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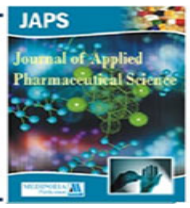

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Chemical constituents of the Egyptian Plant *Anabasis articulata* (Forssk) Moq and its antidiabetic effects on rats with streptozotocin-induced diabetic hepatopathy

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ABSTRACT

This study investigated the possible antidiabetic role and therapeutic crucial action of the saponin fractions of the ethanolic extract of areal parts of the medicinal plant *Anabasis articulata* compared to currently available antidiabetic drug gliclazide (diamicron) against diabetic complications induced tissue injury in rats. Fractionation of hydro alcoholic extract from the aerial parts of *Anabasis articulata* (Chenopodiaceae) led to the isolation of Four known saponins: 3-O-glucopyranosyl of(stigmasterol, ß-sitosterol, sitostanol), 3-O-[ß-D- the glucopyranosyl] oleanolic acid , 3-O-[ß-D-glucopyranosyl -28-O-ß-D - xylopyranosyl] oleanolic acid, in addition to proceric acid. The isolated compounds were identified by means of chemical methods and spectrometric analysis as Rf values, UV Mass, 1H NMR and 13CNMR spectroscopy. Animals were divided into 4 groups. Group1, control rats (not received any medication). Group 2, rats injected intraperitoneally with single dose of streptozotocin (STZ)(40 mg/kg body weight). Group 3, rats orally administered with ethanolic extract of *A. articulata* (400 mg/ kg B.W.) after STZ injection. Group 4, rats orally administered with gliclazide (10 mg/kg B.W.) after STZ injection. Oral administration of the plant modulated the diabetic increase in blood glucose and cortisol levels revealing the antihyperglycemic effect of this medicinal plant. It effectively increases the blood hormone insulin concentration and α- fetoprotein. It is also significantly decrease blood tumor necrosis factor α (TNF-α). The current plant also effectively decreased blood fructosamine to their normal levels as well as the consequence diabetic decrease in the hemoglobin (Hb) and albumin levels. Furthermore, ingestion of the plant effectively modulated hepatic oxidative tissue damage. Supplementation of diabetic animals with gliclazide improved diabetic induced alteration in most of the above studied markers. These results suggest that *Anabasis articulata* has multi-beneficial actions in controlling diabetes and consequence complications induced in pancreas and liver and may candidate as natural antidiabetic drug.

Keywords: Diabetes, Streptozotocin, Anabasis articulata, Saponins, glycation,Gliclazide.

INTRODUCTION

The body′s energy requirements depend on a continuous supply of glucose from the circulation. The regulation of blood glucose concentrations is of critical importance and is regulated by the hormone, insulin. After the ingestion of a carbohydrate, plasma glucose increase quickly, this change is detected by pancreatic β-cells, which in turn, produce insulin.

After binding to its receptors, insulin promotes glucose uptake by skeletal muscle, stimulates glycogen synthesis and suppresses glucose production by the liver, and suppresses lipolysis by adipocytes. Several hours after a meal, as glucose concentrations return to baseline, insulin production is decreased concomitantly. The liver now converts from an organ of net glucose uptake to one of net glucose production and supports circulating glucose concentrations until the next meal (Galerneau and Inzucchi, 2004).

Diabetes mellitus is a chronic metabolic condition that is marked by increased circulating concentrations of glucose, which is associated with the development of long-term vascular complications. There are two predominant forms, type 1 and type2. Type 1 diabetes mellitus (T1DM) is characterized by absolute insulin deficiency that results from autoimmune destruction of pancreatic islet cells, so it referred to "insulin-dependent diabetes mellitus" (Achenbach *et al.,* 2005). Type 2 diabetes mellitus (T2DM) is responsible for 90 to 95% of diabetes worldwide (Fagot-Campagna *et al.,* 2000). T2DM is a disease of dual defects-insulin resistance and relative insulin deficiency (Ferrannini 1998; Gavin *et al.,* 2001). It develops from an initial period of insulin resistance and relatively preserved insulin secretion, as the pancreas attempts to maintain euoglycemia. Pancreatic β--cell function ultimately falters and no longer is able to meet peripheral demands, insulin levels decline and hyperglycemia ensues (Reaven 2004). Hyperglycemia resulting from unregulated glucose level is widely recognized as the causal link between diabetes and diabetic complications (Brownlee, 2001). It was found that hyperglycemia cause tissue damage by mechanisms involving repeated changes in cellular metabolism (Robertson, 2004). One of the key metabolic pathways as being major contributors to hyperglycemia induced cell damage, is the nonenzymatic reaction between excess glucose and several proteins (as hemoglobin and albumin) to form Advanced Glycosylated End (AGE) product (Lapolla *et al.,* 2005). Production of AGE interefers with cell integrity by modifying protein function or by inducing receptors mediated production of Reactive Oxygen Species (ROS) (Thornalley, 2002). Hyperglycemia-evoked oxidative stress plays a crucial role in the development of diabetic complications, including nephropathy, neuropathy, retinopathy and hepatopathy, which is considered to result from augmented reactive oxygen species generation and decreased antioxidant defenses (Brownlee, 2001; Jin *et al.,* 2008). 2DM is often associated with the most commonly occurring metabolic and physiologic problems, including elevated blood pressure, cardiovascular diseases, dyslipidemia and high cholesterol levels. Together with visceral obesity, this clustering of risk factors is known as the metabolic syndrome (Levine, 2006; Araki *et al.,* 2009). In addition, studies have shown that hepatobiliary disorders, such as the inflammation, necrosis or fibrosis of non- alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, hepatitis C, acute liver failure and cholelithiasis can follow diabetes (Tolman, 2004).

