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Research Article
Anti-Diabetic Potential of Common Saudi Medicinal Herbs *Commiphora molmol* and *Astragalus membranaceus* Extracts in Diabetic Rats

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Abstract

Background and Objective: *Commiphora* and *Astragalus* are traditional Mediterranean and Chinese medicine, respectively. In the traditional system of medicine, both are extensively used in the treatment of diabetes. This study examined the antidiabetic potential of different extracts of *Commiphora molmol* and *Astragalus membranaceus* to provide novel information to improve the clinical use of these plants. **Materials and Methods:** Swiss albino mice were used for dose-ranging study utilizing OECD 425 guidelines. The male sprague dawley rats were injected with streptozotocin to induce diabetes. Later, the animals were treated by the alcoholic, hydroalcoholic and aqueous extract of *Commiphora molmol* and *Astragalus membranaceus.* **Results:** A dose-ranging study revealed that at the dose of 2000 mg kg^{-1}, no mortality or any abnormal behaviours were found in tested animals of any treated extract. Significant antidiabetic activity of different extracts was observed by the gain of body weight, reduction of blood glucose level and decrease in serum and tissue biomarkers. This study showed that different extract of *Commiphora* and *Astragalus* contributes in preventing diabetes in rats. The hydroalcoholic extract of *Astragalus membranaceus* showed maximum anti-diabetic potential among all other extracts examined. **Conclusion:** *Commiphora* and *Astragalus* may be useful as an adjuvant remedy in the treatment of type 2 diabetes. However, as a result of the limited number of existing studies, further high-quality research is necessary before decisive conclusions can be made.

Key words: *Commiphora molmol*, *Astragalus membranaceus*, diabetes, medicinal plant, biomarkers, oxidative stress markers

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbal medicines having hypoglycemic properties have been castoff in traditional medicine and traditional healing programs around the world over the years[1]. A huge population of medicinal plants has been cited in the Indian medicine system including traditional medicines for the treatment of diabetes, which work independently or in combination and thus have been of great interest[2].

For medicinal purposes plants have been used since long before the prehistoric era. Evidence provides information that Indian Vaids, Unani Hakims, Mediterranean and European cultures were using naturally derived herbs as medicine for over 4000 years.

Traditional systems of medicine remain to be commonly practised on many occasions. inadequate supply of drugs, rise in population, costly treatments, side effects of numerous synthetic drugs and advance of resistance to currently practised drugs for different diseases have directed to increased prominence on the use of natural origin plant materials as a source of medicines for different category of human ailments[3].

As per statistics available, around three-quarters of the entire world population depend mainly on plants and plant extracts for their daily health care requirements. More than 30% of the total plant species, at a time or additional, were utilized for medicinal purposes. It has been assessed, that in developed nations such as the United States, plant-derived drugs establish as much as 25% of the total medicine, while in rapidly developing countries such as China and India; the contribution of herbal drugs is around 80%. Thus, the commercial importance of medicinal plants is very high in countries for instance India than in the remaining part of the world. These countries offer two-third of the plants utilized in modern system of medicine and the medical care of rural population completely depend on indigenous systems of medicine[4].

Medication with medicinal plants is well-thought-out to be very safe as there are minimal or no side effects. These medications are derived from nature, which serves as the biggest advantage. The unique feature is that the use of herbal medicine is independent of any gender and age group. The basic reason is that herbal medicines are gaining popularity across the world. These herbs which possess medicinal properties provide a rationale for the treatment of many diseases, which are otherwise considered difficult to treat[5]. Today, these herbal medicines are the representing safety when compared to synthetic and semi-synthetic drugs, which are observed as unsafe for the environment and human being[6].

Diabetes is considered to be one of the leading causes of death and morbidity worldwide and its incidence is rising worldwide[7]. According to the International Diabetes Federation, there are currently 246 M people with diabetes, which will increase to 380 M by 2025[8]. Diabetes is represented as a group of physical disorders characterized by hyperglycaemia, the cause of a decrease in insulin production (I) or insufficient insulin use (type-II)[9]. There has been a growing demand for the use of medicinal herbal products with strong antidiabetic activity due to low cost, easy availability and reduced side effects. There has been a huge prevalence in the use of alternative anti-diabetic drugs in many parts of the world[10,11].

Commiphora molmol (CM), also recognized in folk medicine as myrrh. The oleo-gum resin obtained from CM is found in the bark of trees of the burseraceae family. In traditional medicine, they have been used for many years, originally found in North Africa, Arabia and Northern Somalia[12]. The CM has been described to contain alcohol-soluble gum, water-soluble gum and flexible oil. The gum contains proteins and polysaccharides and the essential oils are made up of terpenes, steroids and sterols. Also, the aroma of CM is derived from the Furano sesquiterpenes. Numerous experimental studies have reported the various therapeutic uses associated with the oleo-gum resin of CM. The resin has been used to treat intestinal disorders, wounds, cough and asthma[13]. In addition, the oleo-gum resin of CM is used in the treatment of inflammation, gingivitis and fascioliasis[14-16]. Similarly, studies have shown that myrrh displays antipyretic and hypoglycemic properties[14-17].

Astragalus membranaceus (AM), better known as membranous milk-vetch root in English, Hwanggi in Korean and Huang qi in Chinese, is considered one of the most important ingredients of adaptogenic Qi tonifying Chinese material medica[18,19]. The key elements of AM include saponins, polysaccharides, amino acids, flavonoids and trace elements. The drug has a long medical history for hundreds of years as it has been used in the treatment of chronic diseases, common weakness and increased overall health. The AM has shown the therapeutic approaches in immunodeficiency syndromes, which are associated with cancer treatment, as well as the adaptogenic effect on the kidneys and heart. In addition, ancient Chinese texts have documented the use of AM to augment the spleen and blood. Alternatively, saponins isolated from the AM is been reported to possess strong antioxidant, anti-inflammatory and hepatoprotective properties that benefit the treatment of diabetes[18]. In traditional medicine, CM and AM were widely used as anti-diabetic medications due to their hypolipidemic antioxidant and hypoglycemic properties. In traditional Unani medicine,

a combination of these plants was suggested for diabetic patients[5,20]. The current study aimed to investigate the antidiabetic potential of these 2 plants, namely, CM and AM, based on experimental screening of plants which is a major and important part of the study. Therefore, the objective of this study was to investigate comparative studies on qualitative standards and anti-diabetic activity of different extracts of CM and AM in streptozotocin-induced diabetic rats and to find out which extract has the highest efficacy. In addition, the present study aimed to explore mechanistic intervention to treat hyperglycaemia-related disorders in diabetic individuals to serve mankind.

