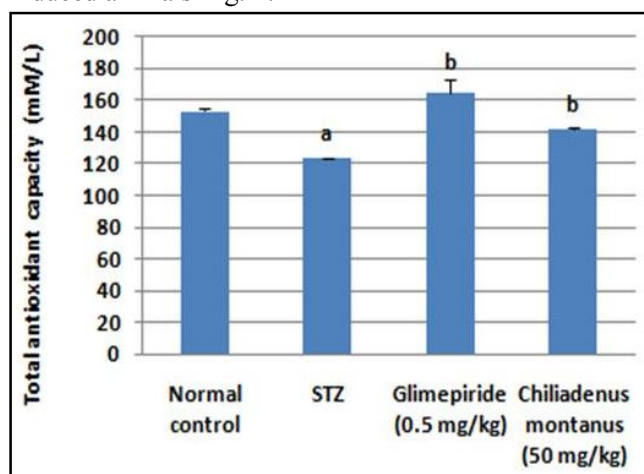

Fig. 2: Effect of *Chiliadenus montanus* on total antioxidant capacity in streptozotocin-induced diabetic rats.
Statistical analysis was carried out by one-way ANOVA followed by LSD test.
a. Significantly different from normal control (Saline) at P<0.05.
b. Significantly different from STZ control group at P<0.05.

Histopathology results

Pancreatic tissue within rats of control group showed ordinary acini. Islets of Langerhan's were numerous, showing regular outline and large size. Prominent cellularity was noticed with organized architecture. Cells showed ample cytoplasm and central, rounded, bland, basophilic nuclei Fig. 3a. Pancreatic tissue in diabetic untreated rat group showed fewer islets of langerhan's; being markedly reduced in size and shrunken. Outline of the islets showed irregularity. Marked reduction in islet cellularity was noticed with disorganized architecture. Constituent cells showed prominent vacuolar degeneration Fig. 3b. Focal pyknotic nuclei were seen.

Focal necrosis was observed as well. Pancreatic tissue in diabetic rat groups treated with each of Glimepiride and *Chiliadenus montanus* showed marked improvement in the islets of langerhan's, size and cellularity; becoming numerous, larger in size and more cellular. Regular outline and organized architecture were restored within islets. No vacuolar degeneration was noticed within constituent cells. Surrounding pancreatic acini were ordinary Fig. 3c, d.
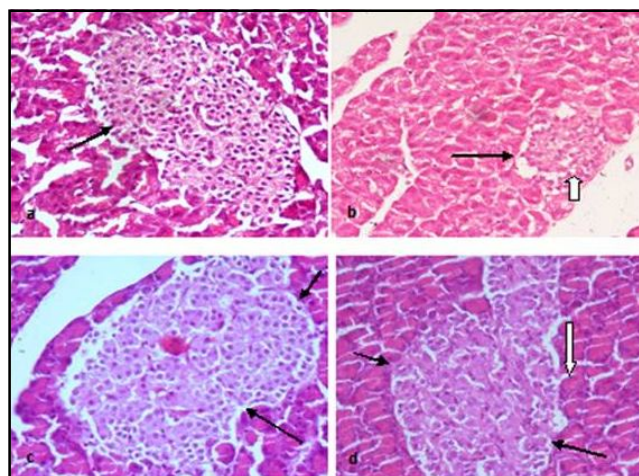

Fig. 3: (a): Tissue section of control rat pancreas showing an ordinary, cellular islet of langerhan's, large in size, regular in contour with organized architecture (black arrow). (H & E X400). (b): Tissue section from pancreas of diabetic untreated rat showing shrunken small sized islet (black arrow) with disorganized architecture, reduced cellularity and vacuolar degeneration within constituent cells (white arrow) (H & E X400). (c) Tissue section from pancreas of diabetic rat treated with reference drug showing restored islet size, cellularity and organization (black arrows). (H & E X400). (d) Tissue section from pancreas of diabetic rat treated with Natural Product showing islet of langerhan's with improved size and cellularity (black arrows) surrounded by ordinary acini (white arrow) (H & E X400).