Conventional drugs used for treatment of diabetes have rigid and multiple dosing regimen, high cost and untoward effects (deMelo Júnior *et al.,* 2002). Thus, it is necessary that we continue to look for new and if possible more efficacious drugs and the vast reserves of phytotherapy may be an ideal target. In some parts of the world, before the advent of insulin injections and other pharmaceutical preparations, healers relied heavily upon medicinal plants and herbs to treat diabetes (Kang *et al.,* 2005). There are 1200 plant species broadly used in the treatment of diabetes and many of these showed effective hypoglycemic activity after laboratory testing (Marles and Farnsworth, 1995). *Anabasis* genus family chenopodiaceae (family: goosefoot*)* were grown in stony and sandy wadies ,heavely browsed by camels and goat (Chopra, 1956) which comprises four genera in Egypt and are widely used as folk medicine (Boulos , 1999). Previously work has been reported belonging to several species of Anabasis. Alkaloids as anabasine, anabasamine (Brutko *et al.,* 1968), Aphylline (Nizamkhodzhaeva *et al.,* 1975). Lupinine and other alkaloids were also detected in *Anabasis aphylla* (Sandberg and Michel, 1962; Sandberg, 1961), jaxartinine has been isolated from *Anabasis jaxartica* (Segal *et al.,* 1969) .Triterpenoidal sapogenins (Sandberg and Shalaby , 1960) have been reported in certain species. Glucosidic and isoflavonoid compounds were isolated from butanol-extract of *Anabasis salsa* and chloroform–extract of *Anabasis brevifolia* (Chen *et al.,* 2005). Six phenolic compounds were identified from ethyl acetate extract of the aerial parts of *Anabasis aphylla* (Du *et al.,* 2009). *Anabasis articulata* locally named as 'ajrem' is a wild plant widely used in folk medicine to treat diabetes, kidney infections, fever, headache and skin diseases such as eczema (Hammiche and Maiza, 2006). It taken orally after decoction in water as a single herb or with other medicinal plants. No scientific investigations concerning its pharmacological properties has been done (Kambouche *et al.,* 2009). The phytochemical constituents of *A. articulata* revealed the presence of saponin. Among them triterpenoid saponin glycosides have been isolated and identified (Segal *et al.,* 1969). Literature data indicates that saponins isolated from the plant significantly reduce blood glucose levels (Kambouche *et al.,* 2009). Several biological activities have been attributed to saponin such as their antidiabetic effect (Abdel-Zaher *et al.,* 2005), immunostimulant effect (Xie *et al.,* 2008), cytotoxicity (Heisler *et al.,* 2005), antitumoral properties (Zheng *et al.,* 2006) as wll as antifibrotic effect (Geng *et al.,* 2010; Mohamed, 2011). Little information about the chemical constituents of the genus under study, also the mechanism underlying the hypoglycaemic effect of *A. articulata* is unclear and the hepatoprotective effect against diabetic hepatopathy have never been investigated in diabetic animals. The present study was also designed to investigate the mechanism(s) of the hypoglycemic effect of the plant extract compared to the currently available antidiabetic drug , gliclazide (diamicron), and to investigate its possible therapeutic beneficial effects against liver oxidative damage associated with diabetic complications in diabetic rats.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. articulta* were collected from Quatamia-Suez desert road in 2010, voucher specimen and was kindly identified by Dr Mohamed El Giblali, researcher of botany NRC and kept in herbarium of National Research Centre, Cairo, Egypt. ^{1}H NMR and ^{13}C NMR spectra were recorded in Varian 500 spectrophotometer at 500 MHz in DMSO-d6. Column chromatography was performed on Merck silica gel (70,230Mesh) and Sephadex LH-20 with various column dimensions, The purity of the samples was checked on TLC presoaked with silica gel GF245 (Merck). Different solvent systems were used.

Extraction & Isolation

Air dried powdered of the aerial parts of *A. articulta* (1 kg) were exhaustively extracted with petroleum ether (40-60 ^{o}C). Then the mark was dried at room temperature and reextracted with 70% EtOH, and the extract was concentrated under vacuum yielded (40 g). The crude ethanolic extract was suspended in H_2O and partitioned with EtOAc, the EtOAc extract concentrated under vacuum afforded (10g) of gummy residue. The EtOAc residue was loaded on silica gel column chromatography (80, 3 cm) eluted with n-hexane, n-hexane - EtOAc , EtOAc – $CHCl_3$, $CHCl_3$- MeOH and finally MeOH . Fractions (20 mL) were collected; similar fractions were combined and separated on TLC silica gel plates. The compounds were purified by HPTLC and on column Sephadex LH-20 . The main pure compounds obtained were identified by different spectral analyses (^{1}H-NMR, MS).

Hydrolysis

Acid hydrolysis was carried out with 2 N HCl (3 hr at 100 ^{o}C) under reflux, the residue was evaporated under reduced pressure and the mixture was dissolved in water and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated under reduced pressure to afford the aglycone. Sugar was identified by co-paper chromatography (BuOH-HOAc-H2O, 4:1:5) and pyridine-EtOAc-HOAc-H2O, 36:36:7:21) and on TLC silica gel plates (EtOH-CHCl3, 1:19) while the aglycone was identified by co-chromatography TLC using solvent system (benzene: ethyl acetate, 8:2 and hexane: ethyl acetate, 5:5)

Compound I

(3-O-[ß-D- glucopyranosyl] oleanolic acid) a white powder (12 mg) was obtained from fraction EtOAc – $CHCl_3$ (17: 3) and further purified on HPTLC and column Sephadex LH-20 using MeOH – H2O (9:1). 5 mg of compound I was hydrolysed, the aglycone was identified as oleanolic acid on TLC in comparison with authentic sample in different solvent systems (Sakakibara *et al.*, 1983). The librated sugar was identified on PC and silica gel TLC through comparison with standard sugar sample and was identified as glucose. From its spectral data positive ion FAB-MS: m/z 617 [M^+], 455(M-Glu) and ^{1}H NMR (CD_3Cl , 300 MHz) δ 5.2 (q,H-12), 8.4(s,H-23), 0.57(s,H-24), 0.72(s,H-25),8.4(s,H-26),1.08(s,H-27),3.74br., (OCH3-C-28), 8.4 (s,H-29), 8.4(s,H-30),4.43(d, J=7.33Hz,H-1),1.24(d, J=6.2 Hz,H-6), the compound was found to be identical as 3-O-[ß-D- glucopyranosyl] oleanolic acid in comparison with the published data (Sahu *et al.*, 1989 and Budzikiewicz *et al.*, 1963).