MATERIALS AND METHODS

Study area: The study was started in September, 2020 and was finished in December, 2020, total duration used in this research was 4 months, in these 4 months, the experiment was conducted and data were processed.

Collection of plant material: Myrrh (C. molmol) resin and AM root was purchased at a commercial market (Alraz herbs Co.) in Buraidah, Saudi Arabia. *C. molmol* was obtained from Farasan Island in the Red Sea. The test material sample was authenticated by the Centre for Plant and Aromatic Medicine in Buraidah, Saudi Arabia, under the specimen number CPAM-2020/36. Plant material was broken into smaller pieces, cleaned with distilled water, dried in an incubator at 37°C and then ground into a coarse powder.

Extraction of plant products

Alcoholic extract: The resins were ground into a coarse powder and the roots were cut into little pieces with a blender. After translation, the resin and roots were extracted separately with 90% ethanol (500 mL) using a Soxhlet apparatus for 6 hrs at 40-60°C and the solution was dried using a rotary evaporation apparatus. The extracts were filtered and the filtrate was evaporated to dryness in a rotating vacuum evaporator at 50-65°C, yielding a dark-coloured molten mass. To get the powdered extract, the semisolid ethanolic extract of CM was lyophilized using a cryodos freeze drier (Adele, Dubai, UAE)[21].

Water extract: The resin and roots were separately homogenized in distilled water (100 mL) in a blender at high speed for 15 min. The homogenized mixture was ltered by using the muslin cloth about three times. The resulting aqueous extract of resin and roots was collected subjected to lyophilisation using a cryodos freeze dryer (Adele, Dubai, UAE) to yield powdered extract[22].

Hydro-alcoholic extract: In a conical flask (200 mL), powdered plant material (resin and root) was kept and 100 mL of solvents such as ethanol and water were added individually. The conical flask's aperture was covered with aluminium foil and placed in a reciprocating shaker for 24 hrs for constant agitation at 150 rev min^{-1} for exhaustive mixing and extensive elucidation of active components to dissolve in the respective solvent. At the time, the extract was filtered using a muslin cloth, followed by filter paper (Whatman no 1) and last by vacuum and pressure pump (Genix, Bahrain). To obtain a powdered extract, the solvent was extracted from the extract using a rotary vacuum evaporator and lyophilized using a cryodos freeze drier (Adele, Dubai, UAE). Finally, all of the residues were collected and used in the experiment[23].

Experimental animals: SPF male Sprague-Dawley rats, weighing 150-180 g for pharmacological screening while female Swiss albino mice (50-80 g) for dose-ranging study, were obtained from the animal house facility of Qassim University, Qassim, Kingdom of Saudi Arabia. The animals were housed in polypropylene cages following standard conditions (maintaining room temperature and 12 hrs light-dark cycle) with freely fed on commercially available pellet laboratory diet and water *ad libitum* all over the experimental period. The animals were acclimatized to laboratory environments for 7 days before preceding the experiment. The Institutional animal ethical committee (379-phuc-2016-1-12-S) has approved the experiment and was completed in compliance with good laboratory practice (GLP) regarding the handling of animals utilized for scientific purposes. All the procedures in the entire experiment were performed according to the guide for the care and use of laboratory animals[24].

Experiment design

Dose-ranging study: The acute oral dose-ranging study of alcoholic extract, water extract and hydro-alcoholic extract of both plants, CM and AM was investigated in Swiss albino mice using the OECD 425 guideline. Each powdered extract was administered to 4 groups (n = 6) of animals at 500, 1000, 1500 and 2000 mg/kg/p.o. On the 1st day, a single female mouse that had fasted for about 4 hrs with water *ad libitum* orally received 500 mg kg^{-1} of the crude extract. The mice were subjected to rigorous observation for the first 4, 12 and 24 hrs and then for 14 days, for general behaviour, hypersensitivity and mortality. The mice were observed for

Table 1: Treatment protocol of normal and diabetic rats with water, alcoholic and hydro-alcoholic extract of *Commiphora molmol* and *Astragalus membranaceus*

Groups	Treatments	Dose
NC	Normal saline	1 mL^{-1} day $p.o^{-1}$.
STZ	Streptozotocin treated	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.
STD	Standard treated	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+glibenclamide (0.5 mg kg^{-1}), p.o. for 21 days
ACM	Diabetic rats with alcoholic extract of *Commiphora molmol*	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+alcoholic extract of *Commiphora molmol* (200 mg/kg/p.o. for 21 days)
WCM	Diabetic rats with water extract of *Commiphora molmol*	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+water extract of *Commiphora molmol* (200 mg/kg/p.o. for 21 days)
HCM	Diabetic rats with hydro-alcoholic extract of *Commiphora molmol*	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+hydro-alcoholic extract of *Commiphora molmol* (200 mg/kg/p.o for 21 days)
AAM	Diabetic rats with alcoholic extract of *Astragalus membranaceus*	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+alcoholic extract of *Astragalus membranaceus* (200 mg/kg/p.o. for 21 days)
WAM	Diabetic rats with water extract of *Astragalus membranaceus*	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+water extract of *Astragalus membranaceus* (200 mg/kg/p.o. for 21 days)
HAM	Diabetic rats with hydro-alcoholic extract of *Astragalus membranaceus*	Streptozotocin (50 mg kg^{-1} in 0.1 mL citrate buffer pH 4.5) once i.p.+hydro-alcoholic extract of *Astragalus membranaceus* (200 mg/kg/p.o. for 21 days)

NC: Normal saline, STZ: Streptozotocin treated, STD: Standard treated, ACM: Diabetic rats with alcoholic extract of *Commiphora molmol*, WCM: Diabetic rats with water extract of *Commiphora molmol*, HCM: Diabetic rats with hydroalcoholic extract of *Commiphora molmol*, AAM: Diabetic rats with alcoholic extract of *Astragalus membranaceus*, WAM: Diabetic rats with water extract of *Astragalus membranaceus* and HAM: Diabetic rats with hydroalcoholic extract of *Astragalus membranaceus*

gross behavioural changes such as hair erection, loss of appetite, tremors, lacrimation, salivation, diarrhoea, hypersensitivity reaction, food and water intake, hyperactivity, lethargy, drowsiness, tremors, convulsions, dizziness, mortality and other signs of toxicity exhibition for 14 days. The same process is repeated with each extract of both plants with an increase in dose as 1000, 1500 and 2000 mg/kg/p.o [25].

