Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: *In vivo* and *in vitro* evaluation of its nutraceutical potential

Adriano Mollica,[1,*] Gokhan Zengin,[2] Marcello Locatelli,[1] Azzurra Stefanucci,[1] Andrei Mocan,[3] Giorgia Macedonio,[1] Simone Carradori,[1] Olakunle Onaolapo,[4] Adejoke Onaolapo,[5] Juliet Adegoke,[4] Marufat Olaniyan,[4] Abdurrahman Aktumsek,[2] and Ettore Novellino[6]

[1] Dipartimento di Farmacia, Università "G. d'Annunzio" di Chieti-Pescara, via dei Vestini 31, 66100, Chieti, Italy

[2]Department of Biology, Science Faculty, Selcuk University Campus, 42250, Konya, Turkey

[3]Department of Pharmaceutical Botany, "Iuliu Hatieganu" University of Medicine and Pharmacy, 8, V. Babes Street, Cluj-Napoca, Romania

[4]Department of Pharmacology, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria

[5]Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

[6]Dipartimento di Farmacia, Università "Federico II" di Napoli, via D. Montesano, Napoli, Italy

*e-mail: a.mollica@unich.it

Abstract

In this study, the nutraceutical potential of *Capparis spinosa* L. for the treatment of hyperglycemic states has been thoroughly investigated. A series of *in vivo* and *in vitro* tests have been conducted on fresh leaf, buds and salty buds (24 h desalted) processed to dry powder. 60% MeOH/H_2O extracts were obtained for HPLC analysis and for α-amylase and α-glucosidase inhibition tests. To estimate the *in vivo* anti-diabetic effect, dry powders of *C. spinosa* leaf and buds were orally administered to streptozocin-induced diabetic rats over a period of 28 days. At the end of the experiment, animals were sacrificed, blood taken for assessment of lipid profile and liver/kidney biochemistry while section of the pancreas, liver and kidneys were processed for general histology. Results showed that the regular administration of *C. spinosa* leaf or buds normalized all the biochemical parameters and reversed the liver/kidney injury with variable degrees of organ protection.

Keywords: α-amylase; α-glucosidase; nutraceuticals; HPLC; polyphenols; flavonoids; *Capparis spinosa* L.; diabetes.

1. Introduction

Herbs, herbal extracts, or phytochemicals are broadly used as foods, dietary supplements and drugs (orthodox or traditional medicines) (Nasri, Baradaran, Shirzad, & Rafieian-Kopaei, 2015; Santini & Novellino, 2014). A number of these herbs have been marketed for their health-promoting effects with multiple biological activities related to their traditional uses. In some cases, the claimed bio-activities are not always scientifically demonstrated therefore their utilities largely rely on the history and ethnopharmacological prospects (Lapenna, Gemen, Wollgast, Worth, Maragkoudakis, Caldeira, 2015; Mocan et al., 2016).

Capparis spinosa L. (Capparaceae) belongs to the genus *Capparis*, which is made up of 250 different species. It is one of the most economically important and well-known member of the genus (Anwar et al., 2016; Tlili, Khaldi, Triki, & Munné-Bosch, 2010). *C. spinosa* is widely distributed in different parts of the globe, in drylands, deserts and rocky areas of the Mediterranean environment, Crimea, Armenia, Iran, as well as India and Pakistan (Anwar et al., 2016). The plant is well known for its showy-white or pinkish-white, hermaphrodite flowers or its fleshy alternate or rarely opposite leaves (Anwar et al., 2016; Rahnavard & Razavi, 2016).

Over the last decades attention was given to determining the chemical composition of *C spinosa* which resulted in the discovery of a number of bioactive compounds, mostly polyphenols. Recently studies have reported its efficacy as a nutraceutical agent due to the antioxidant, antimicrobial, anticancer, antiallergic and hepatoprotective effects (Anwar et al., 2016; Eddouks, Lemhadri, & Michel, 2005; Rahnavard & Razavi, 2016). Traditionally, different parts of the Caper-plant are used in the management of various ailments. The leaves, roots and buds for the treatment of gastrointestinal disorders, skin diseases, earache, kidney and liver diseases amongst the

Arabs, while its fruits to treat diabetes, headache, fever and rheumatism. Furthermore *C. spinosa* has many culinary uses and it is consumed as a source of healthy food worldwide. Buds, unripe or ripe fruits, and young shoots are pickled and added as condiment in salads or in different foods in the Mediterranean region.

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia, resulting from either an absolute or relative lack of insulin or insulin resistance, complicated in a number of cases by microangiopathies and end organ damage. Obesity, poor diet and sedentary life style which are major components of the metabolic syndrome, have been named as the principal causative factors for the prevalence of this disease. Metabolic syndrome is defined as a progressive pathophysiological state, clinically manifested by a cluster of interrelated risk factors (abdominal obesity, dyslipidemia, hypertension and insulin resistance) and it is associated with an increased risk for diabetes mellitus (Huang, Teoh, Lin, Lin, & Roufogalis, 2009; Sperling et al., 2015; Waltenberger, Mocan, Šmejkal, Heiss, & Atanasov, 2016). Worldwide, diabetes mellitus is considered one of the most common non communicable chronic diseases (A. Onaolapo, Onaolapo, & Adewole, 2011). Based on the projections of the International Diabetes Federation and World Health Organization (WHO), the prevalence of diabetes mellitus amongst adults (20-79 years) will increase from 285 million in 2010 to 439 million by 2030 (Shaw, Sicree, & Zimmet, 2010; Waltenberger et al., 2016; Xiao, 2014). These predictions suggest a growing burden of diabetes, necessitating research in to newer, more affordable and less toxic medications for its management, hence this study.

The rationale is based on the imperative necessity to investigate the effects of *C. spinosa* leaf/bud powders as a viable nutraceutical in the management of diabetes mellitus. This was with the aim of: *i*) determining the phytochemical constituents and

in-vitro anti-diabetic potential of *C. spinosa* leaf and bud extracts; *ii*) assessing *in-vivo* the effects of *C. spinose* leaf and bud powders on body weight, blood glucose estimations, lipid profile, biochemical markers of liver and kidney injury and liver, kidney and pancreatic morphology in experimental diabetes. We tested the hypothesis that administration of *C. spinosa* leaf or bud powders can significantly reduce blood sugar, restore plasma lipid balance, and mitigate biochemical and morphologic evidences of renal pancreas and hepatic damages in streptozocin induced diabetes rats.

2. Materials and methods

2.1 Chemicals and drugs

All analytical standards (gallic acid, catechin, chlorogenic acid, *p*-hydroxy-benzoic acid, vanillic acid, epicatechin, syringic acid, 3-hydroxy-benzoic acid, 3-hydroxy-4-methoxybenzaldehyde, *p*-coumaric acid, rutin, sinapinic acid, *t*-ferulic acid, naringin, 2,3-dimethoxy-benzoic acid, benzoic acid, *o*-coumaric acid, quercetin dihydrate, *t*-cinnamic acid, naringenin (all purity 98%)) were purchased from Sigma Aldrich (Milan, Italy).

The standards mix stock solution was prepared by dissolving 1 mg of the compound in 1 mL of methanol (1:1, solid: solvent ratio) and no solubility problems are observed. Working solutions at different concentration levels were daily prepared by appropriate dilution in water. HPLC-grade methanol and acetonitrile were purchased from Sigma-Aldrich (Milan, Italy). For sample analysis, deionized water purified with a Milli-Q system (Millipore, Bedford, USA) was used.

Normal saline, ethanol, Streptozotocin (STZ), α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) and α-amylase from porcine pancreas (E.C. 3.2.1.1) were purchased from Sigma Chemical Co. (Sigma St. Louis, USA). Metformin was obtained

from Bristol-Myers Squibb (UK). All other chemicals and solvents were of analytical grade.

2.2 Plant Material

Capparis spinosa L. samples were collected in July from Lipari Isle, in the Aeolian Archipelago (Italy), and provided to us by Capersud Manufacture. The leaf, fresh buds and salty buds (24 h desalted) were freeze-dried by a VirTis lyophilizer and then powdered.