Compound II

3-O-[ß-D- glucopyranosyl] hydroxy oleanolic acid a white powder (15mg) was eluted from the same column with $CHCl_3$-MeOH (17:3) and was purified by HPTLC, and on column Sephadex LH-20 using MeOH- H_2O (8:2) to give pure sample. Compound II was hydrolyzed, the aglycone was identified as hydroxyl oleanolic acid on TLC in comparison with authentic sample in different solvent systems (Sakakibara *et al.*, 1983). The librated sugar was identified on PC and silica gel TLC through comparison with standard sugar sample and was identified as glucose. From its spectra data positive FAB- MS: m/z 634 [M^+], 617(M -OH), 455(M-OH-Glu) were in accordance with those of 3-O-[ß-D- glucopyranosyl] hydroxy oleanolic acid (Sahu *et al.*, 1989).

Compound III

3-O-[ß-D-glucopyranosyl -28-O-ß-D - xylopyranosyl] oleanolic acid a white powder (20mg) was eluted from the same column with $CHCl_3$- MeOH (15:5) which was recrystallized with EtOH, yielded mp. 280^{o}C. Hydrolysis of compound III was identified as oleanolic acid on TLC in comparison with authentic sample in different solvent systems (Sakakibara *et al.*, 1983). The sugar obtained was identified as glucose and xylose (Sahu *et al.*, 1989). EIMS: m/z 750 [M^+], 616(M-xylose-H), 455(M-xylose–glucose), 438 (aglycone-H2O),426 (aglycone-2Me),410 (aglycone-COOH) 395 (aglycone-COOH+Me),248 and 189,and 203,133,175. This compound was found to be 3-O-[ß-D-glucopyranosyl -28-O-ß-D-xylopyranosyl] oleanolic acid according to their chromatographic and spectral data (MS) and with that of literature (Segal *et al.*, 1969).

Further elution with MeOH, fraction 4 was obtained which was subjected to column Sephadex LH-20 using MeOH – H_2O (7:3) afforded compound IV (25mg).

3-O[β-D-glucopyranosyl]-Sitostanol (1a), 3-O[β-D-glucopyranosyl] Sitosterol(1b), 3-O[β-D-glucopyranosyl] Stigmasterol (1c) .GC showed one major component while MS revealed three ion peaks at m/z 577,575,573[M-H] and 416,414,412 [M-H-Glu] 1HNMR(CDCl3,300MHz): anomeric proton at δ4.45. Partial acid hydrolysis of compound 4 was identified as oleanolic acid through comparison of its spectral data with those reported in ref (Doddrell *et al.*, 1974). The sugar obtained as the result of acid hydrolysis was identified as xylose and D-glucose on TLC by comparison with standard sugar sample (Sahu *et al.*, 1989).

Chemicals

All chemicals used were of high analytical grade, product of Sigma and Merck companies. Kits used for the quantitative determination of different parameters were purchased from Biogamma, Stanbio, West Germany

Animals

Adult female Wistar albino rats weighing 180-200g supplied from the animal house of National Research Center, Dokki, Giza, Egypt. Rats were fed a standard diet and free access to tap water. They were kept for two weeks to acclimatize to the environmental conditions.

Experimental Design

The rats were divided into 4 groups each of ten rats. Group 1: normal healthy control rats (not received any medication). Group2: diabetic group , diabetes was induced by STZ, each rat was injected intraperitoneally with a single dose of STZ (40 mg/kg B.W) dissolved in 0.01 M citrate buffer immediately before use (Milani *et al.,* 2005). After injection, they had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock (Bhandari *et al.,* 2005). After 2 weeks hyperglycemic rats (460-500 mg/dL) were used for the experiment. Group3: diabetic animals treated with ethanol extract of the plant, the extract was given orally in a dose 400mg/KG BW/day for 30 days (Kambouche *et al*., 2009) after 2 weeks of induction of diabetes. Group 4: diabetic animals treated with gliclazide. Gliclazide was given orally (10 mg/kg/day) for 30 days (Dachicourt *et al.,* 1998) after 2 weeks of induction of diabetes. After 30 days of drug treatment, the animals were fasted overnight (12-14 hours).

Sample Preparation

The blood samples were collected from each animal in all groups into sterilized tubes for serum separation and into tubes containing heparin for hemoglobin determination. Serum was separated by centrifugation at 3000 rpm for 10 minutes and used for biochemical serum analysis . After blood collection, rats of each group were sacrificed under ether anesthesia and the liver samples were collected, minced and homogenized in ice cold bidistilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 minutes at 3000 rpm at 4°C and the supernatants were used for different biochemical tissue analysis.

Biochemical assay

Blood analysis

Hb was estimated in the whole heparinized blood by cyanmethaemoglobin method (Drabkin and Austin, 1932).