Induction of diabetes: Diabetes mellitus was induced in overnight fasted male Sprague-Dawley rats by administering a single intraperitoneal injection of streptozotocin (50 mg kg^{-1} b.wt., in 0.1 M citrate-buffered saline, pH 4.5). All the animals were fed with a standard laboratory pellet diet and free access to water after 30 min of administration of STZ. The existence of diabetic symptoms (polydipsia, polyuria and weight loss) verified the induction of diabetes and blood glucose levels were assessed three days after STZ injection. The animals displaying Fasting Blood Glucose (FBG) levels above 300 mg dL^{-1} were selected for the present investigation[26].

Experimental protocol: A total of 54 male Sprague-Dawley rats were divided into 9 groups of 6 animals each. Except for the NC and STZ groups, the dosing of different extracts of each plant in the other groups began when the rats became diabetic, i.e. when their glucose level exceeded 300 mg dL^{-1} for three days in a row. The treatment began on the fourth day of STZ injection, which was deemed day one. Different powdered extracts of CM and AM were provided orally at 200 mg kg^{-1} day in the form of a suspension combined with 1% sodium carboxymethyl cellulose (Na-CMC) in double-distilled water. The extract was delivered through an oral gavage tube daily for 21 days. The dosing was carried out following the protocol outlined in Table 1.

On the 22nd day, the final body weight of each animal was noted and blood glucose levels were measured by tail vein, then after the animals were anaesthetized with thiopentone sodium, blood was collected from the retro-orbital plexus in EDTA and non-EDTA tubes, the former is used in the estimation of different parameters, while the latter was used to obtain serum, rats were dissected by cervical dislocation.

Parameters estimated

Body weight: The body weights of rats of each group were measured on 1, 7, 14 and 21 days of treatment by using an electronic balance (Zontech, Riyadh).

Blood glucose estimation: On the 1, 7, 14 and 21 days of therapy, blood samples were collected through tail vein puncture and examined using a glu check glucometer. Blood glucose levels were measured in milligrams per deciliter (mg dL^{-1}).

Serum collection: The blood was maintained in a standing position for 20 min to allow it to clot and the serum was separated from the clotted blood by centrifuging it at 5000 rpm at 4°C for 15 min before being used to estimate biomarkers.

Biochemical assay of glucose-6-phosphatase activity in the liver: The activity of glucose-6-phosphatase in the liver was determined using a conventional procedure[27].

Biochemical assay of glucose-6- phosphate dehydrogenase in the liver: The activity of the liver glucose-6-phosphate dehydrogenase was measured spectrophotometrically[27].

Assay of hexokinase in the liver: The enzyme activity of hexokinase was measured spectrophotometrically at 340 nm using Chou and Wilson method[27].

Glycated haemoglobin: The standard diagnostic kit (Abcam, USA) method was used to calculate glycated haemoglobin from the whole blood[27].

Serum insulin and tissue glycogen

Biochemical assay of glycogen content: The level of glycogen in the liver was determined biochemically using the standard technique[27].

Serum insulin level: The Enzyme-Linked Immunosorbent Assay (ELISA) method was used to calculate serum insulin[27].

Estimation of oxidative stress markers

Biochemical and enzyme estimation: Lipid Peroxide (LPO), Catalase (CAT), Superoxide Dismutase (SOD) activity, Glutathione Peroxidase (GPx) activity, GSH, Glutathione Reductase (GR) and Glutathione-S-Transfer (GST) was measured[28-33].

Statistical analysis: Results/data were expressed as Mean±SEM. Data were subjected to a one-way analysis of variance along with Dunnett's multiple comparisons test. The treatment groups were compared with the control (STZ) group while the control (STZ) group was compared with the NC group. All statistics were carried out using GraphPad Prism 8 software (v.8; GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Dose-ranging study: The dose-ranging study lasted for 14 days and was designed to estimate the adverse/toxic effects of a single dose of the different extracts of CM and AM in a graded range of doses. Throughout the experimental period, no mortality or adverse effects or alternations such as hair erection, loss of appetite, tremors, lacrimation, salivation, diarrhoea, hypersensitivity reaction, food and water intake, hyperactivity, lethargy, drowsiness, tremors, convulsions, dizziness, mortality and other signs of toxicity were reported by any extract of either drug.

Bodyweight and glucose level: STZ group did show a significant weight loss 168.12±1 g as compared to corresponding values of Normal controls 207.17±2.83 g during the 21 days experimental period while HCM and HAM treated rats gained maximum weight of 196.611±2.4 g (17%) and 198.93±3.41 g 18% ($p<0.0001$) respectively as compared to STZ rats. However, the weight gain in other treated rats was significantly also higher than diabetic controls in Fig. 1a.

As shown in Fig. 1b on day 0 the Plasma glucose levels showed no significant intra-group variation. STZ treated group maintained significantly higher plasma glucose levels as compared to normal control rats. Twenty four hours and 21 days after administration of STZ, the plasma glucose level was increased 241.83±4.56 and 288.21±5.37 mg dL^{-1}, respectively ($p<0.001$) while non-diabetic controls remained unchanged (Fig. 1b). Oral administration of the WCM and HAM extracts showed a maximum decrease in plasma glucose levels to 281.49±3.07 (37%), 148.47±2.94 (52%) and 292.82±3.61 (38%), 130.71±1.98 (55%) mg dL^{-1} on the 14th, 21st day of the experiment, respectively ($p<0.0001$, respectively).

