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Methanolic extract of African mistletoe (*Viscum album*) improves carbohydrate metabolism and hyperlipidemia in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: To justify the use of African mistletoe (AM) *Viscum album* (*V. album*) in folkoric medicine to treat diabetes. **Methods:** In one experiment, the fasting blood glucose (FBG) levels of diabetic rats were monitored for 4 h. Diabetic rats were treated with AM at doses of 50 mg/kg (AM1) and 100 mg/kg (AM2), glibenclamide (GB) (positive control) and saline solution (SS). In another experiment, diabetic rats were treated with AM2, GB and SS daily for 3 weeks. **Results:** AM1 and AM2 elicited significant ($P<0.05$) hypoglycaemic effects within 4 h of extract administration. AM1 and AM2 decreased the FBG by 41% and 49%, respectively, at 2 h. AM2 was found to lower FBG by 51%, relative to baseline, which was comparable to GB at 3 h. In the second experiment, AM2 and GB significantly ($P<0.05$) decreased the FBG by 34% and 51%, respectively. This was followed by marked decrease in levels of HbA1C in AM2- and GB- treated diabetic rats. AM2 significantly ($P<0.05$) decreased the STZ-induced increase in levels of serum triglyceride, urea, lactate dehydrogenase, α-amylase and low-density lipoprotein-cholesterol. Furthermore, diabetic rats treated with AM2 had significantly ($P<0.05$) elevated high-density lipoprotein-cholesterol. In contrast, STZ administration produced insignificant ($P<0.05$) effect on the levels of serum creatinine and total bilirubin. **Conclusions:** Extract of African mistletoe has anti-diabetic and anti-hyperlipidemic effects in STZ-diabetic rats. AM may find clinical application in the amelioration of diabetes-induced lipid disorders.

1. Introduction

Diabetes mellitus is a chronic incurable condition due to insulin deficiency that affects large proportion of the world population. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025[1]. Several attempts were made to reduce the hyperglycemia in diabetes such as treatment by sulfonylureas, which stimulates pancreatic islet cells to secrete insulin; metformin, which acts to reduce hepatic glucose production; α-glucosidase inhibitors, which interfere with glucose adsorption and insulin itself, which suppresses glucose production and augments glucose utilization[2]. These therapies have limited efficacy and side effects, and thus searching for new classes of drugs is essential to overcome the problems posed by diabetes. Nowadays, many indigenous medicinal plants have been found to be useful in managing diabetes and the search is unlimited[3-5].

African mistletoes (*Viscum album* (*V. album*)) (family Loranthaceae), are well known as hemi-parasites of different gymnosperms and angiosperms. They are of great

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economic importance because of the major damages they cause to their host which results to economic losses[6]. Mistletoes have been used in the treatment and management of many diseases in traditional medicine in Africa. It has been reported to be effective in the management of chronic metabolic disorders[7]. A number of biological effects, such as anticancer, anti-microbial, apoptosis-inducing and immune-modulatory activities have been reported for mistletoes[8]. Mistletoe teas and infusions are used for prevention and management of stroke in Nigeria, and it is used to improve the circulatory system and heart function[5]. However, there is dearth of scientific data to justify and support the use of this plant in folkloric medicine. This study was undertaken to investigate the effect of African mistletoe on carbohydrate metabolism and lipids profile of streptozotocin-induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin was purchased from Sigma Chemical Co., Saint Louis, MO USA. Serum biochemical analysis was done by automated system at the Tehran University Teaching Hospital, Iran. Other chemicals were of analytical grade and the purest quality available.

2.2. Plant material and extraction procedure

Samples of fresh African mistletoe (Parasitic on Kola acuminate tree) were obtained from forest at Oke-Ogun area of Oyo State, Nigeria. The botanical identification and authentication was confirmed at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimen (FHI 108411) was kept. The plant was air-dried at room temperature and then powdered. The powdered samples (1 kg) were de-fatted with n-hexane (2 500 mL) and extracted with methanol (2 500 mL) overnight in a soxhlet extractor. The crude methanolic extract was concentrated and evaporated to dryness with a rotary evaporator under reduced pressure. The yield of the crude extract was 6.4%.