Serum analysis

Fasting blood glucose was measured according to method adopted previously by Miwa *et al*.,(1972) using a glucose kit (enzymatic method) (Wako). Insulin was determined using BioSource INS-ELISA kit which is an immunoenzymatric assay for the quantitative measurement of insulin in serum according to Temple *et al.,* (1992). Serum cortisol level was estimated in using ELISA kit (Arakawa *et al.,* 1979). TNF-α was quantified using a commercial ELISA kit (Endogen, Woburn, MA). AFP was assayed by ELISA kit using a goat anti- AFP antibody directed against intact AFP for solid immobilization (Chan and Miao, 1986). Fructosamine (glycated serum protein) was determined using reagents, calibrators and controls from Sigma Diagnostics (St. Louis, MO) and application parameters for the Cobas Mira automated chemistry analyzer. The assay is a modification of the original method of Johnson and colleagues (Parlin *et al.,* 1997) where fructosamine reduces nitro-blue tetrazolium (NBT) under alkaline conditions and forms a purple-colored formazan with an absorption maximum at 530 nm. Albumin was determined using the method of Doumas *et al.,* (1971). ALT and AST activities were determined according to the method described by Bergmeyer *et al.,* (1986).

Hepatic tissue analysis

Enzyme determination

SOD activity was determined by monitoring the decrease in absorbance at 340 nm using the method of Paoletti *et al.,*(1986). The activity was expressed in terms of % inhibition of NADH**.** GR activity was measured by the modified method of Erden and Bor (1984). The reaction mixture contained the following in the final concentration: 4.1 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 5.7 mM EDTA, 60 mM KCl, 2.6 IU GSSG and 0.2 mM of NADPH in final reaction volume of 1 ml. The reaction was started by the addition of tissue extract containing approximately 100 µg of protein. The decrease in absorbance was monitored at 340 nm.

Metabolite determination

Lipid peroxidation was determined by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method **(**Buege and Aust, 1978**).** This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. MDA concentration was calculated using extinction coefficient value(ε) of MDA-thiobarbituric acid complex (1.56×10^5 /M/cm). The reduced glutathione (GSH) was determined using the method of Bentler *et al.,* (1963) based on its reaction with 5,5'-dithiobis (2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm.

Statistical analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.D. The significant differences among values were analyzed using analysis of variance (one-way Anova) coupled with post-hoc (LSD) . Results were considered significant at $P< 0.05$.

RESULTS

Table 1 reveals that injection of rats with STZ induced hyperglycemia indicated by significant increase in diabetic marker, fasted blood glucose level in these animals (G2) as well as cortisol and TNF-α levels compared to the control ones (G1). Oral administration of the drug (G3) down regulated the blood

Table. 1: Effect of ethanol extract of *A articulata* on some serum biochemical parameters in different studied groups .

Parameters / Groups	Control group (G1)	Diabetic group (G2)	*A articulata* -treated group (G3)	Diamicron-treated group (G4)	ANOVA P<
Glucose (mg/dL)	106.0±9.14	482.0 ±22.65	100.33±11.24	86.25±8.75	
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	P< 0.0001
Insulin	20.17±3.55	1.71±0.35	17.85±2.18	13.97±1.99	
(µIU/mL) LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	P<0.0001
Cortisol	163.06±11.62	381.03±21.09	136.1±6.02	109.46±9.17	
(ng/mL) LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	P<0.0001
TNF-α	14.48±1.25	88.78±4.32	25.9±1.35	36.03±2.46	P<0.0001
(pg/mL) LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	
α- fetoprotein	8.32±0.92	2.28±0.36	6.07±0.79	4.51±0.69	P<0.001
(ng/mL) LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	

Data are expressed as mean ±SD of 5 rats in each group.

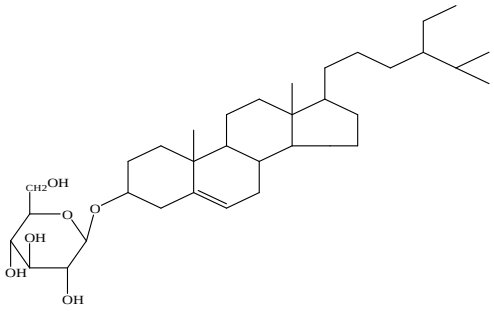
Table. 2: Effect of ethanol extract of *A articulata* on serum fructosamine, hemoglobin and albumin in different studies groups.

Parameters / Groups	Control group (G1)	Diabetic group (G2)	*A articulata* -treated group (G3)	Diamicron-treated group (G4)	ANOVA P<
Fructosamine	0.27±0.01	0.65±0.03	0.34±0.01	0.44±0.03	P<0.001
(mmol/L) LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	
HB (g/dL)	13.22±1.49	7.86±0.91	10.03± 1.85	8.26±1.32	P<0.001
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	
Albumin (g/dL)	4.40 ±0.30	2.60 ±0.11	3.12 ± 0.13	2.71 ±0.12	P<0.001
LSD	(2,3,4)	(1,3)	(1,2,4)	(1,3)	

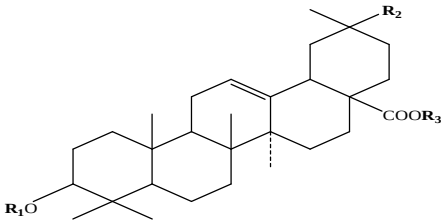
Data are expressed as mean ± SD of 5 rats in each group.

glucose, cortisol and TNF-α within their normal levels .Table 1 also shows a significant decrease in both insulin and AFP concentrations in diabetic animals compared to control group(G1). Oral administration of the plant extract up regulated blood insulin and AFP within their normal ranges. Treatment of diabetic animals with the currently available drug, gliclazide (G4) markedly down regulated blood glucose and improved the other tested parameters. Table 2 shows an increase in blood fructosamine level with concomitant decrease in Hb and albumin levels versus control animals. Oral administration of plant extract (G3) down-regulated blood fructosamine within its normal level . From the table, it can be observed that the tested plant also normalized the decrease in Hb and albumin levels in response to diabetes. Treatment of diabetic animals with the currently available drug, gliclazide (G4) improved the alteration in fructosamine level in relation to diabetic group and ameliorated Hb and albumin within their normal levels. The levels of hepatic oxidative stress markers as well as the levels of some markers of antioxidants are shown in Table 3. Diabetic rats showed marked increase in MDA (index of lipid peroxidation) with parallel decrease in hepatic SOD , GR (enzymatic antioxidants) and GSH (non-enzymatic antioxidant). Ingestion of the plant under investigation effectively normalized the deterioration occurred in these markers in hepatic of diabetic animals. Supplementation of diabetic animals with gliclazide was found to improve the induced deviation in these indices .