Enzymatic analysis: From the results depicted in Table 2, it was observed that Streptozotocin Treated Rats (STZ) showed a highly significant increase ($p<0.001$) in G-6-P 52.1±1.66 mg of IP g^{-1} of tissue and HbA1c 10.89±0.32%, significant decrease($p<0.001$) of in G-6-PD 4.11±0.08 Unit mg^{-1} of tissue, Hexokinase 121.72±2.64 µg mg^{-1} of tissue, Insulin 3.63±0.04 µ IU mL^{-1}, Glycogen 21.09±0.09 µg mg^{-1} of liver tissue level when compared corresponding values of the NC.

The maximum decrease ($p<0.001$) in G-6-P levels was observed in HAM 33.21±1.48 mg of IP g^{-1} and HCM 35.79±1.44 mg of IP g^{-1} groups as compared to the STZ group. Other treatments also showed a similar effect.

An increase in G-6-PD in HCM 11.05±0.44 unit mg^{-1} of tissue ($p<0.001$), HAM 12.88±0.42 unit mg^{-1} of tissue ($p<0.001$), while the marginal increase was observed with other treatments as compared to STZ treated rats.

Hexokinase levels were likewise elevated in HCM (170.11±2.61 g mg^{-1} of tissue) and HAM (171.54±2.31 g mg^{-1} of tissue) ($p<0.001$), whereas WCM showed a minor rise of 28% (156.37±2.49 g mg^{-1} of tissue) ($p<0.05$).

When compared to the STZ treated group, all treatments exhibited a substantial decrease in HbA1c levels, with the HAM treated group (5.01±0.19%, $p<0.001$) and ACM (55.41±0.24%, $p<0.001$) showing the greatest decrease, while the WCM group (6.89±0.29% $p<0.01$) showing the least.

Plasma insulin levels were considerably higher in the CM and AM extract treated groups compared to STZ treatment, ACM (14.94±0.05 µIU mL^{-1} $p<0.001$), HAM

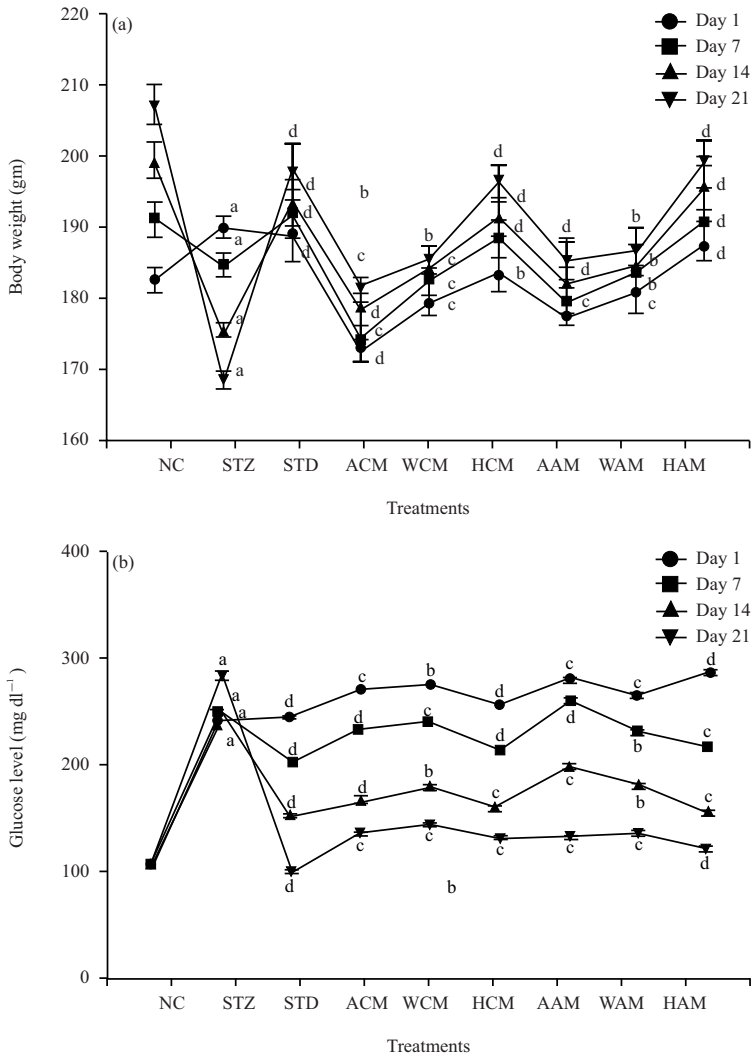

Fig.1(a-b): Effect of alcoholic extract, water extract and hydro-alcoholic extract of CM and AM on Mean, (a) Body weight level (n=6) and (b) Body weight and glucose level (n=6), in STZ induced diabetic rat

Values are expressed as Mean±SEM. [a]p<0.001 when STZ is compared with NC while [b]p<0.05, [c]p<0.01 and [d]p<0.001, when treated groups were compared to STZ group, NC: Normal saline, STZ: Streptozotocin treated, STD: Standard treated, ACM: Diabetic rats with alcoholic extract of *Commiphora molmol*, WCM: Diabetic rats with water extract of *Commiphora molmol*, HCM: Diabetic rats with hydroalcoholic extract of *Commiphora molmol*, AAM: Diabetic rats with alcoholic extract of *Astragalus membranaceus*, WAM: Diabetic rats with water extract of *Astragalus membranaceus* and HAM: Diabetic rats with hydroalcoholic extract of *Astragalus membranaceus*

(15.62±0.06 μIU mL^{-1} p<0.001) and WCM (13.09±0.05 μIU mL^{-1} p<0.05).

Tissue glycogen levels were found to be significantly higher in HCM (48.87±0.26 μg mg^{-1} of liver tissue, p<0.001), HAM (48.32±0.19 μg mg^{-1} of liver tissue group p<0.001) and WCM (42.21±0.23 μg mg^{-1} of liver tissue p<0.05) as compared to the STZ group.

Inflammatory cytokines: Streptozotocin Treated Rats (STZ) showed a highly significant increase (p<0.001) in IL-6 (37.04±0.64 pg mL^{-1}), TNF- α (62.29±0.45 pg mL^{-1}), TGF-β1 (97.04±1.13 pg mg^{-1} protein), tissue FN (72.38±1.67 pg mg^{-1} protein) when compared with the Normal Control (NC).