2.3 Preliminary phytochemical screening of plant fraction

C. spinosa samples (500 g) were extracted by three different techniques: microwave extraction, soxhlet extraction and decoction by 60% MeOH/H_2O. The extracts were subjected to lyophilization. The dry extract obtained was analyzed by HPLC and tested for enzymatic assays

2.3.1 HPLC analysis

Extracts of *C. spinosa* were analyzed for the quantitative and qualitative determination of polyphenols and flavonoids, which were performed by means of HPLC-PDA reported in literature (Zengin et al., 2016, Locatelli et al., 2017). Analyses were carried out by using a Waters liquid chromatograph equipped with a photodiode array detector, a C18 reversed-phase column (Prodigy ODS-3, 4.6x150 mm, 5 μm; Phemomenex, Torrance, CA), an *on-line* degasser, a column oven set at 30°C (±1°C). The gradient elution was achieved by a solution of water-acetonitrile (93:7 ratios, with 3% of acetic acid) as initial settings. The complete separation was achieved in 60 minutes. No matrix interferences were observed for the

2.4. *In vitro* enzyme inhibition assays

α-Amylase inhibitory activity was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method (Zengin, 2016). Sample solution (25 μL, 2

mg/mL) was mixed with α-amylase solution (porcine pancreas, EC 3.2.1.1, Sigma, Saint Louis, Mo., USA) (50 µL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37°C. After pre-incubation, the reaction was initiated with the addition of starch solution (50 µL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution. The reaction mixture was incubated 10 min at 37°C then stopped by adding HCl (25 µL, 1 M). This is followed by the addition of the iodine-potassium iodide solution (100 µL). The sample and blank absorbances were recorded at 630 nm. The absorbance of the blank was subtracted from that of the sample and the α-amylase inhibitory activity was expressed as millimoles of acarbose equivalents (mmol ACE/g extract).

α-Glucosidase inhibitory activity was performed as previously described by Zengin (2016). Sample solution (50 µL) was mixed with glutathione (50 µL, 2 mg/mL), 50 µL α-glucosidase solution (from *Saccharomyces cerevisiae*, EC 3.2.1.20, Sigma) in phosphate buffer (pH 6.8), and 50 µL of 10 mM PNPG (4-N-trophenyl-α-D-glucopyranoside) (Sigma-Aldrich, Darmstadt, Germany) solution in a 96-well microplate and incubated for 15 min at 37°C. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution. The reaction was stopped with the addition of sodium carbonate (50 µL, 0.2 M). The sample and blank absorbances were recorded at 400 nm. The absorbance of the blank was subtracted from that of the sample and the α-glucosidase inhibitory activity was expressed as millimoles of acarbose equivalents (mmol ACE/g extract).

2.5 Animals

Healthy male Wistar rats from Empire Breeders, Osogbo, Osun State, Nigeria were used. Rats were housed in plastic cages measuring 24 x 18 x 12 inches (6 rats in each cage). General housing is a temperature-controlled (22.5°C ±2.5°C) quarters with

12 hours of light. Animals had free access to food and water. All animals were fed commercial standard chow (calories: 29% protein, 13% fat, 58% carbohydrate). All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.6 Acute Toxicity Test

Acute oral toxicity studies were conducted using the Organization for Economic Co-operation and Development (OECD) guidelines (2008) (Oecd, 2008).

2.7 Induction of diabetes mellitus

Diabetes mellitus was experimentally induced in the animals by a single intraperitoneal injection of 70 mg/kg/body weight of streptozotocin (STZ), dissolved in ice-cold physiologic saline and given at a volume of 2 mL/kg. The non-diabetic control animals were injected intraperitoneally with an equivalent volume of cold saline (vehicle). Rats were fasted overnight prior to administration of STZ or vehicle. 72 hours following STZ injection, the rats were fasted for 8 hours and blood taken from the tail veins for glucose estimation. Rats having hyperglycemia (that is, with blood glucose of ≥ 10.56 mmol/L) were considered diabetic and included in the diabetic experimental groups.

2.8 Experimental methodology

Fifty-four, male, 3-month old Wistar rats weighing between 80 and 100 g each were randomly assigned into nine groups of six rats each. Animals received vehicle, distilled water at 10 mL/kg [non-diabetic control (VEH) and diabetes control (STZ)], metformin (MET), a standard antihyperglycaemic agent at 1.8 mg/kg and one of three doses of *C. spinosa* leaf (L) or bud (B) at 100, 200 and 400 mg/kg. Vehicle, standard drug or herbs were administered daily by gavage starting at 9.00 am; gavage was used as

method of drug delivery to simulate administration in humans. Animals were weighed weekly. All treatments were administered for a period of 28 days. Blood samples from the tail vein were taken weekly after an overnight fast for glucose estimation by the glucose oxidase method. At the end of the experimental period (day 28) animals were fasted overnight and serum used for estimation of lipid profile, cholesterol and biochemical parameters of liver/renal injury.

2.8.1 Sacrifice of animals

At the end of the experimental period, rats were observed for changes in their physical characteristics, and then sacrificed by decapitation following anaesthesia with diethyl-ether. Pancreas liver and kidneys were dissected out, observed grossly and fixed in 10% neutral buffered formalin. Sections of the pancreas, liver and kidney were processed for paraffin-embedding, cut at 5 μm and stained with haematoxylin and eosin for general histological study.

2.8.2 Biochemical assays

Blood was collected from each rat on the 28th day via intracardiac puncture after an overnight fast. Samples were collected into lithium heparinized bottles, blood was allowed to clot and serum separated by centrifugation at 3,500 rpm for 10 minutes using a general centrifuge (Uniscope SM112, Surgifriend Medicals, England). The serum was assayed either immediately or stored at -20˚C.

Alanine transaminase (ALT) and aspartate transaminase (AST) levels were determined according to the spectrophotometric method described using appropriate assay kits. Serum creatinine and Urea levels were determined by a colorimetric reaction (Jaffe's Method), and (DAM Method) respectively using an autoanalyser (Astra 8 autoanalyzer; Beckman Instruments, Fullerton, CA) (A. Onaolapo & Onaolapo, 2012; A. Y. Onaolapo, Onaolapo, & Adewole, 2012). Total cholesterol, triglycerides, HDL-C, LDL-C,

and VLDL-C in serum were analyzed using commercially available kits following the instructions of the manufacturer.

2.9 Photomicrography

Sections of the pancreas were examined using a Sellon-Olympus trinocular microscope (XSZ-107E, China) with a digital camera (Canon Powershot 2500) attached, and photomicrographs taken.

2.10 Statistical analysis

Data was analysed using Chris Rorden's ezANOVA for windows. Hypothesis was tested using analysis of variance (ANOVA). We tested the hypothesis that oral administration of *C. spinosa* leaf or bud significantly alter body weight, blood sugar, lipid profile, biochemical parameters of hepatic and renal function and morphology of the pancreas, liver and kidneys in streptozocin induced diabetic rats. Two factor ANOVA was used to test effects of *C. spinosa* dose and repeated administration on body weight, and glucose control, while one factor ANOVA was used to assess the effect of *C. spinosa* on lipid profile and liver/kidney biochemistry. Tukey (HSD) test was used for within and between group comparisons. Results were expressed as mean ± S.E.M, and $p < 0.05$ considered significant.

3. Results

3.1. Phytochemical composition and *in* vitro enzyme inhibitory effects of *Capparis spinosa*

The extracts of *C. spinosa* have revealed a large amount of flavonoids and other important bioactive compounds. Particularly, rutin was determined as major flavonoid in the analyzed extracts (Table 1). In accordance with our results, rutin was detected also as predominant phenolic compound in *C. spinosa* harvested from different areas (Siracusa et al., 2011; Tlili et al., 2010). Another major constituent is *p*-coumaric acid,

which is present in all examined extracts. From multi-component analyses it can be observed that soxhlet procedure extracts more components than the other techniques, while microwave is a valid methodology to obtain catechin and epicatechin, two compounds not detected in the other extracts. As reported in Table 1, gallic acid can be found only when fresh buds were analyzed, furthermore the total quantities (μg/mg) of the herein considered phenolic components are higher in leaf extracts than in the commercial salted buds.

The enzyme inhibition assays have shown a strong inhibition activity on α-amylase and α-glucosidase which are involved in glucose metabolism (Table 2). Fresh buds' decoction and salted buds microwave extracts revealed the greatest inhibitory effect on α-amylase and α-glucosidase, respectively. The observed remarkable enzyme inhibitory effects may be explained with the higher levels of rutin. These findings were supported by some researchers; for example, Fernandes et al. (2010) suggested that rutin could be a valuable agent for preventing diabetes mellitus. Rutin may be useful as an antidiabetic modulator in streptozotocin-induced diabetic rats. In this direction, our findings could provide a precious contribution in the connection of rutin and diabetes mellitus.

3.2. Acute toxicity test of *Capparis spinosa*

Acute toxicity studies of powdered leaf and bud of *C. spinosa* were carried out in rats by using the Organization for Economic Co-operation and Development (OECD) (2008) guideline. Rats were tested up to a dose of 5000 mg/kg body weight. Then they were observed for general behavioral changes, symptoms of toxicity and mortality after treatment for the first four (critical) hours, then over a period of 24 hours, and thereafter for 14 days. There were no mortalities, or observable evidence of behavioural or tissue toxicity.