Liver tissue within normal rat group showed preserved architecture. Lobules of liver cells were seen. Central vein was noticed. Hepatocytes were disposed in cords, one cell thick each, radiating from central veins. Sinusoids were seen separating between liver cords. Hepatocytes were polyhedral with ample eosinophilic cytoplasm and central, rounded, vesicular, bland nuclei Fig. 4a. Liver tissue within diabetic untreated rat group showed marked congestion and dilatation within central veins. Bands of fibrosis were seen; particularly in perivascular sites together with few aggregates of chronic inflammatory cells. Hepatocytes showed marked microvesicular vacuolar degeneration Fig. 4b, c, d. Liver tissue within diabetic rat group treated with each of Glimepiride Fig. 4e and *Chiliadenus montanus* Fig. 4f showed marked improvement. No bands of fibrosis were seen. Central veins showed no dilatation and no congestion. Hepatocytes were ordinary, approximating control; except for few microvesicles within scattered hepatocytes in case of the diabetic rat group treated with the Glimepiride.

Discussion

Diabetes mellitus is a life threatening chronic metabolic disorder and associated with carbohydrate and fat disturbances as well as and protein metabolism due to defects in action or secretion of insulin (Imam 2012). In the present study STZ injection exhibited severe hyperglycemia when compared with the normal group. This hyperglycemia is due to selective destruction of β-cells by STZ with focal necrosis. Islets of Langerhan's showed marked reduction in number, size, diameter and volume together with inflammatory cellular infiltrate as shown in our histopathology. Koshy *et al.,* (2012) reported that STZ diabetic untreated rats, showed high blood glucose level with diffused necrotic changes in the islets of langerhans (El-Baz *et al.,* 2020; Koshy *et al.,* 2012). On the other hand, *Chiliadenus montanus* administration showed a reduction of the elevated blood glucose levels in diabetic rats via increased uptake of glucose in skeletal muscle and adipocytes, as well as insulin secretion in pancreatic cells, this result supported by our histopathology which showed improvement in the pancreas; in the form of increase in number, size and volume of the islets, as well as increase within the density of cells inside the islets. These findings are consistent with a previous study which reported that a mixture containing *Chiliadenus montanus* had antihyperglycemic properties in alloxan-induced diabetic rats(Helal *et al.,* 2015). In another study, Gorelick *et al.,* (2011) revealed the anti-diabetic activity of another species named *Chiliadenus iphionoides* (*Gorelick et al., 2011*).

Chiliadenus montanus extract might exhibit anti-diabetic action through several mechanisms. Our prediction was confirmed by significant inhibition of α-amylase serum activity that was elevated by STZ. Salama *et al.,* (2017) showed an elevation of á-amylase serum activity by injecting STZ when compared with the normal group (Salama *et al.,* 2017); α-amylase enzyme hydrolyses alpha-bonds of starch to yield high levels of glucose and maltose (Ajayi *et al.,* 2109). So *Chiliadenus montanus* extract prevent starch destruction and glucose elevation.

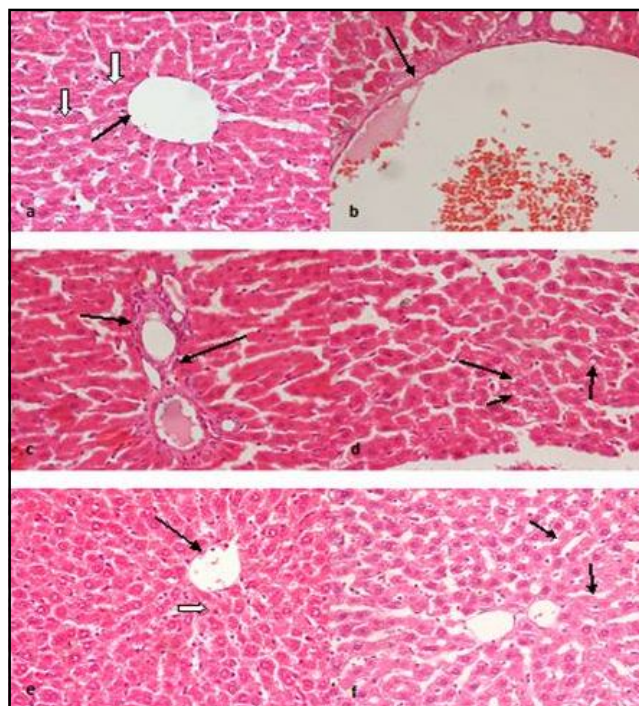Our data, also, showed a increase in cholesterol and decrease in HDL-cholesterol serum levels in STZ rats