As shown in Fig. 2a, all treatments reduced IL-6 levels significantly, but the maximum decrease was observed in the HAM treated group (17.62±0.22 (p<0.001) and HCM 19.36±0.2 pg mL^{-1} (p0.001), while the WAM and WCM groups showed slightly lower decreases of 26.79 ±0.21 (p<0.01) and 25.21±0.18 pg mL^{-1} (p<0.05) respectively.

When compared to STZ, all treatments resulted in a considerable drop in TNF-. HCM had the greatest effect (29.42±0.15 pg mL^{-1} p<0.001), followed by HAM

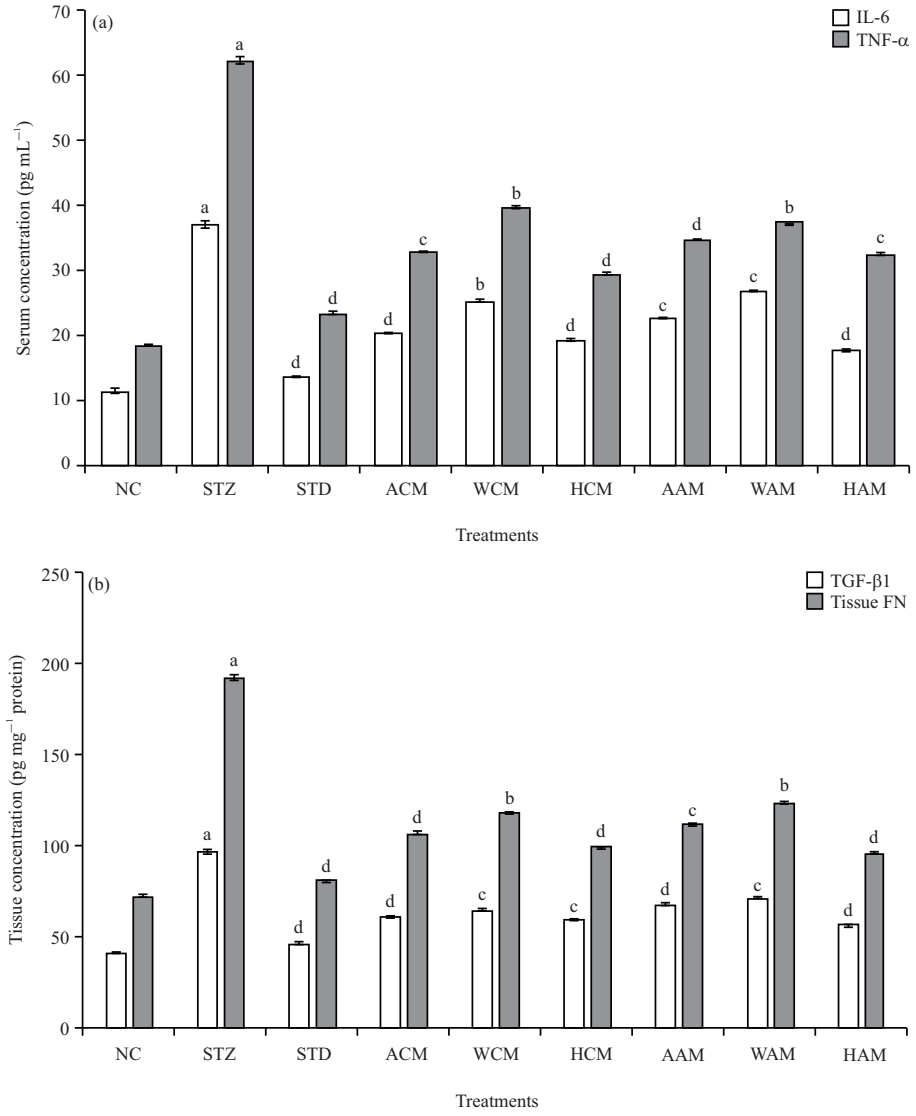

Fig. 2(a-b): Effect of alcoholic extract water extract and hydro-alcoholic extract of CM and AM on inflammatory cytokines (a) IL-6 and TNF-α level (n = 6) and (b) Tissue TGF-β1 and FN level (n = 6), is STZ induced diabetic rat

Values are expressed as Mean±SEM [b]P<0.001 When STZ is compared with NC while [a]p<0.05, [c]p<0.01 and [d]p<0.001 when treated groups where compared to STZ group, NC: Normal Saline, STZ: Streptozotocin treated, STD: Standard treated, ACM: Diabetic rats with alcoholic extract of *Commiphora molmol*, WCM: Diabetic rats with water extract of *Commiphora molmol*, HCM: Diabetic rats with hydroalcoholic extract of *Commiphora molmol*, AAM: Diabetic rats with alcoholic extract of *Astragalus membranaceus*, WAM: Diabetic rats with water extract of *Astragalus membranaceus* and HAM: Diabetic rats with hydroalcoholic extract of *Astragalus membranaceus*

(32.53±0.15 pg mL^{-1} p<0.001), while WCM had the least effect (39.79± 0.13 pg mL^{-1} p<0.05) (Fig. 2a).

Figure 2b showed that hydro-alcoholic extracts of AM and CM had a highly significant decrease in TGF-1 level in HAM (56.31±0.55 pg mg^{-1} protein p<0.001), HCM (59.85±0.57 pg mg^{-1} protein p<0.001), while water extracts of both CM and AM had a slightly lower decline in TGF-1, WCM (64.21±0.59 pg mg^{-1} protein p<0.01) and WAM Tissue FN decreased significantly in the HAM (96.2±0.83 pg mg^{-1} protein p<0.001), HCM (99.41±0.35 pg mg^{-1} protein p<0.001), WAM (123.73±0.72 pg mg^{-1} protein p<0.05), AAM (42%) and WCM (118.95±0.71 pg mg^{-1} protein p<0.05) groups.