3.3. Effects of *Capparis spinosa* on body weight

Figure 1C represents the effect of *C. spinosa* on body weight. Two factor ANOVA with repeated measures assessing the effect of treatment on initial (taken on day 1) and final (taken on day 28) body weight revealed a significant main effect of treatment ($F(8,40) = 10.4$ $p<0.001$), and body weight ($F(1,5) = 55.6$ $p<0.006$), with significant interactions between treatment x body weight ($F(8,40) = 13.1$ $p<0.001$). Pairwise comparisons of diabetic-control (STZ), metformin (MET) or *C. spinosa* and vehicle (non diabetic-control) revealed a significant decrease in body weight with STZ ($p<0.001$, $p<0001$), MET ($p<0.001$, $p<0.001$) and with *C. spinosa* leaf at 100 ($p<0.003$, $p<0.002$), 200 ($p<0.045$, $p<0.001$) and 400 mg/kg ($p<0.001$, $p<0.001$) and *C. spinosa* bud at 100 ($p<0.001$, $p<0.002$), 200 ($p<0.004$, $p<0.002$) and 400mg/kg ($p<0.001$, $p<0.003$) compared to vehicle with either initial or final body weight readings. Comparisons between MET, *C. spinosa* leaf/bud and diabetic-control (STZ) revealed no significant difference in body weight with either MET, *C. spinosa* leaf or bud with initial body weight reading, while with the final body weight readings, there was a significant increase in body weight with MET ($p<0.001$, $p<0.001$), *C. spinosa* leaf and bud at 100 ($p<0.001$, $p<0.001$), 200 ($p<0.001$, $p<0.001$) and 400 mg/kg ($p<0.002$, $p<0.001$) respectively. Comparisons between *C. spinosa* leaf or bud and MET revealed no significant difference in body weight at any of the doses of *C. spinosa* leaf or bud with the initial body weight readings while with the final body weight readings there was a significant increase in weight with *C. spinosa* leaf at 100 ($p<0.002$) and 200 mg/kg ($p<0.045$) and with *C. spinosa* bud at 200 mg/kg ($p<0.045$).

3.4. Effect of *Capparis spinosa* on blood glucose levels

Figure 1A represents the effect of *C. spinosa* body glucose levels in diabetic rats.

Two factor ANOVA with repeated measures assessing the effect of treatment on initial (taken on day 1) and final (taken on day 28) blood glucose levels revealed a significant main effect of treatment ($F(8, 40) = 20.2$, $p<0.001$), and blood glucose ($F(1,5) = 1336$ $p<0.001$), with significant interactions between treatment x blood glucose ($F(8, 40) = 18.3$ $p<0.001$). Pairwise comparisons of diabetic-control (STZ), metformin (MET) or *C. spinosa* and vehicle (non diabetic-control) revealed a significant increase in blood glucose with STZ ($p<0.001$, $p<0001$) with initial and final blood glucose readings respectively, a significant increase in blood glucose was seen with MET ($p<0.001$), *C. spinosa* leaf and bud at 100 ($p<0.001$, $p<0.001$), 200 ($p<0.001$, $p<0.001$) and 400 mg/kg ($p<0.001$, $p<0.001$) respectively with initial blood glucose readings, while with respect to the final blood glucose readings no significant difference was seen compared to vehicle. Comparisons between MET, *C. spinosa* leaf or bud and diabetic-control (STZ) revealed no significant difference in blood glucose levels with either MET, *C. spinosa* leaf or bud with initial blood glucose reading, while with the final blood glucose readings, there was a significant decrease in blood glucose with MET ($p<0.001$), *C. spinosa* leaf and bud at 100 ($p<0.001$, $p<0.001$), 200 ($p<0.001$, $p<0.001$) and 400 mg/kg ($p<0.001$, $p<0.001$) respectively. Comparisons between *C. spinosa* leaf or bud and MET revealed no significant difference in blood glucose levels at any of the doses of *C. spinosa* leaf or bud with the initial blood glucose estimation while with the final blood glucose values there was a significant decrease in blood glucose with *C. spinosa* leaf at 200 ($p<0.027$).

3.5. Effect of *Capparis spinosa* on liver enzymes

Figure 1B shows the effect of *C. spinosa* on liver enzymes in diabetic rats. Aspartate transaminase (AST) levels increased significantly ($F(8,45) = 71.2$, $p<0.001$) in the diabetic control (STZ) and with *C. spinosa* leaf at 100, 200 and 400 mg/kg compared to non diabetic-control (vehicle), while there was a significant decrease in

AST levels with metformin (MET), a standard antihyperglycaemic agent. Compared to STZ group there was a significant decrease in AST with MET, *C. spinosa* leaf and bud at 100, 200 and 400mg/kg respectively. While compared to MET group administration of *C. spinosa* bud resulted in a significant increase in AST levels at 100, 200 and 400mg/kg.

Alanine transaminase levels (ALT) increased significantly ($F(8,45) = 25.3$, $p<0.001$) with STZ compared to vehicle while with MET, *C. spinosa* leaf or bud no significant difference was seen. Compared to the STZ group there was a significant decrease in ALT levels with MET and with *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively, whilst compared to MET there was no significant difference in ALT levels with *C. spinosa* leaf or bud.

3.6. Effect of *Capparis spinosa* on kidney function tests

Table 3 shows the effect of *C. spinosa* leaf or bud on urea and creatinine levels in STZ-induced diabetic rats. There was a significant increase in urea levels ($F(8,45) = 146$, $p<0.001$) in the STZ group. Compared to STZ group there was a significant decrease in urea levels with MET and *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively. Compared to MET, no significant difference was seen in urea levels with *C. spinosa* leaf or bud.

Creatinine levels increased significantly ($F(8,45) = 46.3$, $p<0.001$) in the STZ group compared to vehicle. Compared to the STZ group there was a significant decrease in creatinine with metformin and *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively. Compared to metformin, a significant increase in creatinine was seen with *C. spinosa* leaf at 200 and 400 mg/kg and bud at 100, 200 and 400 mg/kg.

3.7. Effect of *Capparis spinosa* on lipid profile

Table 3 also shows the effect of *C. spinosa* on lipid profile in diabetic rats. Total cholesterol (TC) increased significantly ($F(8,45) = 40.9$ $p<0.001$) in the STZ group and

with *C. spinosa* leaf at 100 mg/kg, and decreased with metformin compared to vehicle. Compared to STZ group there was a significant decrease in TC with metformin and *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively. Compared to metformin, TC increased significantly with *C. spinosa* leaf at 100, 200 and 400 mg/kg.

Triglyceride (Tg) levels increased significantly ($F(8,45) = 74.3$, $p<0.001$) in the STZ group and with *C. spinosa* leaf at 400 mg/kg and decreased significantly with metformin compared vehicle. Compared to the STZ group Tg decreased significantly with metformin and with *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively. Compared to metformin there was a significant increase in Tg with administration of *C. spinosa* leaf at 200 and 400 mg/kg and bud at 100, 200 and 400 mg/kg.

High density lipoprotein (HDL) levels decreased significantly ($F(8,45) = 5.48$, $p<0.007$) in the STZ group and increased significantly with capparis spinosa bud at 100, 200 and 400 mg/kg compared to vehicle. Compared to the STZ group there was significant increase in HDL with metformin and with *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively. Compared to metformin, HDL levels increased significantly with *C. spinosa* bud at 100, 200 and 400mg/kg.

Low density lipoprotein (HDL) levels increased significantly ($F(8,45) = 132$, $p<0.001$) in the STZ group, and with *C. spinosa* leaf at 100 and 200 mg/kg, and decreased significantly with metformin and with *C. spinosa* bud at 200 and 400 mg/kg compared to vehicle. Compared to the STZ group, LDL levels decreased significantly with metformin and with *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively. Compared to metformin, LDL levels increased significantly with *C. spinosa* leaf at 100, 200 and 400 mg/kg and a significant decrease with bud at 200 and 400 mg/kg.