Table 4 shows the activities of serum ALT and AST as two markers evaluate liver function in normal and diabetic animals in different experimental groups. From the table it can be noticed that induction of diabetes developed significant liver damage as observed from elevated levels of hepatospecific enzymes, serum ALT and AST. Administration of the candidate plant successively down-modulate the increase in these serum enzymes within their normal activities, however treatment of diabetic animals with the currently antidiabetic drug, gliclazideshows a slight improvement in the alteration in these enzymes. Figure 1 shows that column of EtOAc result in four saponin compounds1 to 4 (fig 1) .The structure of the isolated compounds was established throught chromatography as well as chemical tests and spectroscopic analysis as (FAB-mass, 1H NMR , 13CNMR) .The four compounds was isolated from *A.articulata* for the first time.



Compounds. 1:



2- R1=Glu R2=CH3 R3=H
3- R1=Glu R2=CH2OH R3=H
4- R1=Glu R2=CH3 R3=Xylose

Fig. 1: Four saponin compounds (1 to 4) resulted from EtOAc column.

Table. 3: Effect of ethanolic extract of *A articulata* on some antioxidant enzymes in different studies groups.

Parameters / Groups	Control group (G1)	Diabetic group (G2)	*A articulata* -treated group (G3)	Diamicron-treated group (G4)	ANOVA P<
MDA (µmol/g tissue)	0.74±0.13	5.54±1.31	1.86±0.19	2.71±0.87	P<0.001
LSD	(2,4)	(1,3,4)	(1,2,4)	(1,2,3)	
GSH (µmol /g tissue)	2.6 ±0.12	1.87 ±0.06	2.06±0.15	1.90±0.063	P<0.001
LSD	(1,2,3)	(1,3)	(1,2,4)	(1,3)	
GR(nmol NADH oxidized/min/mg protein)	12.25±1.98	2.86±0.09	7.83±1.12	5.12±0.87	P<0.001
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	
SOD(nmol N ADH oxidized/min/mg protein)	7.36±1.35	1.33± 0.15	5.42± 0.76	3.7 2±0.54	P<0.001
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	

Data are expressed as mean ± SD of 5 rats in each group.

Table. 4: Activities of some serum liver function enzymes in normal, diabetic and treated groups.

Parameters / Groups	Control group (G1)	Diabetic group (G2)	*A articulata* -treated group (G3)	Diamicron-treated group (G4)	ANOVA P<
AST (U/L)	28.59±3.67	68.09±13.97	29.38±3.59	38.29±4.18	P<0.001
LSD	(2,4)	(1,3,4)	(2,4)	(1,2,3)	
ALT (U/L)	21.11±3.52	74.29±14.11	27.23±2.19	43.12±5.12	P<0.001
LSD	(2,3,4)	(1,3,4)	(1,2,4)	(1,2,3)	

Data are expressed as mean ± SD of 5 rats in each group.

DISCUSSION

Diabetes is a serious complex chronic condition that is a major source of ill health worldwide. The number of people in the world with diabetes has increased dramatically in recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world (Kim *et al.*, 2006). Due to high prevalence of diabetes worldwide, extensive research is still being performed to develop new antidiabetic agents and determine their mechanisms of action, consequently, a number of diabetic animal models have been developed and improved over the years (Islam and Loots, 2009). Little scientific evidence exists to support the numerous herbs used to improve diabetes-related metabolic disorders. It is known that considerable medical resources have been invested on the prevention and control of the diabetes-related complications.

The present study has demonstrated that injection of rats with STZ resulted in a significant elevation in blood glucose in diabetic group as compared with normal animals indicating establishment of diabetic state. Administration of diabetic animals with *A articulata* extract and the current drug diamicron markedly regulated the blood glucose level, but the used plant showed a better hypoglycemic effect than the drug diamicron. This is consistant with Shokeen *et al.,* (2008) who estimated that administration of the ethanolic extract of the root of *Ricinus communis* effectively decrease fasting blood glucose to normal level. Indeed, Kamobouche *et al.,* (2009) demonstrated that aquous extract of *A articulata* leaves reduced the hyperglycemia level and improved glucose tolerance in alloxan diabetic mice. The authors attributed the antidiabetic activity of the extract to saponin components through the release of insulin from the pancreas. Also, eremanthin isolated from *Costus specious* rhizome significantly decreased blood glucose level. In addition, Mohamed *et al,*. (2009) found that the powder of *Curcuma longa* L rhizome and *Nigella sativa* seeds successfully modulated the diabetic increase in blood glucose to its normal level, indicating their potential antidiabetic effects. Recently, Sohn *et al.,* (2010) reported that the treatment of the diabetic rats with chlorogenic acid and 3,5-di-O-caffeoylquinic acid extracted from the aerial parts of the plant *Aster koraiensis* (Asteraceae) for 13 weeks was effective in reducing blood glucose.

Insulin is highly homologous growth hormone found in animal serum, which can generate similar biological responses in a wide variety of cell types (Kenner *et al.*, 1993). Insulin initiate these responses by binding to their specific cell surface receptors, this interaction induces autophosphorylation of the receptor through intrinsic tyrosine kinase activity (White and Kahn, 1994). The increase in receptor-associated tyrosine kinase activity leads to cascade of phosphorylation events necessary to generate the intracellular second messenger signal required for biological cellular responses (Ullrich and Schlessinger, 1990).