Oxidative stress markers: As depicted in Table 3 Streptozotocin Treated Rats (STZ) showed a highly significant decrease (p<0.001) in tissue SOD (5.18±0.11 µmol/min/mg protein), GPx (7.26±0.09 nmol/min/mg

Table 2: Effect of alcoholic extract, water extract and hydro-alcoholic extract of CM and AM on glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, hexokinase, serum insulin, tissue glycogen level (n=6) in STZ induced diabetic rat

Treatment groups	G-6-P (mg of IP g^{-1} of tissue)	G-6-PD (U mg^{-1} of tissue)	Hexokinase (µg mg^{-1} of tissue)	HbA1c (%)	Insulin (µIU Ml^{-1})	Glycogen (µg mg^{-1} of liver tissue)
NC	27.04±1.27	17.03±0.73	180.34±3.61	3.24±0.21	18.84±0.11	54.61±0.26
STZ	52.11±1.66[a]	04.11±0.08[a]	121.72±2.64[a]	10.89±0.32[a]	03.63±0.04[a]	21.09±0.09[a]
STD	29.63±1.21[d]	16.57±0.45[d]	183.90±3.11[d]	3.71±0.22[d]	17.99±0.06[d]	52.38±0.24[d]
ACM	37.21±1.59[b]	10.88±0.34[d]	167.62±2.63[d]	5.41±0.24[b]	14.94±0.05[d]	46.01±0.27[c]
WCM	40.02±1.49[c]	08.72±0.29[c]	156.37±2.49[b]	6.89±0.21[c]	13.09±0.05[b]	42.21±0.23[b]
HCM	35.79±1.44[d]	11.05±0.44[d]	170.11±2.61[d]	5.92±0.27[d]	15.04±0.07[b]	48.87±0.26[d]
AAM	36.44±1.21[d]	10.12±0.21[c]	168.07±2.38[d]	5.83±0.24[c]	15.32±0.07[d]	46.01±0.18[c]
WAM	38.22±1.72[b]	09.61±0.28[c]	159.84±2.44[d]	6.42±0.23[c]	14.73±0.05[d]	44.94±0.23[c]
HAM	33.21±1.48[d]	12.88±0.42[d]	171.54±2.31[d]	5.01±0.19[d]	15.62±0.06[d]	48.32±0.19[d]

Values are expressed as Mean±SEM, [a]p<0.001 when STZ is compared with NC while [b]p<0.05, [c]p<0.01 and [d]p<0.001, when treated groups were compared to STZ group NC: Normal Saline, STZ: Streptozotocin treated, STD: Standard treated, ACM: Diabetic rats with alcoholic extract of *Commiphora molmol*, WCM: Diabetic rats with water extract of *Commiphora molmol*, HCM: Diabetic rats with hydroalcoholic extract of *Commiphora molmol*, AAM: Diabetic rats with alcoholic extract of *Astragalus membranaceus*, WAM: Diabetic rats with water extract of *Astragalus membranaceus* and HAM: Diabetic rats with hydroalcoholic extract of *Astragalus membranaceus*

Table 3: Effect of alcoholic extract, water extract and hydro-alcoholic extract of CM and AM on oxidative stress markers level (n=6) in STZ induced diabetic rat

Treatment Groups	SOD(µmole/min/ mg protein)	GPx (nmol/min/ mg protein)	GST (µmol/min/ mg protein)	GR (nmol/min/ mg protein)	CAT (µmol/min/ mg protein)	GSH (µmol g^{-1} tissue)	LPO (nmol MDA formed h/g tissue)
NC	11.33±0.43	14.41±0.21	23.04±0.13	07.25±0.11	212.29±6.14	54.51±1.17	03.49±0.06
STZ	05.18±0.11[a]	07.26±0.09[a]	11.45±0.04[a]	03.29±0.03[a]	107.71±2.77[a]	21.29±0.49[a]	09.24±0.12[a]
STD	10.09±0.36[d]	13.22±0.16[d]	21.44±0.09[d]	07.02±0.10[d]	203.14±6.02[d]	51.63±1.27[d]	03.85±0.04[d]
ACM	08.39±0.28[d]	10.34±0.18[d]	16.37±0.07[c]	06.27±0.08[d]	178.38±5.41[d]	42.18±1.23[c]	04.88±0.04[d]
WCM	07.82±0.26[c]	09.27±0.13[c]	16.22±0.08[c]	05.73±0.09[b]	163.05±5.70[c]	33.07±1.19[b]	04.17±0.04[d]
HCM	08.63±0.25[d]	10.46±0.17[d]	17.85±0.08[d]	06.31±0.07[c]	183.59±5.83[c]	40.64±1.21[c]	05.31±0.05[c]
AAM	07.74±0.27[d]	10.28±0.16[d]	17.01±0.06[d]	05.44±0.07[d]	172.49±5.37[c]	37.21±1.16[d]	04.63±0.03[d]
WAM	08.29±0.25[d]	08.94±0.14[c]	15.83±0.07[b]	06.15±0.07[d]	170.83±5.18[b]	35.79±1.18[c]	04.72±0.03[d]
HAM	09.13±0.23[d]	11.73±0.16[d]	18.44±0.08[d]	06.25±0.08[d]	189.36±5.56[d]	43.82±1.16[d]	05.26±0.04[c]

Values are expressed as Mean ± SEM. [a]p<0.001, when STZ is compared with NC while [b]p<0.05, [c]p<0.01 and [d]p<0.001, when treated groups were compared to STZ group, NC: Normal saline, STZ: Streptozotocin treated, STD: Standard treated, ACM: Diabetic rats with alcoholic extract of *Commiphora molmol*, WCM: Diabetic rats with water extract of *Commiphora molmol*, HCM: Diabetic rats with hydroalcoholic extract of *Commiphora molmol*, AAM: Diabetic rats with alcoholic extract of *Astragalus membranaceus*, WAM: Diabetic rats with water extract of *Astragalus membranaceus* and HAM: Diabetic rats with hydroalcoholic extract of *Astragalus membranaceus*

protein), GST (11.45±0.04 µmol min^{-1} mg^{-1} protein), GR (3.29±0.03 nmol/min/mg protein), CAT (107.71±2.77 µmol/min/mg protein) and GSH (21.29±0.49 µmol g^{-1} tissue) while highly significant increase (p<0.001) in LPO (9.24±0.12 nmol MDA formed/h/g tissue) level when compared with the normal control.