3.8. Effect of *Capparis spinosa* on the morphology of the pancreas

Figure 2 and 3 show representative photomicrographs of hematoxylin and eosin stained sections of the pancreas in normal and STZ-induced diabetic Wistar rats administered increasing doses of *C. spinosa* leaf (Figure 2 a-e) or bud powder (Figure 3 a-e). Examination of slides from rats that received vehicle (Figure 2a, 3a) revealed normal pancreatic islet cells with deeply staining nuclei, normal sized blood vessels and pancreatic ducts. The exocrine pancreas also showed normal glandular cells with deeply staining nuclei. Slides from the STZ control groups (Figure 2b, 3b) of rats showed oedematous pancreatic tissue with widening of the interglandular spaces and engorged blood vessels. The islets of Langerhans appeared shrunken with numerous pale staining nuclei. In groups administered Metformin (Figure 2c, 3c) a standard antihyperglycaemic agent, there was minimal swelling of the pancreatic tissue evidenced by decrease in the spaces between individual glands. The pancreatic duct showed some dilation while only a few engorged blood vessels were seen. In groups administered *C. spinosa* leaf powder at 100 (Figure 2d), 200 (Figure 2e) and 400 mg/kg (Figure 2f), a dose related effect was seen as evidenced by oedematous pancreatic tissue and minimally engorged blood vessels, shrunken islet of Langerhans cells with a mixture of pale and deeply staining nuclei at 100 (Figure 2d) and 200 mg/kg (Figure 2e) while at 400 mg/kg (Figure 2f) the pancreatic islet cells appear almost normal with deeply staining nuclei and very few pale staining nuclei. The blood vessels are not engorged and the pancreatic ducts are not dilated. In groups administered *C. spinosa* bud powder at 100 (Figure 3d), 200 (Figure 3e) and 400 mg/kg (Figure 3f) a dose related effect was also seen, similar to the effects seen with *C. spinosa* leaves although oedema at 100 (Figure 3d) and 200 mg/kg (Figure 3e) are markedly reduced and the engorgement of blood vessels was minimal. The islet of Langerhans cells were shrunken with numerous pale and few deeply

staining nuclei at 100 mg/kg while at 200 mg/kg, the pancreatic islet of Langerhans had numerous deeply staining nuclei and very few pale staining nuclei. At 400 mg/kg (Figure 3f) the pancreatic islet of Langerhans was not as well preserved as seen with the leaf powder.

3.9. Effect of *Capparis spinosa* on the morphology of the liver

Examination of haematoxylin and eosin-stained sections of the liver in rats that were administered vehicle (Figure 4a, 5a, see Supporting Information) revealed sheets of radially-arranged hepatocytes around the central vein, with sinusoidal spaces demarcating cords of hepatocytes. Also obvious were deeply-staining hepatocyte nuclei and nuclei of a few Kupffer cells scattered surrounding the central vein; these features are in keeping with normal histology. Slides from sections of the liver of the STZ group (Figure 4b, 5b see Supporting Information) showed marked disruption of hepatocyte parenchyma, with loss of intervening sinusoidal spaces and swollen hepatocytes; with numerous pale-staining shrunken hepatocyte nuclei and numerous Kupffer cells were scattered throughout the hepatic parenchyma. Also obvious is a marked dilated central vein, these features are in keeping with hepatic injury. Examination of liver slides from the metformin group (Figure 4c, 5c see Supporting Information) revealed sheets of radially-arranged hepatocytes with intervening sinusoids and a mixture of deeply-staining nuclei and few pale staining shrunken hepatocyte nuclei. Numerous inflammatory cells and/or kupfer cells are seen scattered around a dilated central vein and hepatic vein; overall features are in keeping with some protection against liver injury. In the groups administered *C. spinosa* leaf and bud at 100, 200 and 400 mg/kg respectively, varying degrees of protection from liver injury are seen, as evidenced by mild to moderate loss of normal liver architecture with some radially-arranged cords of

hepatocytes and normal nuclei interspersed between normal hepatocytes with deeply staining hepatocyte nuclei and a few hepatocytes with pale staining shrunken nuclei and a few kupfer cells. A mildly dilated central vein is seen with *C. spinosa* leaf at 100 (Figure 4d see Supporting Information), 200 (Figure 4e see Supporting Information) and 400 mg/kg (Figure 4f see Supporting Information) and *C. spinosa* bud at 100 (Figure 5d see Supporting Information) and 200 (Figure 5e see Supporting Information). *C. spinosa* bud at 400 mg/kg showed normal sized central vein with liver protection approaching normal histology.

3.10. Effect of *Capparis spinosa* on the morphology of the kidney

Examination of slides taken from sections of the right kidney of animals administered vehicle (Figure 6a, 7a see Supporting Information) revealed well demarcated cortex and medulla. Glomeruli, Bowman's capsule, Bowman's space, proximal and distal renal tubules and blood vessels all appear normal. Deeply staining-nuclei of the glomeruli and tubular epithelium were seen. Examination of slides of the STZ group (Figure 6b, 7b see Supporting Information) showed disruption of normal kidney architecture with markedly contracted renal glomeruli, degenerating crumpled glomeruli, widening of the Bowman's space and swollen renal tubules. Nuclei of the glomeruli and renal tubular epithelial cells ranged from numerous pale-staining shrunken renal cell nuclei to a few deeply staining normal renal cell nuclei, numerous inflammatory cells were seen scattered all over the renal parenchyma. In animals administered metformin (Figure 6c, 7c see Supporting Information), swelling of the renal glomeruli, with dilation of the Bowman's space. Also obvious was swelling of the renal tubules, as evidenced by constriction of lumen of both the proximal and distal tubules. The nuclei of the renal glomeruli and renal tubular epithelium were a mixture of both deeply and pale-staining; features in keeping with some protection from kidney

injury. The groups administered *C. spinosa* leaf and bud at 100 (Figure 6d, 7d see Supporting Information), 200 (Figure 6e, 7e see Supporting Information) and 400 mg/kg Figure 6, 7f see Supporting Information) respectively showed varying levels of protection from kidney injury. Administration of *C. spinosa* leaf at 100 (Figure 6d), 200 (Figure 6e) and 400 mg/kg (Figure 6f) showed loss of normal renal architecture, with very few degenerating or shrunken renal cell nuclei, and mild to moderate swelling of the tubules. Bowman's space was minimally dilated, with CS bud at 100 (Figure 7d), 200 (Figure 7e) and 400 mg/kg (Figure 7f) preservation of the renal parenchyma is almost total with numerous deeply staining nuclei with their respective nucleoli, very few of the swollen renal glomeruli are seen, renal tubules are normal-sized, and nuclei of the renal tubular epithelial cells are deeply-staining.

4. Discussion

A number of studies have been conducted to investigate the antidiabetic potential of various extracts of *C. spinosa* (Eddouks, Lemhadri, & Michel, 2004; Eddouks et al., 2005; Kazemian, Abad, reza Haeri, Ebrahimi, & Heidari, 2015; Mohammadi, Mirzaei, Delaviz, & Mohammadi, 2012). In this study, we set out to assess (possibly for the first time) the effects of *C. spinosa* leaf/bud powders as a potential nutraceutical in the management of diabetes mellitus. The antidiabetic potential of *C. spinosa* leaf/bud was examined both *in vitro* and *in vivo*. *In vitro* analysis of plant samples revealed large quantities of phenolics and flavonoids, especially quercetin-3-O-rutinoside (rutin) which is a glycoside of the flavonoid quercetin. Rutin was the predominant flavonoid, in accordance with the already published literature (Siracusa et al., 2011; Tlili et al., 2010). Moreover, rutin has been associated with health-promoting effects; studies have shown that rutin inhibits aldose reductase activity (Reddy, Muthenna, Akileshwari, Saraswat, & Petrash, 2011), preventing the deposition of sorbitol, which has been associated with

474 diabetic end–organ injury. Rutin possess significant antioxidant potential in preventing
475 oxidative stress, a major component of diabetes related end organ damage
476 (Kamalakkannan & Prince, 2006). The results of the enzyme inhibition assays
477 demonstrated strong inhibition of α-amylase and α-glucosidase, in particular by the
478 bud. α-Amylase and α-glucosidase are key enzymes in carbohydrate metabolism.
479 Inhibition of these enzymes have been associated with regulation of blood glucose level
480 following a carbohydrate meal, which is an important component of diabetes
481 management. The observed enzyme inhibitory effects have been attributed to the
482 presence of a number of phytochemical constituents like hydrolysable tannins,
483 flavonoids, xanthones, fatty acids, terpenoids, procyanidins and caffeoylquinic acid
484 derivatives (Chang et al., 2013). In this case, the presence of flavonoids, especially a high
485 content of rutin might be responsible for the enzyme inhibitory effects reported
486 (Fernandes et al., 2010) .

487 *In vivo* studies were performed to test the effect of *C. spinosa* leaf or bud powder
488 on blood glucose, lipid profile, biochemical/morphological indicators of liver and kidney
489 injury, and morphological changes in the pancreas in streptozotocin induced diabetes in
490 Wistar rats. These changes were compared against the effects of a standard
491 antihyperglycaemic agent (Metformin). The results showed that administration of
492 increasing doses of *C. spinosa* leaf or bud resulted in 1) a reversal of diabetes-induced
493 weight loss 2) significant reduction in blood glucose 3) reversal of diabetes induced
494 dyslipidaemia and biochemical parameters of liver and kidney injury 4) varying levels
495 of protection of renal, hepatic and pancreatic tissues against the deleterious effects of
496 diabetes mellitus.