2.3. Animals

Male Wistar rats, 220-230 g, were used for the study. The rats were 10-12 weeks of age at the time of this study. They were bred and housed in the Central Animal House, Faculty of Pharmacy, Tehran University of Medical Sciences, Iran. The animal house was well ventilated with a 12 h light-dark cycle. They were fed on normal laboratory chow and allowed free access to water for two weeks before the commencement and during the period of the experiment. Handling of animals and other protocols conform to the guidelines of the National Institutes of Health (NIH publication 85-23, 1985). The study was approved by the Animal Ethical committee of University of Tehran, Iran.

2.4. Study design

2.4.1. Experiment 1

The effect of extract of African mistletoe (AM) on FBG levels of STZ-diabetic rats was studied within 4 h of extract administration. The procedure described by Sharma *et al*[9] was adopted in this study. Rats were fasted overnight and made hyperglycemic by a single intraperitoneal injection of STZ dissolved in 0.05 M of citrate buffer (pH 4.3), at a dose of 35 mg/kg[10]. The FBG of these rats were estimated 72 h after STZ administration, and moderately diabetic rats having FBG level above 250 mg/dL were selected and divided into four groups of 6 animals each. One group served as negative control (received vehicle), two groups were given AM at doses of 50 and 100 mg/kg (AM1 and AM2) and the last group received glibenclamide (GB) (positive control) at a dose of 5 mg/kg. A group of non-diabetic rats was also included during this study. The FBG per rat in all groups were estimated just before a single dose of GB or AM was administered and, the FBG monitored at 30, 60 and 90-240 min after drugs administration.

2.4.2. Experiment 2

The effect of extract of African mistletoe administered daily for 3 consecutive weeks was studied in STZ-diabetic rats. Another set of STZ-diabetic rats were randomly divided into three groups of 6 rats each. One group received saline solution (vehicle) (STZ only). The second and third groups received African mistletoe at 100 mg/kg (STZ+AM2), and glibenclamide at 5 mg/kg (STZ+GB), respectively, daily for 3 consecutive weeks. The fourth group consists of non-diabetic rats (Normal). After the last dose of the drugs, rats were fasted overnight and sacrificed by cervical dislocation. Visceral organs were obtained by dissection and immediately weighed, while blood was collected from the heart of the animals into plain centrifuge tubes

2.5. Preparation of serum

Blood samples were allowed to stand for 1 h and then centrifuged at 3 000 g for 15 min in an MSC bench centrifuge to obtain serum. The clear supernatant (serum) was used for the estimation of urea, creatinine, enzymes, lipid profile and other parameters.

2.6. Biochemical assays

Glucose levels were determined by the glucose oxidase method of Sharma *et al*[9]. The protein contents in the samples were assayed by the method of Lowry *et al*[11] using bovine serum albumin as standard. Serum urea and creatinine levels were determined by the method of Talke and Schubert[12] and Jaffe[13], respectively. The activities of alanine and aspartate aminotransferases (ALT and AST) were assayed by the combined methods of Mohun and Cook[14] and, Reitman and Frankel[15]. Serum γ-glutamyl transferase (GGT) activity was assayed by the method of Fossati *et al*[16]. The activities of α-amylase and lactate dehydrogenase (LDH) were determined by the methods of Gella *et al*[17] and, Zimmerman and Weinstein[18], respectively. The levels of HbA1c were determined by the enzymatic method of Hirokawa *et al*[19]. The estimation of alkaline phosphatase (ALP) activity was based on the method

of Williamson[20]. Total bilirubin level was assayed by the method of Rutkowski and Debaare[21]. Serum triglyceride and cholesterol levels were assayed using commercial diagnostic kits (Randox). For the determination of HDL level, VLDL and LDL lipoproteins were precipitated by addition of phosphotungstic acid and magnesium chloride. After centrifugation, the supernatant containing the HDL fraction was assayed for cholesterol using Randox diagnostic kit. LDL - cholesterol (LDL-C) was calculated using the formula of Friedewald *et al*[22].