The current work showed a significant decrease in insulin concentration in STZ-treated rats compared to normal animals. These data are in harmony with Yamamoto *et al.*,(1981) who found that STZ administration in rats led to destruction of pancreatic cells by inducing DNA strand breaks and fragmentation leading to cell death, that was followed by partial and limited regeneration. Neonatal STZ-induced diabetic rats have been reported to have lower basal insulin levels, higher basal glucose levels and lower insulin response to glucose load, compared to normal rats (Weir *et al.,* 1981). Also, Guillot *et al.*, (1995) stated that impairment of insulin secretion represents key and early characteristics of diabetes mellitus in humans but the factors

implicated in the development of this secretory disorder have not yet clearly defined. In addition, Wuarin *et al.,* (1996) demonstrated that insulin levels are reduced in type1 diabetes, while resistance to insulin is coupled with a partial reduction of insulin production in type 2 diabetes. The authors reported also that insulin growth factor (IGF) gene expression is reduced in STZdiabetic rat brain and liver. Moreover, Lee and Park (2000) found that most β-cells were destroyed by treatment with STZ, only few of them shows a weak secretory activity of insulin. Recently, Serbedzija *et al.,* (2009) reported that substantial evidence showed that patients have diminished brain insulin and IGF.

Treatment of STZ-diabetic rats with the current extract showed a significant elevation in insulin level compared to normal and diamicron-treated group. These results are in line with Yadav *et al.,* (2004) who reported that seed powder of the plant *Trigonella foenum* graecum prevented the development of hyperglycemia through modulation of insulin secretion which may attributed to its saponins. Also, Singab *et al.,* (2005) found that oral administration of the active constituents (flavonoids and triterpens) isolated from the Egyptian *Morus alba* plant protect pancreatic β-cells from degeneration with concomitant increase in the insulin level. In addition, Shokeen *et al.,* (2008) reports that administration of the ethanolic extract (containing flavonoids, alkaloids, carbohydrates, tannins and saponins) of the root of *Ricinus communis* effectively increased serum insulin level of the diabetic rats. Moreover, Eliza *et al.,* (2009) stated that oral administration of sesquiterpenoids, eremanthin isolated from *Costus speciosus* rhizome markedly increased plasma insulin level to near normal level. Nutritional studies performed in animals and intervention studies with humans suggest that the ingestion of fermented *Glycine max* (Soybeans, Fabaceae) products, isoflavones, such as doenjang, kochujang, and chungkookjang improved insulin resistance and secretion (Kwon *et al.,* 2010).

Cortisol has been proposed to play a role in blunting neuroendocrine and metabolic responses (Davis *et al.,* 1997). The brain may be an important site of action for cortisol to blunt responses to subsequent stress (Sandoval *et al.,* 2003). Dysregulation of the hypothalamic-pituitary-adrenocortical axis such as elevation of corticotropin (ACTH) and glucocorticoids (cortisol) is observed in diabetes of both humans and rats (Roy *et al.,* 1990; Chan *et al.,* 2003).

Our data revealed a significant increase in cortisol concentration in STZ-diabetic rats. The present data are in harmony with Chan *et al.,* (2001) who stated that in STZ-diabetic rats, corticosterone and ACTH are elevated and restored to normal levels by treatment with insulin. Indeed, many reports demonstrated that serum cortisol levels were elevated in human type 2 diabetes mellitus (Roy *et al.,* 1990; Chan *et al.,* 2003). The mechanism of increment of cortisol are not known yet. However, chronic hyperglycemia in diabetic patients is associated with a defect in the glucocorticoid receptor function that may include negative feedback by cortisol in the central nervous system and pituitary, so far, glucocorticoid is generally increased in human type 2 diabetes mellitus (Landmark *et al.,* 2006). Treatment of STZ-diabetic rats with the current plant extract improved cortisol level when compared to control and diamicron drug.

Cells of the monocyte/macrophage lineage play a central role in inflammatory cytokine production and the most abundant product of activated macrophages is Cachectin or Tumor Necrosis Factor (TNF) (Beutler and Cerami, 1989). It is now believed that TNF is one of the main proinflammatory cytokines that plays a central role in initiating and regulating the cytokine cascade during an inflammation response and is involved in local systemic events attendant an inflammation (Makhatadze, 1998). High affinity binding of TNF to their receptors mediated a wide range of biological functions including immunological responses, inflammatory reactions, anti-tumor and anti-viral activities (Smith *et al.,* 1990).

It has been reported that TNF-α production is increased under chronic hyperglycemia in diabetic animals (Sagara *et al.,* 1994). The increased TNF-α production has been indicated to mediate some pathological conditions in diabetes mellitus such as insulin resistance (Hotamisligil and Spiegelman, 1994). Insulin resistance, the" traditional " cornerstone defect of type 2 diabetes, leads to an array of adverse effects on β-cells, including hypertrophy, apoptosis and those caused by lipotoxicity and glucotoxicity (Chen *et al.,* 2007). With respect to the loss of β- cell function which is associated with the progression from normal glucose tolerance to impaired glucose tolerance and overt diabetes, researches reported that glucotoxicity hamper insulin synthesis through inhibiting the expression of insulin gene and inducing β-cells apoptosis and reduce the secretion of insulin through lowering β- cells sensitivity to glucose by hexosamine pathway (Weyer *et al.,* 2001). TNF-α induces insulin resistance by suppressing the insulin transduction in muscles and adipose tissues (Feinstein *et al.,* 1993).