497 In this study experimentally induced diabetes was associated with significant
498 weight loss compared to non-diabetic controls, this is consistent with the results of

other studies (A. Onaolapo et al., 2011; Patel & Sharma, 2015; Zafar & Naqvi, 2010) that have reported weight changes with experimental diabetes. Weight loss in diabetic animals have been attributed to breakdown of tissue proteins. Metformin administration resulted in lesser weight gain compared to *C. spinosa* in agreement with studies in either experimental animals (A. Onaolapo & Onaolapo, 2012; Pournaghi, Sadrkhanlou, Hasanzadeh, & Foroughi, 2012; Yanardag, Ozsoy-Sacan, Bolkent, Orak, & Karabulut-Bulan, 2005) or humans that have reported reduced weight gain or weight loss with metformin use. Metformin's effect on body weight have been linked to its ability to reduce adipose tissue mass (Bailey & Turner, 1996). Treatment with *C. spinosa* resulted in a dose-related weight gain compared to both metformin and diabetic control, with leaf powder showing greater weight gain compared to buds. Studies using extracts of different parts of the *C. spinosa* plant have also reported weight gain in diabetic animals (Kazemian et al., 2015; Patel & Sharma, 2015).

Glycaemic control is the cardinal goal for the management of diabetes mellitus. In the present study, both plant parts were able to control blood glucose after daily administration; although, glycaemic control after administration of leaf powder appeared to be better, when compared to that of powdered-bud. Blood glucose control following treatment with *C. spinosa* has been attributed to the effects of a number of its biologially active constituents. Phytochemicals identified from *C. spinosa* leaves or buds include lipids, alkaloids, Quercetin-3-rutinoside (rutin), glucocapperin, polyphenols, phenolic acids and flavonoids (Manikandaselvi & Brindha, 2014). Extracts of different parts of the *C. spinosa* plant have been reported to be highly effective in the control of blood glucose (Eddouks et al., 2004, 2005; Kazemian et al., 2015; Mohammadi et al., 2012; Patel & Sharma, 2015; Rahmani, Mahmoodi, & Salehi, 2013) in experimental diabetes. Its antihyperglycemic potential has been linked to the ability to slow down

digestion and absorption of carbohydrate, thereby preventing a sudden rise in blood glucose after food intake (Eddouks et al., 2004). Eddouks et al. (2004) also reported that *C. spinosa*'s antihyperglycemic activity was insulin independent. The results of the *in vitro* enzyme analysis suggest that the glucose lowering effect is related to the presence of large quantities of flavonoids like quercetin and/or rutin which could inhibit intestinal brush-border α-glucosidase and pancreatic α-amylase.

Rutin is a powerful antioxidant which may help in the control of blood sugar via the preservation of pancreatic beta cells (as was seen with pancreatic morphology in this study) from oxidative damage, thereby ensuring continued secretion of insulin. *C. spinosa* leaf at 200 and 400 mg/kg/day and buds at 400 mg/kg /day reduced blood glucose significantly more than metformin. Metformin lowers blood glucose primarily by suppressing hepatic gluconeogenesis (Kirpichnikov, McFarlane, & Sowers, 2002), which is markedly increased with diabetes and by activating adenosine monophospate–activated protein kinase (AMPK), which is an insulin-signal for the maintenance of energy balance, glucose and fats metabolism (Towler & Hardie, 2007). At higher doses, *C. spinosa* may have effects that are similar to that seen with metformin.

Control of plasma lipids levels and maintaining a balance in their relative composition are goals of management of diabetes mellitus. Diabetes mellitus is associated with dyslipidaemia, which is a sequelae of mobilization of free fatty acids from the peripheral fat stores (Ahmed, Lakhani, Gillett, John, & Raza, 2001). Derangement in blood glucose is usually accompanied by dyslipidaemia (rise in plasma cholesterol, triglycerides and LDL and reduction in HDL levels), these have been attributed to the activation of hormone sensitive-lipases which release free fatty acids from adipose tissue following insulin deficiency. In this study, induction of diabetes resulted in derangement of the blood lipid profile which was reversed by metformin

549 and increasing doses of *C. spinosa* leaf and bud. The lipid lowering effect of capparis
550 powder are similar to that seen with studies that utilised extracts of plant parts
551 (Eddouks et al., 2005; Kazemian et al., 2015).

552 *C. spinosa* contains high quantities of phytosterols which have been reported to
553 mediate the reduction in cholesterol levels. It has been suggested that it exerts
554 antilipidaemic effects by decreasing intestinal absorption of cholesterol acting on bile
555 acids; decreasing cholesterol biosynthesis by reducing the activity of 3-hydroxy-3-
556 methyl-glutaryl coenzyme A reductase (Sharma, Nasir, Prabhu, Murthy, & Dev, 2003). It
557 could also enhance uptake of low density lipoproteins by increasing the activity of
558 lecithin cholesterol acyl transferase (LCAT) which may contribute to the regulation of
559 blood lipids. LCAT aids the incorporation of free cholesterol into HDL which may be
560 responsible for increase in HDL levels seen with *C. spinosa* bud more than leaf.

561 Hyperglycemia and hyperlipidemia resulting in the formation of advanced
562 glycation, lipoxidation end products, increased production of free radicals and
563 reduction in antioxidant activity have been implicated in the pathogenesis of diabetes–
564 induced end-organ damage (Cheville, 2000; Robertson, Harmon, Tran, & Poitout, 2004).
565 Histological examination of the pancreas, liver and kidneys in diabetic rats showed
566 features that were consistent with organ injury in the diabetes control, which was
567 similar to those reported in a number of other studies (Mohammadi et al., 2012; A.
568 Onaolapo & Onaolapo, 2012; A. Y. Onaolapo et al., 2012). This was ameliorated to
569 varying degrees with metformin or increasing doses of *C. spinosa* leaf or bud.

570 Biochemical parameters of liver (ALT and AST) and kidney (urea and creatinine)
571 injury were also markedly elevated in the diabetic control, but showed significant
572 reduction with treatment (Metformin or *C. spinosa*). Administration of *C. spinosa*
573 resulted in dose dependent decrease in ALT, AST levels, urea and creatinine, with bud

574 powder showing better results than leaf. The effects of *C. spinosa* leaf and bud on the
575 pancreas, liver and kidneys can be attributed to the presence of flavonoids like
576 quercetin and rutin which have antioxidant properties (Manikandaselvi & Brindha,
577 2014) and help to either reduce oxidative damage in the pancreas and liver or prevent
578 the formation of glycation products in the kidneys. Phytosterols prevent fatty liver,
579 metabolism of cholesterol and triglycerides reducing hyperlipidaemia and the
580 formation of free radicals.

581 **Conclusions**

582 This study shows that *C. spinosa* fresh leaf and buds powders (deriving from the
583 commercial salted buds), contain phytochemicals useful in the management of
584 experimental diabetes mellitus in rats. In particular, rutin is the major flavonoids in all
585 three preparations of leaves and buds. Enzymatic inhibition tests performed on α-
586 glucosidase and α-amylase support the existence of a molecular basis for the activity of
587 the herb, which may be related to the rutin content. *In vivo* administration of *C. spinosa*
588 leaf or buds revealed the normalization of all the biochemical parameters and reversed
589 the liver/kidney injury with variable degrees of organ protection. Therefore, it could be
590 a potentially-viable adjunct in the day to day management of humans with diabetes
591 mellitus and in the prevention of the raising of prediabetes by the systematic use of *C.*
592 *spinosa* in the diet.

593 **Conflict of interest**

594 Declared none.

595 **References**

596 Ahmed, I., Lakhani, M., Gillett, M., John, A., & Raza, H. (2001). Hypotriglyceridemic and
597 hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in
598 streptozotocin-induced diabetic rats. *Diabetes research and clinical practice, 51*(3), 155-161.

Anwar, F., Muhammad, G., Hussain, M. A., Zengin, G., Alkharfy, K. M., Ashraf, M., & Gilani, A.-H. (2016). *Capparis spinosa* L.: a plant with high potential for development of functional foods and nutraceuticals/pharmaceuticals. *International Journal of Pharmacology, 12*(3), 201-219.

Bailey, C. J., & Turner, R. C. (1996). Metformin. *N Engl J Med, 334*(9), 574-579. doi: 10.1056/nejm199602293340906

Chang, C. L., Lin, Y., Bartolome, A. P., Chen, Y.-C., Chiu, S.-C., & Yang, W.-C. (2013). Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. *Evidence-Based Complementary and Alternative Medicine, 2013*.

Cheville, N. (2000). Response to cellular injury *Ultrastructural Pathology*. Ames, Iowa, USA: Willey- Blackwell,.

Eddouks, M., Lemhadri, A., & Michel, J.-B. (2004). Caraway and caper: potential anti-hyperglycaemic plants in diabetic rats. *Journal of ethnopharmacology, 94*(1), 143-148.

Eddouks, M., Lemhadri, A., & Michel, J.-B. (2005). Hypolipidemic activity of aqueous extract of *Capparis spinosa* L. in normal and diabetic rats. *Journal of ethnopharmacology, 98*(3), 345-350.

Fernandes, A. A. H., Novelli, E. L. B., Okoshi, K., Okoshi, M. P., Di Muzio, B. P., Guimarães, J. F. C., & Junior, A. F. (2010). Influence of rutin treatment on biochemical alterations in experimental diabetes. *Biomedicine & Pharmacotherapy, 64*(3), 214-219.