2.7. Statistical analysis

All values were expressed as the mean ± S.D. of six animals per group. Data were analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0). Values were considered statistically significant at $P<0.05$.

3. Results

3.1. Phytochemical analysis of crude methanolic extract of African mistletoe (AM)

The phytochemical screening of crude methanolic extract of AM revealed the presence of bioactive compounds in the plant. Seven bioactive constituents were tested, out of which two tested positive. Analysis of tannins and flavonoids were positive while alkaloids, phlobatannins, saponins, anthraquinones and cardiac glycosides were completely absent in the extract.

3.2. Effect of extract of African mistletoe administered at 50 mg/kg (AM1) and 100 mg/kg (AM2) on blood glucose levels of STZ-diabetic rats within 4 h of administration

In STZ-diabetic rats, AM1 was found to decrease FBG by 41%, 42% and 41%, while AM2 decreased FBG by 49%, 51% and 50%, after 120, 180 and 240 min of extract administration, respectively. Similarly, glibenclamide (GB) progressively lowered the FBG of the rats by 50%, 51% and 49%, at 120, 180 and 240 min after GB administration. Importantly, the hypoglycemic effects of GB were statistically similar to AM2 at 180 and 240 min after administration. In addition, the FBG levels in diabetic rats that received saline solution, that is negative control (STZ only) remain statistically unchanged ($P>0.05$) during the study (Table 1).

3.3. The effect of AM2 on body weight and FBG of STZ-diabetic rats

Table 2 shows the effect of AM2 on body weight and relative weight of organs of STZ-diabetic rats. The final body weight of STZ-diabetic rats decreased significantly ($P>0.05$) when compared with normal. Also, the change in body weight was highest for untreated STZ-diabetic rats when compared with GB- and extract- diabetic rats. Similarly, the relative weight of liver in untreated STZ group was significantly ($P>0.05$) higher than others. At the end of this study, the FBG levels of STZ-diabetic animals treated with AM2 and GB decreased significantly ($P>0.05$) when compared with untreated diabetic group (Figure 1). In addition, both AM2 and GB significantly ($P>0.05$) decreased the HbA1c levels of the treated diabetic rats when compared to untreated diabetic group (Table 3).

Table 1
Effect of extract from African mistletoe on the level of fasting blood glucose in streptozotocin-diabetic rats.

Time (min)	Fasting blood glucose (mg/dL)				
	Normal (non-diabetic)	STZ only	STZ+AMI	STZ+AM2	STZ+GB
0	68.5±4.3	368.2±34.6	385.1±32.3	383.2±35.5	369.0±27.4
30	65.3±6.1	360.7±28.6	353.6±39.8	361.3±37.2	375.1±37.7
60	66.3±3.8	365.3±31.8	332.0±41.0	311.2±26.7	228.5±27.3*
90	68.1±5.4	361.2±41.0	227.2±18.5*	214.1±19.3*	189.3±19.0*
120	67.5±7.3	370.0±39.6	225.1±21.6*	196.7±15.3*	183.3±21.6*
180	69.2±6.8	368.5±37.2	223.7±24.1*	187.4±16.9*	185.6±18.0*
240	68.0±5.2	364.8±35.1	228.0±18.4*	190.5±21.8*	187.9±17.5*

Values are means±S.D. of 5 rats per group. *Significantly differeent from normal at $P<0.05$. STZ=Streptozotocin, AMI=African mistletoe at a dose of 50 mg/kg, AM2=African mistletoe at a of 100 mg/kg, GB=Glibenclamide.

Table 2
Effect of extract from African mistletoe on the body weight and relative weight of organs of streptozotocin-diabetic rats.