Our results showed that a marked increase in TNF-α concentration in STZ-diabetic rats. The present data are in accordance with Hotamisligil *et al.,* (1993a) who reported the overexpression of the cytokine TNF-α in adipose tissue of different rodent genetic modeles of obesity. This overexpression led to the downregulation of the signal transduction of insulin receptor via the increased serin phosphorilation of insulin receptor substrate turning it to an inhibitor of the receptor (Hotamisligil *et al.,*1993b). Also, Makhatadze, reported that insulin dependent diabetes is characterized by a lymphocyte infiltration of the pancreatic islet cells-insulitis that may result in the progressive destruction of the insulin-secreting β-cells.The authors studies on non-obese diabetic mice showed that TNF is deeply involved in the pathogenesis of the disease. In addition, Winkler *et al.,* (1998) found elevated serum concentrations and bioactivity of TNF-α in males with type 2 diabetes and obese patients as compared to the controls. The authors suggested the higher biological activity of TNF-α observed in these circumstances can either be the consequence of the production of a TNF-α variant with increased cytotoxic activity or the decrease of the activity of physiological TNF-α inhibitors. Moreover, Donath *et al.,* (2003) and Gwozdziewiczova *et al.,*

(2005) found that in type 2 diabetes, the adipocyte-derived cytokines such as TNF-α which causes an inflammatory response and insulin resistance are elevated in the blood. The data derived from cultured cell systems showed that TNF-α inhibits insulin synthesis and secretion, induces β-cell apoptosis and may take part in the development of β-cell dysfunction. Furthermore, Moriwaki *et al.,* (2003) reported that circulating levels of TNF-α elevated in patients with metabolic syndrome, regarded as a prediabetic state, and in obese or nephropathic patients with type 2 diabetes and such patients are therefore considered at high risk of cardiovascular events. Recently, Hirota *et al.,* (2008) reported that TNF-α is a pridector of both coronary artery calcification and arterial stiffness in type 2 diabetic patients. Our current plant showed a significant reduction in TNF-α concentration in serum of rats when compared to control and available drug diamicron.

Alpha fetoprotein (AFP) is a glycoprotein, of unknown function, normally produced during neonatal development by the liver and in a small concentrations by the gastrointestinal tract (Abelev *et al.,* 1963). The present study revealed a significant decrease in AFP concentration in STZ- induced diabetic rats. These results are in accordance with Milunsky *et al.,* (1982) who reported that maternal serum alpha- fetoprotein decreased in insulin dependent diabetes. It seems possible that metabolic changes associated with poorly controlled diabetes result in a functional deficiency of fetal protein. However, a net decrease in functioning fetal protein could occur with increased catabolism or a production of hypoglycosylated and functionally incompelent molecules that, in turn, would be less efficiently. Whatever the actual mechanism, the net effect would be to decrease the availability of fetal protein and inhibit the fetal growth rate (Baumgarten *et al.,* 1988). The authors found an inverse relationship between the concentrations of maternal glycosylated Hb and maternal serum AFP and they hypothesized that reduced maternal serum AFP in poorly controlled diabetes may indicate an interference with the synthesis or effectiveness of fetal protein which, in turn, can produce fetal growth retardation, fetal malformation, or both, depending on the stage of gestation in which glycemic control fails. Recently, Thornburg *et al.,* (2008) supported the previously reported findings that diabetic patients have lower maternal serum AFP levels than non-diabetic patients.

Treatment of diabetic rats with the ethanolic extract of the used plant showed a marked improvement in AFP levels compared to control and the diamicron drug.

In line with previous studies, the current investigation also revealed significant elevation in serum fructosamine level with concomitant decrease in Hb and albumin levels in diabetic animals in relation to normal ones (Montilla *et al.,* 2004). Serum fructosamine, is a glycated protein which has the crucial role in the progression of many pathological conditions (Misciagna *et al.,* 2004). It results from spontaneous nonenzymatic condensation of excess glucose present in blood and a number of proteins including Hb and albumin due to uncontrolled or poorly controlled diabetes, therefore the total Hb and albumin levels are decreased (Sudnikovich *et al.,* 2007; Lapolla *et al.,* 2005). The amount of increase in fructosamine, was found directly proportional to the fasting blood glucose level (Jackson *et al*., 1979). Albumin is the most abundant plasma protein and is a powerful extracellular antioxidant (Bourdon *et al.,* 1999). It contains 17 disulphide bridges and has a single remaining cysteine residue which is responsible for the capacity of albumin to react with and neutralize peroxyl radicals (Young and Woodside, 2001). Decreased in albumin due to its glycation during diabetes may consider one of the important factors responsible for oxidative stress related to diabetes (Jin *et al.,* 2008).

Ingestion of the current plant effectively normalized the deterioration in fructosamine, Hb and albumin levels which may attributed to its potential glycemic control together with it may has an important role in preventing protein glycation. Our results are documented by previous investigation has shown that oral administration of sesquiterpenoids, eremanthin, isolated from Cotus speciosus rhizome significantly decreased glycosylated hemoglobin level to near normal(Eliza *et al.,* 2009). The antidiabetic role as well as the therapeutic beneficial action of Curcuma longa L rhizome and Nigella sativa seeds against diabetic complications induced liver injury in rats were investigated by Mohamed *et al.*,(2009). The authors reported that the powder of either plant successfully modulated the diabetic increase in serum fructosamine to its normal level as well as the consequence diabetic decrease in the Hb and albumin levels, indicating their potential antidiabetic and antiglycating abilities. Moreover, recently, Sohn *et al.*,(2010) stated that treatment of diabetic rats with chlorogenic acid and 3,5-di-O-caffeoylquinic acid extracted from the aerial parts of *Aster koraiensis* (Asteraceae) for 13 weeks was effective in reducing glycated hemoglobin and albumin, the authors revealed that this plant extract prevented AGEs deposition suggesting that it has an inhibitory effect on AGE accumulation. Administration of diabetic animals with gliclazide was also found to have an inhibitory effect on the glucose-induced glycation during diabetic state indicated by improvement of fructosamine level and normalization of Hb and albumin levels. This was ensured by previous study provides the first evidence of the antiglycation effect of gliclazide on *in vitro* AGE formation from glucose and methylglyoxal (Li *et al.,* 2008).