Germano, M. P., De Pasquale, R., D'angelo, V., Catania, S., Silvari, V., & Costa, C. (2002). Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. *Journal of agricultural and food chemistry, 50*(5), 1168-1171.

Huang, T. H.-W., Teoh, A. W., Lin, B.-L., Lin, D. S.-H., & Roufogalis, B. (2009). The role of herbal PPAR modulators in the treatment of cardiometabolic syndrome. *Pharmacological research, 60*(3), 195-206.

622 Kamalakkannan, N., & Prince, P. S. M. (2006). Antihyperglycaemic and antioxidant effect of rutin,
623 a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. *Basic & clinical*
624 *pharmacology & toxicology, 98*(1), 97-103.

625 Kazemian, M., Abad, M., reza Haeri, M., Ebrahimi, M., & Heidari, R. (2015). Anti-diabetic effect of
626 Capparis spinosa L. root extract in diabetic rats. *Avicenna journal of phytomedicine, 5*(4), 325.

627 Kirpichnikov, D., McFarlane, S. I., & Sowers, J. R. (2002). Metformin: an update. *Annals of internal*
628 *medicine, 137*(1), 25-33.

629 Lapenna, S., Gemen, R., Wollgast, J., Worth, A., Maragkoudakis, P., & Caldeira, S. (2015). Assessing
630 Herbal Products with Health Claims. *Critical reviews in food science and nutrition, 55*(13), 1918-
631 1928.

632 Locatelli, M. , Zengin, G., Uysal, A., Carradori, S., De Luca, E., Bellagamba, G., Aktumsek, A.,
633 Lazarova, I. (2017). Multicomponent pattern and biological activities of seven Asphodeline taxa:
634 Potential sources of natural-functional ingredients for bioactive formulations. *Journal of Enzyme*
635 *Inhibition and Medicinal Chemistry*, 32, 60-67.
636 Manikandaselvi, S., & Brindha, P. (2014). Chemical standardization studies on *Capparis spinosa*
637 L. *6*, 47-54.

638 Mocan, A., Zengin, G., Uysal, A., Gunes, E., Mollica, A., Degirmenci, N. S., Alposoy, L., Aktumsek, A.
639 (2016). Biological and chemical insights of *Morina persica* L.: A source of bioactive compounds
640 with multifunctional properties. *Journal of Functional Foods, 25*, 94-109.

641 Mohammadi, J., Mirzaei, A., Delaviz, H., & Mohammadi, B. (2012). Effects of hydroalcoholic
642 extract of *Capparis spinosa* on histomorphological changes of pancreas in diabetic rats model.
643 *Journal of Birjand University of Medical Sciences, 19*(3), 235-244.

644 Nasri, H., Baradaran, A., Shirzad, H., & Rafieian-Kopaei, M. (2015). New concepts in
645 neutraceuticals as alternative for pharmaceuticals. *International journal of preventive medicine,*
646 *5*(12), 1487-1499.

647 Oecd. (2008). *OECD Guidelines for the Testing of Chemicals*: Organization for Economic.

648 Onaolapo, A., & Onaolapo, O. (2012). *Ocimum Gratissimum* Linn causes dose dependent
649 hepatotoxicity in streptozotocin-induced diabetic Wistar rats. *Macedonian Journal of Medical*
650 *Sciences, 5*(1), 17-25.

651 Onaolapo, A., Onaolapo, O., & Adewole, S. (2011). Ethanolic extract of *Ocimum grattissimum*
652 leaves (Linn.) rapidly lowers blood glucose levels in diabetic Wistar rats. *Macedonian Journal of*
653 *Medical Sciences, 4*(4), 351-357.

654 Onaolapo, A. Y., Onaolapo, O. J., & Adewole, S. O. (2012). *Ocimum Gratissimum* Linn Worsens
655 Streptozotocin-Induced Nephrotoxicity in Diabetic Wistar Rats. *Macedonian Journal of Medical*
656 *Sciences, 5*(4), 382-388.

657 Patel, V., & Sharma, V. (2015). Antioxidant and antidiabetic effect of *capparis spinosa* linn. In
658 streptozotocin induced diabetic rats. *European Journal of Biomedical and Pharmaceutical*
659 *Sciences, 2*, 308-318.

660 Pournaghi, P., Sadrkhanlou, R.-A., Hasanzadeh, S., & Foroughi, A. (2012). *An investigation on body*
661 *weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformin-*
662 *treated diabetic female rats.* Paper presented at the Veterinary Research Forum.

663 Rahmani, R., Mahmoodi, M., & Salehi, M. (2013). Effect of hydroalcoholic extract of *Capparis*
664 *spinosa* fruit on blood sugar and lipid profile of diabetic and normal rats. *Zahedan Journal of*
665 *Research in Medical Sciences, 15*(11), 34-38.

666 Rahnavard, R., & Razavi, N. (2016). A review on the medical effects of Capparis spinosa L.
667 *Advanced Herbal Medicine, 2*(1), 44-53.

668 Reddy, G. B., Muthenna, P., Akileshwari, C., Saraswat, M., & Petrash, J. M. (2011). Inhibition of
669 aldose reductase and sorbitol accumulation by dietary rutin. *Current Science, 101*(9), 1191-
670 1197.

671 Robertson, R. P., Harmon, J., Tran, P. O. T., & Poitout, V. (2004). β-cell glucose toxicity,
672 lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes, 53*(suppl 1), S119-S124.

673 Santini, A., & Novellino, E. (2014). Nutraceuticals: Beyond the Diet Before the Drugs. *Current*
674 *Bioactive Compounds, 10*(1), 1-12.

675 Sharma, S., Nasir, A., Prabhu, K., Murthy, P., & Dev, G. (2003). Hypoglycaemic and hypolipidemic
676 effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits.
677 *Journal of Ethnopharmacology, 85*(2), 201-206.

678 Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for
679 2010 and 2030. *Diabetes research and clinical practice, 87*(1), 4-14.

680 Siracusa, L., Kulisic-Bilusic, T., Politeo, O., Krause, I., Dejanovic, B., & Ruberto, G. (2011). Phenolic
681 composition and antioxidant activity of aqueous infusions from *Capparis spinosa* L. and
682 *Crithmum maritimum* L. before and after submission to a two-step in vitro digestion model.
683 *Journal of agricultural and food chemistry, 59*(23), 12453-12459.

684 Sperling, L. S., Mechanick, J. I., Neeland, I. J., Herrick, C. J., Despres, J. P., Ndumele, C. E., . . . Grundy,
685 S. M. (2015). The CardioMetabolic Health Alliance: Working Toward a New Care Model for the
686 Metabolic Syndrome. *J Am Coll Cardiol, 66*(9), 1050-1067. doi: 10.1016/j.jacc.2015.06.1328

687 Tlili, N., Khaldi, A., Triki, S., & Munné-Bosch, S. (2010). Phenolic compounds and vitamin
688 antioxidants of caper (*Capparis spinosa*). *Plant foods for human nutrition, 65*(3), 260-265.

689 Towler, M. C., & Hardie, D. G. (2007). AMP-activated protein kinase in metabolic control and
690 insulin signaling. *Circulation research, 100*(3), 328-341.

691 Waltenberger, B., Mocan, A., Šmejkal, K., Heiss, E. H., & Atanasov, A. G. (2016). Natural products
692 to counteract the epidemic of cardiovascular and metabolic disorders. *Molecules, 21*(6), 807.

693 Xiao, J. (2014). Natural polyphenols and diabetes: understanding their mechanism of action.
694 *Current medicinal chemistry, 22*(1), 2-3.

695 Yanardag, R., Ozsoy-Sacan, O., Bolkent, S., Orak, H., & Karabulut-Bulan, O. (2005). Protective
696 effects of metformin treatment on the liver injury of streptozotocin-diabetic rats. *Human &*
697 *experimental toxicology, 24*(3), 129-135.

698 Zafar, M., & Naqvi, S. N.-u.-H. (2010). Effects of STZ-Induced Diabetes on the Relative Weights of
699 Kidney, Liver and Pancreas in Albino Rats: A Comparative Study. *International Journal of*
700 *Morphology, 28*(1), 135-142.

701 Zengin, G. (2016). A study on in vitro enzyme inhibitory properties of *Asphodeline anatolica*:
702 New sources of natural inhibitors for public health problems. *Industrial Crops and Products, 83*,
703 39-43.