There was a significant increase in tissue SOD level in all treatments as compared to STZ. Notably HCM (08.63±0.25 µmol/min/mg protein p<0.001), HAM (09.13±0.23 µmol/min/mg protein p<0.001) showed maximum effect. The WCM (07.82±0.26 µmol/min/mg protein) and AM (07.74±0.27 µmol/min/mg protein) groups had a marginally low increase in SOD levels. All treatments led to an increase in tissue GPx level (p<0.001) notably HAM and HCM had a maximum increase of 11.73±0.16 and 10.46±0.17 nmol/min/mg protein respectively while other treatment groups also had a similar response.

The GST levels were significantly increased in all treatments as compared to STZ. Hydroalcoholic extracts of CM (17.85±0.08 µmol/min/mg protein) and AM (18.44±0.08 µmol/min/mg protein) (p<0.001) showed a highly significant increase in GST levels. On the other hand, WAM (15.83±0.07 µmol/min/mg protein) groups showed a significant increase (p<0.05) level.

When compared to the STZ group, all treatments demonstrated a significant rise in tissue GR levels. The HCM had a highly significant rise of 70% (06.31±0.07 nmol/min/mg protein p<0.001), while HAM had a significantly lesser increase (06.25±0.08 nmol/min/mg protein p<0.001).

When compared to STZ, catalase levels were significantly greater in all treatments. The HAM treated group (189.36±5.56 µmol/min/mg protein) showed a highly significant rise in catalase (p<0.001), while the WCM treated group (163.05±5.70 µmol/min/mg protein p<0.01) showed a marginally lower but substantial increase.

The GSH levels increased with all treatments, with HAM showing the greatest increase (43.82±1.16 μmol g^{-1} tissue $p<0.001$) and WCM showing the least significant increase (33.07±1.19 μmol g^{-1} tissue, $p<0.05$).

Tissue lipid peroxidase levels were significantly lower in all treatments as compared to STZ The water extract of CM (WCM) showed the greatest decrease (04.17±0.04 nmol MDA formed/h/g tissue $p<0.001$), while the hydroalcoholic extracts of AM (HAM) showed the least drop (05.26±0.04 nmol MDA formed/h/g tissue $p<0.001$).

DISCUSSION

Diabetes is a metabolic disease that has already become a Global Health threat, characterized by polyphagia, polydipsia, polyuria, blurred vision followed by weight loss[34]. The liver plays a major role in regulating glucose homeostasis, which raises evidence of the pathogenesis of glucose intolerance in diabetes. Studies have shown that diabetes is caused by oxidative stress that leads to the production of Reactive Oxygen Species (ROS), which are major causes of cell damage. Thus, diabetes is closely related to cirrhosis, hepatitis, apoptosis, β-cell malfunction and ultimately causes liver dysfunction[35].

The STZ, an antibiotic formed by Streptomyces achromogenes, is commonly used to supplement DM models to study the effect of hypoglycemic agents. The STZ-induced diabetes mellitus involves selective damage of pancreatic β-cells, which makes the cells less efficient and leads to improper insulin sensitivity to glucose uptake into tissues and results from inhyperglycaemia[36].

In extract-treated mice, there was a decrease in blood glucose levels compared with diabetes-controlled rats (STZ). Similarly, the effect of lowering blood sugar was observed, which was associated with both pancreatic and extra-pancreatic activity when the extracts were administered[37]. Increased insulin levels in the extract-treated diabetes mellitus may be due to the small regeneration of pancreas cells but a definite mechanism for recovery is yet to be found. The diabetes control group has shown damage to the arterial islet cells of Langerhans, caused by the administration of STZ. This indicates the protective role of different extracts of both the plants on the pancreas.

Weight loss in diabetic rats indicates that the loss or degeneration of structural protein occurs due to diabetes and structural proteins are known to contribute to body weight. Another possible reason is the inability to digest carbohydrate fuel sources leading to a change in dependence on fats and this results in fat stores depletion and weight loss and a reduction in weight gain related to age-related controls[38]. There has been a significant increase in body weight of CM and AM treated rodents probably due to the combination of protein constructs and may be related to increased insulin levels simultaneously. Insulin displays an anabolic effect on protein metabolism and stimulates the synthesis of protein and diminishes protein degradation[39]. Another significant action of insulin is to suppress adipose tissue lipolysis, which may lead to inhibition of muscular tissue lipolysis and proteolysis[40]. Reduction of lipolysis and proteolysis may therefore increase body weight in extract-treated rats.

Glucose-6-Phosphate Dehydrogenase (G6PD) is the primary enzyme and limits the pentose phosphate pathway, leading to the production of ribose-5-phosphate and NADPH[28,29]. Thus, a reduction in G6PD activity leads to a reduction in NADPH and makes cells more sensitive to oxidant damage. Chronic hyperglycaemia instigated by STZ leads to a reduction in G6PD in tissues, leading to increased oxidative stress. The treatment group displayed that the inhibition of acquired G6PD in tissue which may partly be because of a decrease in G6PD expression and an increase in G6PD phosphorylation caused by PKA production.

In diabetic rats, there was an amplified activity of glucose 6-phosphatase which provided hydrogen binding to NADP+ in the form of NADPH and from carbohydrate, it enhances the synthesis of fats, i.e., lipogenesis[30] and ultimately incorporates to increased blood sugar levels. Administration of CM and AM significantly reduced glucose 6-phosphatase enzyme activity in diabetic rats.

Hexokinase inadequacy in diabetic mice can lead to a decrease in glycolysis and a decrease in glucose utilization in energy production[31]. Administration of CM and AM in diabetic rats improved hexokinase activity and elevated blood glucose uptake by liver cells and increased glycolysis.

HbA1c often acts as an indicator of glycaemic control. During diabetes, excessive glucose in the blood reacts with haemoglobin to form HbA1C[41]. Diabetic rats showed higher levels of HbA1C compared with those found in normal rats, demonstrating their poor glycemic control. Current studies show that the release of CM and AM effectively regulates glycemia as evidenced by low levels of glycosylated haemoglobin (HbA1c) in experimental rodents[33].