Oxidative stress is known to play a pivotal role in development of diabetes An imbalance of oxidant/antioxidant in favour of oxidants contributes to the pathogenesis of diabetes (Jin *et al.,* 2008; Sudnikovich *et al.,* 2007). Data presented in our investigation indicate that induction of diabetes results in augmentation of oxidative stress in livers of diabetic rats accompanied by impaired antioxidative defence, as indicated by significant elevation in the levels of liver oxidative stress marker, MDA (index of lipid peroxidation) with parallel depletion of free-radical scavenging antioxidants, SOD, GR and GSH. These results may be due to hyperglycemia induced auto-oxidation of lipids and glycation of protein/glucose, result in formation of reactive oxygen species (ROS) and nitrogen species (RNS) which have the major role in diabetic complications (Sudnikovich *et al.,* 2007; Szkudelski, 2001). Lipid peroxidation can damage protein, lipids,

carbohydrates and nucleic acids. Plasma membranes are the critical targets of lipid peroxides (Freeman and Crapo, 1982). It also has been found that lipid peroxidation is one of the risk factor of protein glycation (Selvaraj *et al.,* 2006). On the other hand, decline in the activities of free-radical scavenging enzyme, SOD may be due to inactivation caused by excess reactive oxygen species production. This damages the first line of enzymatic defense against superoxide anion and hydrogen peroxide. The significant depletion of GR and GSH indicating damage to the second line of antioxidant defense. This probably further exacerbates oxidative damage by adversely affecting critical GSH-related processes such as free-radical scavenging, detoxification of electrophilic compounds, modulation of cellular redox status and thiol-disulphide status of proteins, and regulation of cell signalling and repair pathways. Reduced antioxidant levels as a result of increased ROS production in experimental diabetes has been previously reported (Lee *et al.,* 2005; Liu *et al.,* 2008).

Supplementation of the plant under investigation, effectively ameliorate the deviation in the oxidative stress and antioxidant markers in rats liver, implying its beneficial antioxidant ability. These data are in harmony with Singab *et al.,* (2005) who reported that oral administration of the active constituents (richin, flavonoids and triterpens) isolated from the Egyptian plant *Morus alba* root bark for 10 days diminished lipid peroxidation indicated by significantly decreased serum lipid peroxides. Also, Ciocoiu *et al.,* (2009) reported that the natural polyphenol compound extract from fruits of *Sambucus nigra* (Family, Adoxaceae) reduced the lipid peroxides, neutralized the lipid peroxil radicals and significantly increased serum activity of glutathione peroxidase and superoxide dismutase indicating its antioxidant effect against oxidative stress and metabolic disorders in diabetic rats. In support, Kim *et al.,* (2009) found that oral administration of aqueous extract markedly reduced the level of malondialdhyde (MDA), a lipid peroxide product used as an indicator of oxidative stress in both serum and hepatic tissue, to normal level. In addition, these data are in accordance with Mohamed *et al.,* (2009) who reported that ingestion of *C. longa* and *N.sativa* seeds effectively modulated diabetes induced hepatic oxidative tissue damage suggesting their antioxidant potential actions. Moreover, Althunibat *et al.,* (2010) demonstrated that the phenolic compounds extracted from the plant Punica granatum peel significantly enhanced or maintained the antioxidant defense capacity with liver, kidney, RBC and serum of diabetic rats and inhibited lipid peroxidation in rat tissues suggesting that the extract could exert a beneficial action against pathological effects of oxidative stress in diabetic patients. Also, similar effect was obtained with supplementation of diabetic animals with gliclazide suggesting that it may protect against the oxidative stress-related chronic diabetes complications (Sliwinska *et al.,* 2008).

In parallel with previous report , the present work revealed that in diabetic rats the activities of serum AST and ALT (markers of liver tissue damage) were significantly increased relative to their normal levels (Hickman *et al.,* 2008). Supporting our finding, it has been found that hyperglycemia (25 days) resulted in hepatolysis reflected by increased blood plasma aminotransferase as one of the consequences of diabetic complication (Mansour *et al.,* 2002). The increment of such markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream. Treatment of the diabetic rats with the tested plant caused normalization in the activities of these serum enzymes, indicating its potential ability in inhibiting liver damage induced by diabetic status. However, treatment with gliclazide, although it improved the diabetic increase in these liver enzymes but they still above their normal levels which is coincided with previous published data revealed that patients receiving gliclazide had relatively abnormal liver functions (Belcher and Schernthaner, 2005).

These data are in accordance with Shokeen *et al.,* (2008) who reported that the administration of the ethanolic extract of the root of *Ricinus communis* effectively improve the altered liver and kidney function of the diabetic rats. Also, Eliza *et al*., (2009) stated that oral administration of sesquiterpenoids, eremanthin isolated from *Costus speciosus* rhizome restored the altered plasma enzymes (aspartate and alanine aminotransferases, lactate dehydrogenase, alkaline and acid phosphatases) levels to near normal. In addition Farswan *et al.,* (2009) stated that β-sitosterol and tannis isolated from Cassia glauca leaves are responsible for the hepatoprotective activity of the plant in STZ-induced type 2 diabetes in rats.

CONCLUSION

In conclusion, the present study demonstrated that the saponin fractions of ethanolic extract of the current plant *A articulata* has beneficial glycemic control as well as it has principle role in preventing different metabolic disorders and liver damage caused by hyperglycemia. In the light of our findings, the antidiabetic activity of the current extract could be attributed to saponoin components, and its antihyperglycemic activity is through the release of insulin from the pancreas that is, it exerts a direct insulinotropic effect, or it could be due to the insulin like effect of the active principle (saponin) present in the extract. Hence, with its antidiabetic, antiglycating and antioxidant features, treatment with the used plant, at the safely effective therapeutic dose used in the current study, can be effective in the recovery of liver tissue and other organs from the damage and complications induced by diabetes and may candidate as natural antidiabetic drugs.

Authors' statements

Competing interest: The authors declare no conflict of interest.

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