Treatment	body weight (g)			Weight (g)			Relative weight (% body weight)		
	Initial	Final	Change	Liver	Kidney	Heart	Liver	Kidney	Heart
Normal	225.1±5.3	241.3±6.2	16.2±5.6	6.8±0.5	1.3±0.1	0.8±0.1	2.8±0.3	0.50±0.09	0.30±0.05
STZ only	226.3±4.7	178.0±9.1*	-48.3±8.3	7.1±0.8	1.2±0.2	0.8±0.1	4.0±0.5*	0.60±0.07	0.40±0.05
STZ+AM2	231.6±6.2	208.5±7.4	-23.1±6.0	7.0±0.5	1.3±0.1	0.9±0.2	3.4±0.5	0.60±0.10	0.40±0.05
STZ+GB	228.4±4.4	204.6±8.0	-23.8±7.3	7.2±0.8	0.3±0.2	0.8±0.1	3.5±0.4	0.60±0.08	0.40±0.08

Values are means±S.D. of 6 rats per group. * Significantly different from normal at $P<0.05$. STZ=Streptozotocin, AM2= African mistletoe at 100 mg/kg, GB=Glibenclamide.

Table 3
Effect of extract from African mistletoe on some biochemical indices in streptozotocin-diabetic rats.

Treatment	Serum		Red cell HbA_{1c} (%)
	Protein (mg/dL)	LDH (IU/L)	
Normal	1.82±0.46	579.8±31.2	4.20±0.28
STZ only	1.63±0.38	1 137.0±51.3*	8.30±0.36*
STZ+AM2	1.74±0.35	728.4±39.7**	6.10±0.41**
STZ+GB	1.86±0.44	824.2±43.2**	6.20±0.22**

Values are means±S.D. of 6 rats per group. * Significantly different from normal at $P<0.05$. ** Singnificantly different from STZ only at $P<0.05$. STZ=Streptozotocin, AM2=African mistletoe at 100 mg/kg, GB=Glibenclamide, HbA1c=Glycosylated hemoglobin, LDH= Lactate dehydrogenase.

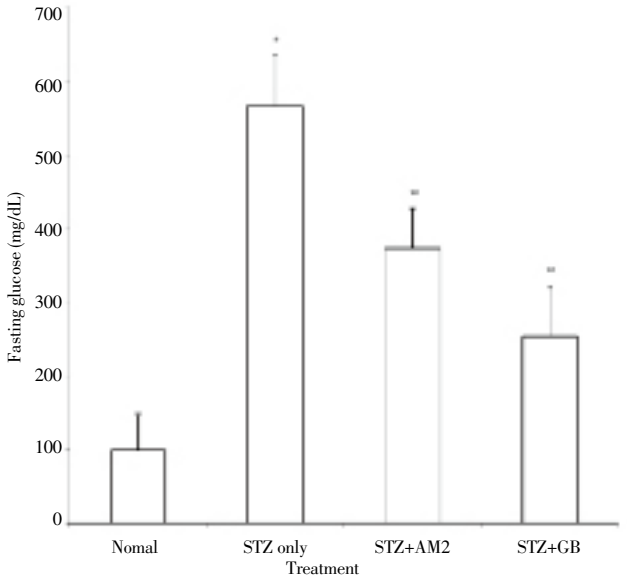

Figure 1. Effect of extract from African mistletoe on the levels of fasting glucose of streptozotocin (STZ)-diabetic rats.

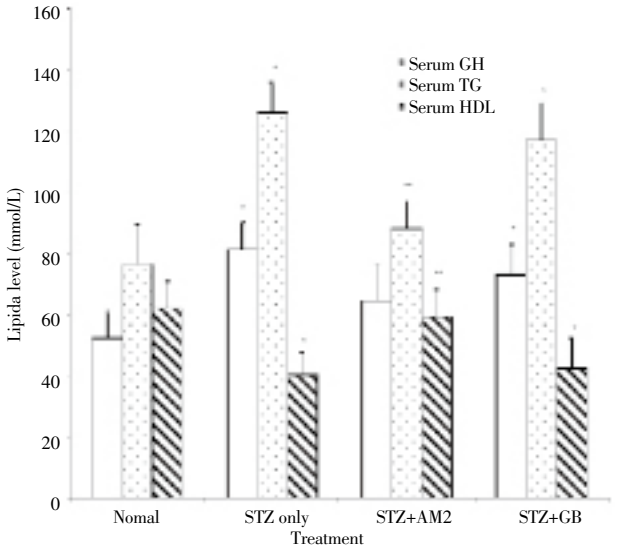

Figure 2. Effect of extract from African mistletoe on the levels of serum cholesterol, triglyceride and HDL-cholesterol of streptozotocin (STZ)-diabetic rats.