704 Zengin, G., Locatelli, M., Ceylan, R., Aktumsek, A. (2016). Anthraquinone profile, antioxidant and
705 enzyme inhibitory effect of root extracts of eight Asphodeline taxa from Turkey: can
706 Asphodeline roots be considered as a new source of natural compounds? *Journal of Enzyme*
707 *Inhibition and Medicinal Chemistry*, 31, 754-759.
708

709 **Table 1.** Phenolic components of *Capparis spinosa* (μg/mg extract) *

Compounds	***C. spinosa* leaf extracts**			***C. spinosa* commercial salted buds extracts**			***C. spinosa* fresh buds extracts**		
	Soxhlet	**Decoction**	**Microwave**	**Soxhlet**	**Decoction**	**Microwave**	**Soxhlet**	**Decoction**	**Microwave**
Gallic acid	nd	nd	nd	nd	nd	nd	0.20±0.05	0.14±0.02	0.15±0.03
Catechin	0.14±0.02	nd	2.75±0.37	nd	nd	1.98±0.24	0.47±0.06	nd	nd
Chlorogenic acid	0.34±0.04	nd	1.01±0.21	nd	nd	nd	nd	nd	nd
p-OH benzoic acid	0.24±0.07	0.10±0.04	nd	0.76±0.12	0.35±0.05	nd	0.27±0.02	0.27±0.03	0.27±0.02
Vanillic acid	0.19±0.05	nd	0.14±0.01	0.13±0.02	nd	nd	nd	nd	nd
Epicatechin	nd	nd	0.36±0.05	nd	nd	3.87±0.41	nd	0.11±0.01	0.12±0.02
Syringic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-OH benzoic acid	nd	nd	nd	0.13±0.01	nd	nd	0.19±0.01	0.39±0.04	0.44±0.02
3-OH-4-MeO benzaldehyde	nd	nd	nd	0.39±0.05	0.08±0.01	0.72±0.12	0.07±0.01	0.10±0.01	0.08±0.01
p-coumaric acid	0.39±0.03	0.12±0.02	0.17±0.02	0.41±0.06	0.21±0.06	0.09±0.01	nd	nd	0.09±0.01
Rutin	32.32±5.45	19.93±2.02	37.69±4.25	17.00±1.98	26.23±3.21	1.96±0.23	15.81±1.87	18.92±1.54	18.73±1.28
Sinapic acid	0.07±0.01	nd	nd	0.08±0.01	nd	nd	0.09±0.01	0.10±0.02	nd
t-Ferulic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd
Naringin	0.49±0.09	0.34±0.04	0.59±0.06	0.42±0.05	nd	0.07±0.01	nd	0.17±0.01	0.17±0.02

2,3-diMeO benzoic acid	1.09±0.14	0.39±0.03	0.63±0.09	nd	0.52±0.07	nd	0.41±0.03	0.24±0.04	0.21±0.02
Benzoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd
o-Coumaric acid	nd	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin	0.40±0.11	0.15±0.02	0.13±0.03	nd	nd	nd	nd	nd	nd
t-Cinnamic acid	0.18±0.08	nd	nd	0.22±0.06	nd	0.14±0.01	0.15±0.02	0.17±0.03	0.15±0.01
Naringenin	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total (μg/mg extract)	**35.85**	**21.03**	**43.46**	**19.53**	**27.37**	**8.82**	**17.69**	**20.61**	**20.41**

nd: not detected; *Values expressed are means ±SD of three experiments

721 **Table 2.** Enzyme inhibitory potentials of *Capparis spinosa* extracts

Samples	**Amylase Inhibition (mmolACAE/g extract)**	**Glucosidase Inhibition (mmolACAE/g extract)**
Fresh buds decoction	0.210±0.01*	2.63±0.07
Fresh buds soxhlet	0.171±0.01	10.31±0.36
Fresh buds MW	0.190±0.01	1.31±0.12
Leaf decoction	0.190±0.01	2.76±0.10
Leaf soxhlet	0.155±0.01	12.57±0.45
Leaf MW	0.170±0.01	1.76±0.05
Salted buds Decoction	0.102±0.01	11.89±0.22
Salted buds soxhlet	0.161±0.01	19.53±0.16
Salted buds MW	0.116±0.01	21.09±0.48

722 * Values expressed are means ±SD of three experiments. ACAE: acarbose equivalents.

723

724

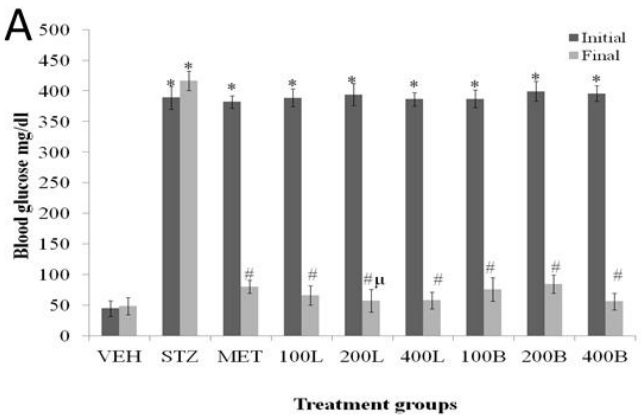
725

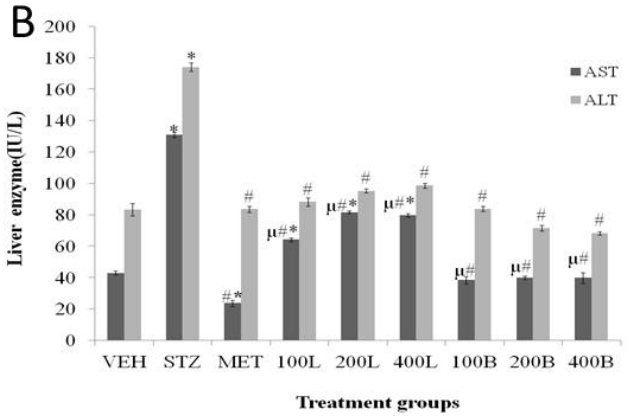
726

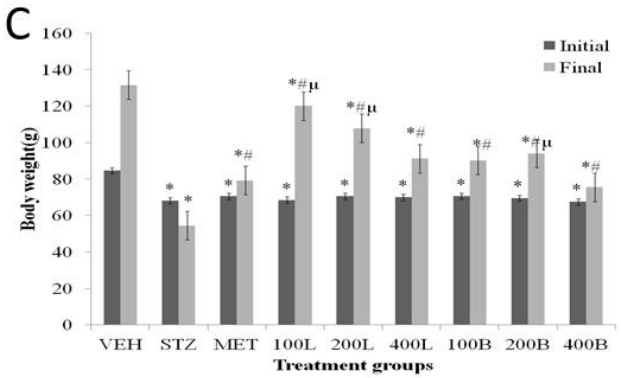
727 **Table 3.** Urea and creatinine levels, lipid profile parameters.

Groups	Urea (mmol/L)	Creatinine (µmol/L)	TC (mmol/L)	Tg (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
VEH	4.48±0.15	52.00±1.83	4.14±0.15	1.78±0.12	1.20±0.17	2.74±0.20
STZ	19.27±0.17*	173.17±1.72*	10.61±0.15*	11.89±0.10*	0.38±0.05*	12.83±0.34*
MET	6.55±0.21#	43.33±1.38#	2.58±0.15*#	1.04±0.15#	0.80±0.17#	1.01±0.10*#
100L	4.06±0.08#	53.17±1.41#	5.37±0.52*#µ	2.05±0.43#	0.95±0.06#	3.77±0.28*#µ
200L	6.250.09#	68.67±1.81#µ	4.99±0.35#µ	3.09±0.64#µ	1.13±0.07#	3.82±0.26*#µ
400L	5.42±0.19#	68.00±1.58#µ	3.97±0.59#µ	3.58±0.42*#µ	1.15±0.11#	3.48±0.28#µ
100B	7.00±0.18#	67.17±1.26#µ	3.86±0.27#	2.32±0.31#µ	1.96±0.14*#µ	2.24±0.43#µ
200B	6.59±0.16#	73.00±1.30#µ	3.51±0.36#	2.74±0.45#µ	1.91±0.11*#µ	1.61±0.20#
400B	5.9±0.11#	74.67±1.68#µ	3.58±0.29#	2.22±0.40#µ	1.93±0.13*#µ	1.69±0.11#

728 Mean ±SE.M. Comparisons are: *p < 0.05 versus VEH, #p < 0.05 versus STZ, µp < 0.05 versus MET VEH: Vehicle, STZ: Streptozocin, MET: Metformin,
729 TC: Total cholesterol, Tg: Triglyceride, HGS: High density lipoprotein, LDL: Low density lipoprotein, L: *C. spinosa* leaf, B: *C. spinosa* bud, number of
730 animals per group=6.