Fig. 4: (a): Tissue section of control rat liver showing ordinary architecture with central vein(black arrow); from which are radiating ordinary hepatocytes (white arrows) in cords (H & EX400). (b): Tissue section of liver from diabetic untreated rat showing markedly dilated and congested central vein (black arrow) (H & E X400). (c) Tissue section from liver of diabetic untreated rat showing bands of fibrosis within perivascular tissues (black arrows) (H & E X400). (d): Liver section from liver of diabetic untreated rat showing microvesicular degeneration within hepatocytes (black arrows) (H & E X400). (e): Liver section from liver of diabetic rat treated with reference drug showing hepatocytes disposed in cords radiating from central vein (black arrow) with residual few microvesicles (white arrow) (H & E X400). (f): Liver section from diabetic rat treated with Natural Product showing ordinary hepatocytes (black arrows) approximating control (H & E X400).

when compared with the normal group. This higher cholesterol level may be attributed to exacerbated lipase activity which led to reduced glucose utilization (Pinheiro *et al.,* 2017) and indicated metabolic changes in the liver as confirmed in our histopathological study which showed dilated and congested central vein with fibrosis within perivascular tissues and degeneration within hepatocytes. Treatment with *Chiliadenus montanus* exhibited a decrease in cholesterol and an increase in HDL serum levels. Otherwise, the present histopathological study showed ordinary hepatocytes reversing metabolic changes in the liver. Our results agree with a previous study that mentioned that *Chiliadenus montana administration* showed reduced levels of triglycerides (TG), total cholesterol and low density lipoprotein (LDL) in obese diabetic rats fed high-fat diet (Hussein 2011).

The ability of *Chiliadenus montanus* to reduce plasma lipids in diabetic animals could be explained by its capacity for releasing the insulin, this effect is due to high amount of polyphenols, essential oils, flavonoids and quercitrin. Ovaskainen *et al.,* (2008) showed the capacity of quercetin to release insulin from isolated islets of Langerhans (Ovaskainen *et al.,* 2008). Other mechanisms of the hypolipidemic activity of the *Chiliadenus montanus* ethanolic extract are the decrease in the intestinal cholesterol absorption (Crozier *et al.,* 2009), inactivation of hepatic hydroxyl methyl glutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme, in cholesterol synthesis (Raz *et al.,* 2005) and finally increased expression of peroxisome proliferator-activated receptors (PPAR) that promotes insulin sensitivity decreasing plasma lipids (Feige *et al.,* 2006).

Diabetes is associated with a failure of various organ systems and liver complications. In diabetic rats, serum AST and ALT levels were elevated as any abnormality in amino acid metabolism leads to the oxidation of the amino acid that is known as aminotransferases (Visweswara Rao *et al.,* 2013), while these levels of both enzymes for *the first time* were estimated and considerably decreased in diabetic rats treated with *Chiliadenus montanus* to a similar extent as Glimepiride. Diabetes and its complications are linked to oxidative stress that generates reactive oxygen species (ROS) causing coronary micro-vascular dysfunction and injury in diabetic nephropathy. In the present work, STZ injection produced liver dysfunction with a significant reduction in serum total antioxidant capacity when compared with the control group. These results may be confirmed with another study that showed that induction of diabetes by STZ provoked oxidative stress as elevated lipid peroxidation and reduced glutathione which in turn impairs

glucose uptake in adipose tissue and muscle and reduces insulin secretion from pancreatic B cells (Hussein *et al.,* 2019; Maddux *et al.,* 2001). While treatment with *Chiliadenus montanus* exerted antioxidant activities through elevating serum total antioxidant capacity and protected the tissues from lipid peroxidation due to phenolic compounds. *In an in vitro* oxidative stress model as an astrocytoma cell line induced by exogenous H_2O_2, *Chiliadenus montanus* hydroalcoholic extracts possessed good antioxidant properties via high quantities of phenolic compound as gallic acid (Eissa et al. 2013). Moreover, polymethoxylated flavonoids of *C. montans* aerial parts have NAD(P) H: quinone oxidoreductase1 (NQO1) inducer activity scavenging superoxide and ameliorated oxidative recycling (Hamed *et al.,* 2016).

Conclusion

the treatment with alcoholic extract of *Chiliadenus montanus* arial parts given to diabetic rats exhibited a hypoglycemic and hypoloidaemic effects as well as lowered hepatic microsomal enzymes in diabetic animals, through different mechanisms that include the inhibition of glucose elevation and á-amylase activity, improvement of antioxidant capacity and protection of β-cell of islet of pancreas and liver tissues against destruction.

Data Availability

The data used to support the findings of this study are available from the corresponding author (Abeer Salama) upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest

Acknowledgements

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