3.4. Effects of AM2 on the lipid profiles of STZ-diabetic rats

STZ-intoxication caused a significant increase ($P>0.05$) in the serum total cholesterol and triglycerides of untreated diabetic animals when compared with normal (Figure 2). Precisely total cholesterol and triglycerides were increased by 56% and 65%, respectively in untreated diabetic rats. Furthermore, serum LDL-cholesterol level of untreated STZ-diabetic rats increased by 75% relative to normal (Figure 4). In contrast, untreated STZ-diabetic rats had significantly ($P>0.05$) lowered serum HDL-cholesterol level when compared with normal (Figure 2). Administration of AM2 significantly reduced ($P>0.05$) the hypercholesterolemia and hypertriglyceridemia in these diabetic rats (Figures 2 and 4). Also, AM2 significantly ($P>0.05$) reversed the adverse effect of STZ on HDL-cholesterol levels in the animals (Figure 2). The reversal effect of AM2 on the lipid profile of STZ-diabetic rats was much stronger than GB-treated diabetic rats.

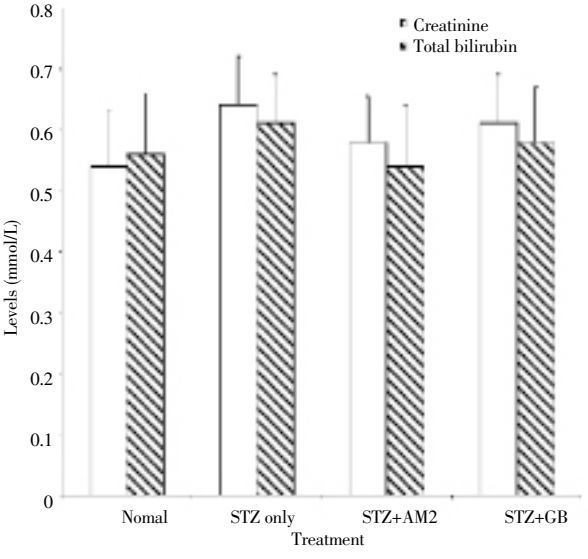

Figure 3. Effect of extract from African mistletoe on the levels of serum creatinine and total bilirubin of streptozotocin (STZ)-diabetic rats.

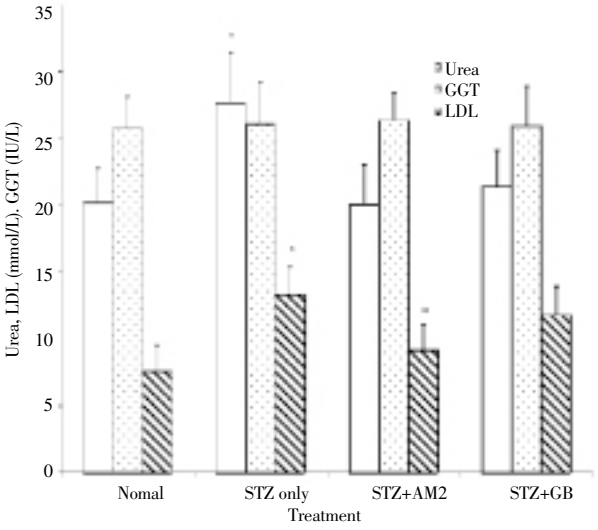

Figure 4. Effect of extract from African mistletoe on the levels of serum urea, gamma glutamyl transferase and LDL-cholesterol of streptozotocin (STZ)-diabetic rats.