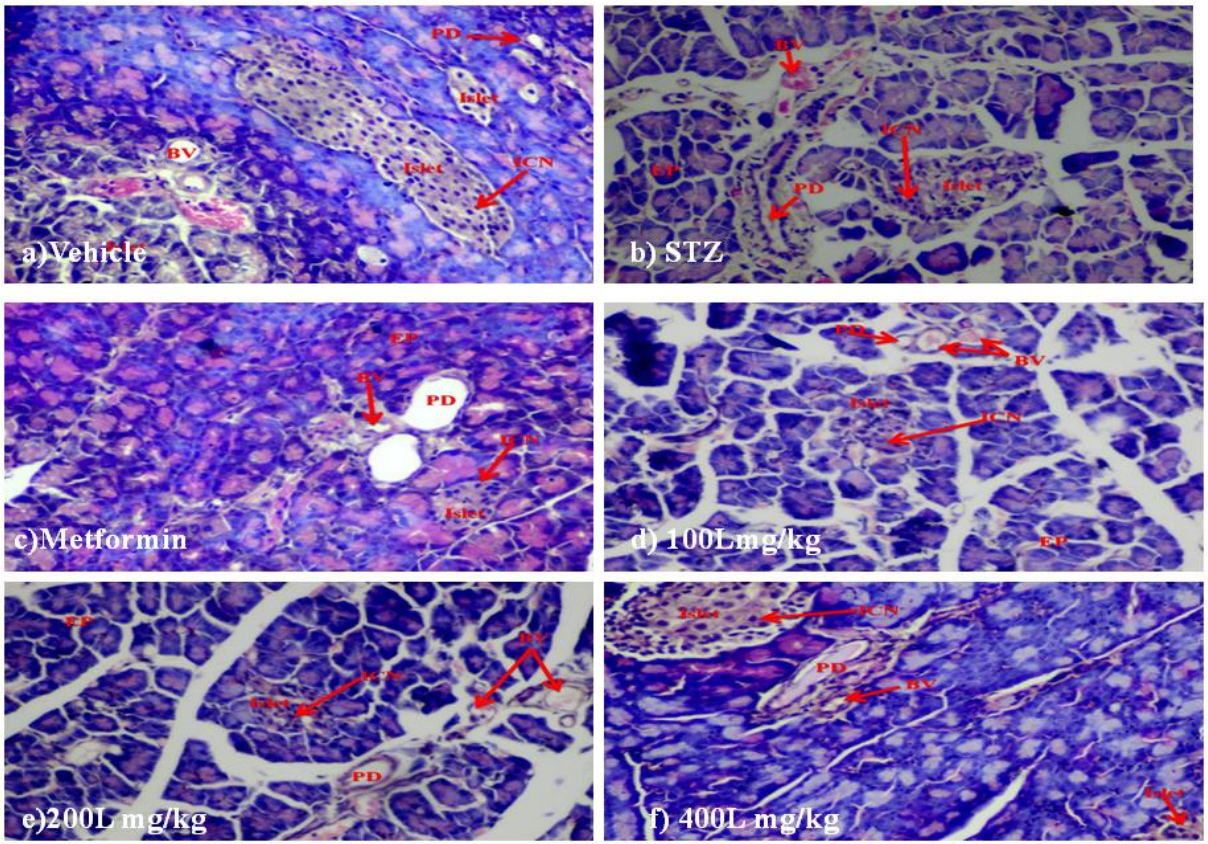

A
Blood glucose mg/dl
Initial
Final
VEH STZ MET 100L 200L 400L 100B 200B 400B
Treatment groups


B
Liver enzyme(IU/L)
AST
ALT
VEH STZ MET 100L 200L 400L 100B 200B 400B
Treatment groups


C
Body weight(g)
Initial
Final
VEH STZ MET 100L 200L 400L 100B 200B 400B
Treatment groups

731

Figure 1. (**A**) Effect of *C. spinosa* on body glucose in streptozocin-induced diabetic rats. Each bar represents Mean ±S.E.M, Differences between experimental groups (STZ, MET, L or B) and normal control (VEH) marked by * (post hoc test: $p < 0.05$), differences between MET, L or B and STZ marked by # ($p < 0.05$), differences between L or B and MET marked by μ ($p<0.05$), number of rats per treatment group = 6; VEH: Vehicle, STZ: Streptozocin, MET: Metformin, L: *C. spinosa* leaf, B: *C. spinosa* bud. (**B**) Effect of *C. spinosa* on liver enzymes in streptozocin-induced diabetic rats. Each bar represents Mean ±S.E.M, Differences between experimental groups (STZ, MET, L or B) and normal control (VEH) marked by * (post hoc test: $p < 0.05$), differences between MET, L or B and STZ marked by # ($p < 0.05$), differences between L or B and MET marked by μ ($p<0.05$), number of rats per treatment group = 6; VEH: Vehicle, STZ: Streptozocin, MET: Metformin, L: *C. spinosa* leaf, B: *C. spinosa* bud. **(C):** Effect of *C. spinosa* on body weight in streptozocin-induced diabetic rats. Each bar represents Mean ±S.E.M, Differences between experimental groups (STZ, MET, L or B) and normal control (VEH) marked by * (post hoc test: $p < 0.05$), differences between MET, L or B and STZ marked by # ($p < 0.05$), differences between L or B and MET marked by μ ($p<0.05$), number of rats per treatment group = 6; VEH: Vehicle, STZ: Streptozocin, MET: Metformin, L: *C. spinosa* leaf, B: *C. spinosa* bud.



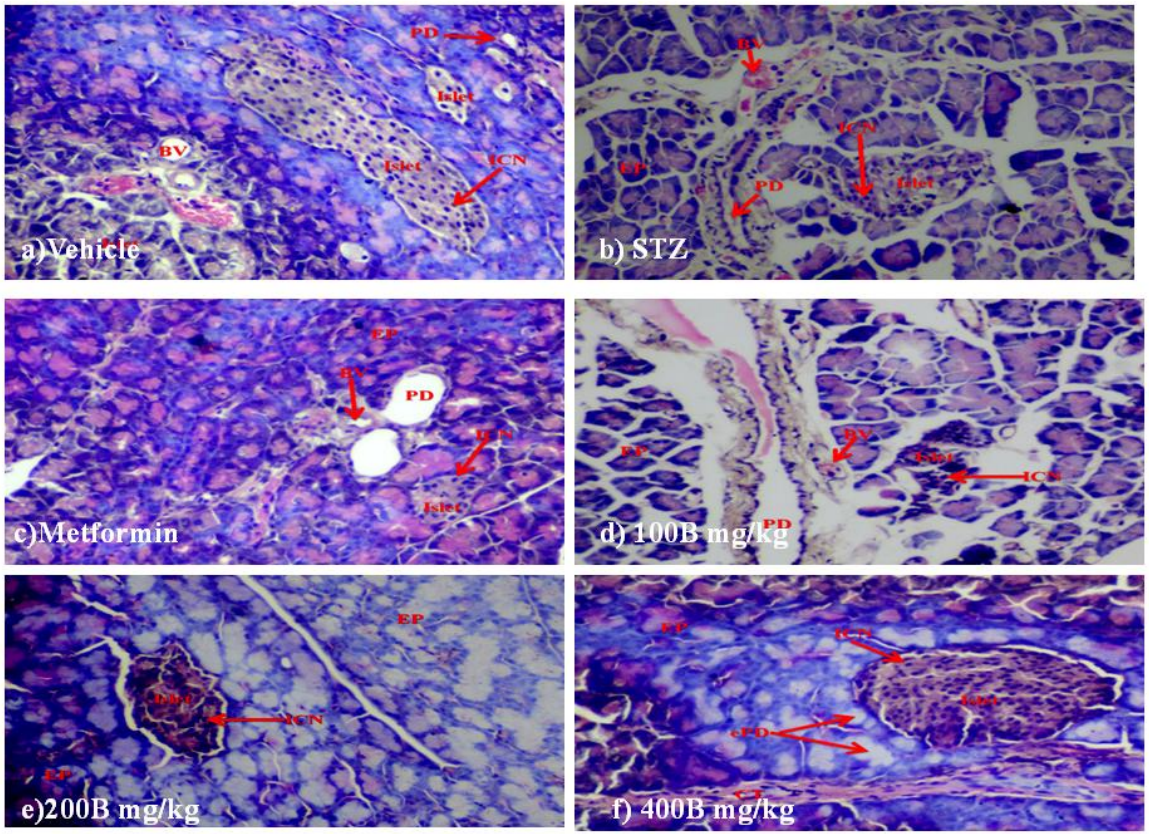
747

748 **Figure 2a-f.** Pancreas of rats showing islet of Langerhans (Islet) with deep staining nuclei (ICN), exocrine pancreas (EP), blood vessels (BV), and

749 pancreatic duct (PD), *C. spinosa* leaf (L), H&E x160, Scale bar- 29µm.

750

751



752

753 **Figure 3a-f**. Pancreas of rats in the *C. spinosa* bud group, showing islet of Langerhans (Islet) with shrunken pale staining nuclei (ICN) exocrine

754 pancreas (EP), engorged blood vessels (BV) and pancreatic duct (PD), *C. spinosa* bud (B) H&E x160, Scale bar- 29µm.

755