In normal people, insulin is secreted endogenously in a pulsatile manner from the pancreas[42]. Hyperinsulinemia and hyperglycemia specify irreversible destruction of islets of Langerhans. A significant increase in serum insulin levels in extract-treated rats was observed when compared to STZ-induced diabetes rats indicating beta cells-reactivation, which

may be due to the stimulating effect of the bio-active phytoconstituents present in plant extracts[43].

The rats of the treatment group also exhibited an increase in liver glycogen content. It is proposed as the hypoglycemic effect of CM and AM extract was due to the promotion of glycogen synthesis leading to suppression of glycogen phosphorylase and other gluconeogenic enzymes and improved hepatic glycogen content[44]. Increased liver glycogen content may be due to increased glycogen synthesis in the liver and decreased glucose uptake by peripheral tissues due to augmented insulin secretion and action.

Insulin usually has an anabolic effect on protein metabolism because it stimulates protein synthesis and slows protein degradation[39].

Diabetes is associated with polyuria, proteinuria[45]. Maintaining normoglycaemia is important in delaying the progression of diabetic complications including kidney disorders[46].

The available evidence suggests that progressive hyperglycaemia improves the formation of AGES. The interaction of AGES with its RAGE kidney receptor has activated a variety of signalling mechanisms related to fibrosis, inflammation and oxidative stress[47].

Increased levels of AGES were observed in STZ group rats. Reduction of AGES levels by CM and AM extract has suggested the anti-fibrotic mechanism of these extracts.

TNF-α and IL-6 are two of the most significant pro-inflammatory serum cytokines[48]. High serum levels of IL-6 and TNF-α are related to the development of diabetes and both cytokines can suppress insulin action by disrupting insulin-receptor signalling[49]. Increased levels of IL-6 have been shown to induce diabetes in rats leading to the onset of inflammation and increasing IL-6 secretion, such as an inflammatory cytokine. The current findings were consistent with previous studies, which exhibited that diabetes can cause inflammation. In addition, Haidari *et al.*[50] have shown that high plasma levels of inflammatory cytokines, such as IL-6 and TNF-α, are associated with type II diabetes and insulin resistance.

Transforming Growth Factor (TGF) exerts effects on kidney tissue that are very similar to those of high sugar content[51,52]. Diabetes is linked with the stimulation of the renal TGF- β early at the onset of the disease, leading to an increase in matrix gene expression and progress of diabetes renal hypertrophy. TGF-β increases in glomeruli all through the early stages of rapid kidney disease in diabetes. These results suggest that TGF-β may be a key contributor to the pathogenesis of external matrix accumulation and thickening of the basement membrane. Fibronectin (FN) is a unique extracellular matrix (ECM) glycoprotein that plays an important role during tissue repair[53]. In interstitial and glomerular fibrosis, there is a marked significant increase in total FN levels in different regions of kidney and fibrosis areas[54,55]. In this study, there was an increase in the level of TGF-β, IL-6, TNF-α and FN in the STZ group, the different extract of CM and AM prevents excessive expression of TGF-β, IL-6, TNF-α and FN thus reducing the accumulation of extra cell-matrix, promoting wound healing and tissue repair.

The SOD and CAT are the 2 major free radical rummaging enzymes. The SOD serves as the primary enzymatic defence mechanism against the superoxide anion. This enzyme clears the toxin's superoxide anion, thereby converting them into water and H_2O_2. CAT is a heme protein that contributes to the reduction of hydrogen peroxides and defends tissues from hydroxyl radicals[56]. The GPx, enzyme-containing selenium, works in conjunction with GST in the metabolism of organic hydroperoxides and H_2O_2 in non-toxic products at the cost of GSH[57]. Reduced GPx activity may be caused by enzyme glycation dysfunction[58]. The GR is an enzyme that lowers glutathione disulfide (GSSG) into the form of sulfhydryl GSH, an essential cellular antioxidant. GST plays a key role in the detoxification and digestion of many endobiotic and xenobiotic compounds[59]. GST also has isomerase and peroxidase activity. It binds in combination with active metabolites formed from xenobiotics and does not bind to lipophilic molecules, thus protecting oxidative stress[60]. The GSH is a direct counterpart of free radicals along with co-substrate for the detoxification of peroxide by GPx and GST. The reduction in GSH in diabetic rats may be due to its increased use by hepatocytes to combat the increased formation of lipid peroxides on the exposure of STZ. The high level of LPO is due to the increased production of ROS. In the present study, it was found a significant increase in the formation of MDA, an LPO index in the heart of type 2 diabetes mice. The activity of SOD, GPx, GST, GR, CAT and GSH is reduced while the level of LPO in the liver is increased in diabetic rats, possibly due to enzyme glycation due to hyperglycaemia. The administration of various releases of CM and AM has shown an increase in SOD, GPx, GST, GR, CAT and GSH in liver function thus restoring antioxidant immune systems.

Diabetes not only leads to many metabolic syndromes but can also cause serious DNA damage. Due to the hyperglycaemic status and metabolism alteration of non-enzymatic glycation of nucleic acids, lipids and proteins occur which leads to the augmented release of high reactive free radicals[61]. The generated Reactive Oxygen Species (ROS) such as H_2O_2, O_2^- and OH^- can degrade DNA.

CONCLUSION

The current study suggests that different extract of CM and AM contributes in preventing the damage of kidneys, pancreas and liver in diabetic rats. Since CM and AM extracts possess antioxidant potential thus preventing STZ induced damage caused by ROS production due to stress. CM and AM extract can be used for further development as a therapeutic agent for the treatment of diabetes as a chemical or drug or can be combined with herbal plants or others. The hydroalcoholic extract of AM showed maximum anti-diabetic potential among all other extracts examined. However, further research will be needed, to better understand the mechanism of action of CM and AM at the cellular and molecular level to treat liver and kidney damage in diabetes.

SIGNIFICANCE STATEMENT

This study recommends using AM's hydroalcoholic extract, which has the highest anti-diabetic potential of various extracts evaluated in the treatment of diabetes mellitus. Stress-induced ROS formation produces STZ-induced damage, which can be reduced by extracts of CM and AM, also, these extracts increase insulin and glycogen levels. For the treatment of DM, further research is needed to identify and isolate specific active molecules.

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