3.5. Effects of AM2 on the kidney and liver function indices of STZ-diabetic rats

Figures 3-6 and Table 3 show that the STZ intoxication produced insignificant ($P>0.05$) effect on the levels of serum total bilirubin, creatinine, gamma glutamyl transferase, alanine and aspartate aminotransferases and alkaline phosphatase in the rats when compared with normal. In contrast, STZ-diabetic rats had significantly ($P>0.05$) increased levels of serum urea, lactate dehydrogenase (LDH) and α-amylase relative to normal. Treatment with AM2 significantly ($P>0.05$) ameliorated the STZ-induced elevation in these biochemical indices.

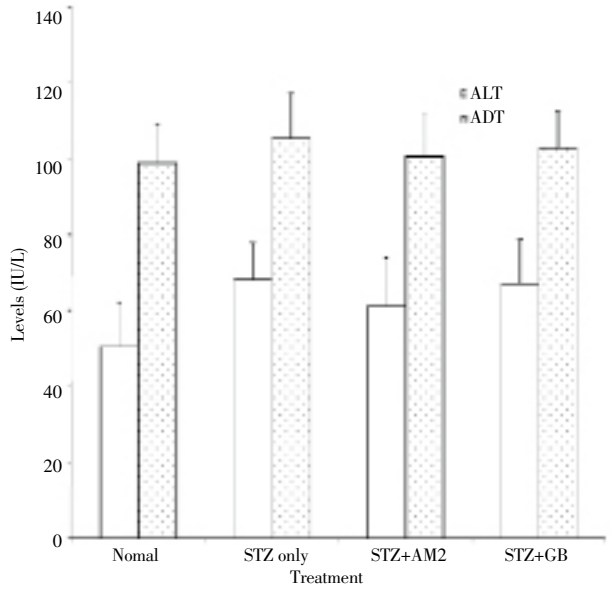

Figure 5. Effect of extract from African mistletoe on the activities of serum alanine and aspartate aminotransferases (ALT and AST) of streptozotocin (STZ)-diabetic rats.

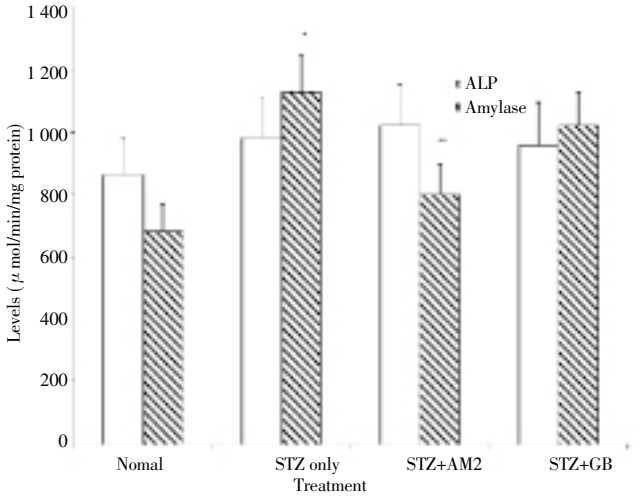

Figure 6. Effect of extract from African mistletoe on the activities of serum alkaline phosphatase and α-amylase of streptozotocin (STZ) diabetic rat.

4. Discussion

Research conducted over decades, and the on-going studies have shown that plants and plant- based therapies may be potent means of controlling and treating diabetes and its complications[23-25]. Appropriate nutritional management is essential for restoring and maintaining a normal metabolic state. Therefore, diet remains a cornerstone in diabetic management. Spices and cereals such as Fenugreek, curry patta (Murraya koenigii), Brassica juncea, commonly used as dietary constituents have been found to have beneficial effect on carbohydrate metabolism experimentally, as well as clinically[26,27].

The major findings of the present study are that AM exhibited marked hypoglycemic effects and improved serum lipid profile of STZ-diabetic animals. In addition, AM elicited better glycemic control evidenced by the HbA_{1c} values of the treated diabetic rats when compared with untreated group. Furthermore, AM attenuated the increased α-amylase and lactate dehydrogenase activities, and reduced the levels of serum urea in the diabetic rats.

In this study, STZ was used to induce hyperglycemia in the rats. The use of STZ to induce diabetes mellitus in experimental animal models is widely accepted since STZ-induced diabetes appears to resemble human hyperglycemic non-ketotic diabetes mellitus[28] and is often associated with kidney hypertrophy which may contribute to end stage renal damage, hepatotoxicity, oxidative stress and hypercholesterolemia[29,30]. It was noticed that AM exhibited hypoglycaemic activities in fasted STZ-diabetic rats up till 4 h after extract administration. When the treatment period was extended to 3 weeks, AM further elicited significant hypoglycemic activities which were similar in action to the effects induced by glibenclamide. The significant hypoglycaemic effect of this extract may be indicative that AM exerts their effect by both direct and indirect mechanisms in the rats[31]. If the extract acted only as

indirect hypoglycaemic agents, no effect would be observed when they were administered to STZ-diabetic rats, since STZ administered at a dose of 35 mg/kg[10] should have caused severe destruction of β-cells of the pancreas[9]. The likely indirect mechanism suggests that AM probably act by stimulating the few surviving β-cells to release more insulin rather than by aiding the regeneration of necrotic β-cells of the pancreas.

In addition, the anti-diabetic activity of the extract was further confirmed with the normalization of HbA1c values in the diabetic rats treated with AM. HbA_{1c} is an index of diabetic control that is used to assess the effectiveness of therapy in diabetes[32]. Furthermore, diabetic control was assessed by determining the activities of α-amylase of the diabetic rats[33]. In this study, the serum α-amylase activities of STZ diabetic rats were almost 2-fold higher than the normal rats. However, treatment with AM significantly attenuated the activities of this enzyme, which confirms better glycemic control by AM.

Still lingering for solutions are the secondary complications of diabetes such as nephropathy, cardiovascular disorders, hepatic damage, fatty liver, oxidative stress, etc. In this study, marked elevation of serum urea was observed in the STZ-diabetic rats, although the creatinine levels were insignificantly affected. This observation is consistent with the findings of Alderson *et al*[34] and Adisa *et al*[4] which reported elevated urea levels in serum of STZ-diabetic rats. Serum creatinine and urea levels are sensitive and reliable biochemical indices for evaluation of renal function in animal model[35]. The increased serum urea levels indicate impairment to the kidney function such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis[36]. Our data indicate that crude methanolic extract of African mistletoe at a dose of 100 mg/kg reduced the levels of serum urea in STZ-diabetic rats to near normalcy. This result indicates the possible protective effect of AM against diabetes-induced renal dysfunction in the animals. ALT and AST are reliable maker enzymes for liver function in animals. It is established that AST can be found in the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes and erythrocytes, whereas ALT is predominantly present in the liver[37]. The increased levels of AST and ALT indicate an increased permeability and damage and/or necrosis of hepatocytes[38]. The membrane bound enzymes like ALP and GGT are also released into bloodstream depending on the pathological phenomenon[39]. In our study, we found that the activities of AST, ALT, ALP and GGT in STZ-diabetic rats were insignificantly different from normal. This observation may be due to the short duration (3 weeks) of the study. It is therefore reasonable to suggest that to ascertain the effect of diabetes on the integrity of liver marker indices, the duration of study could be extended to 6-8 weeks. In addition to its hypoglycaemic effect, AM was able to improve lipid metabolites including total cholesterol, triglyceride, HDL- and LDL-cholesterol levels in the diabetic rats. Diabetes is associated with profound alterations in lipid and lipoprotein profiles[40]. Therefore, lowering of plasma or tissue lipid levels may lead to a decrease in the risk of micro- or macrovascular disease and related complications[41]. It can therefore be suggested that AM may improve lipid profiles directly or indirectly through reducing blood glucose in diabetic animals as observed in this study.

In conclusion, these results confirm antidiabetic and antihyperlipidemic effects of African mistletoe in STZ diabetic rats and further substantiated the claim of its use in folklore medicine as anti-diabetic agent. The beneficial effect of AM on lipid profile of diabetic rats suggests its possible amelioration of secondary complications of diabetes. Further detailed studies are required to establish the toxicity and active component of AM responsible for the observed effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Zimmet P, Alberti KGMM, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**(6865): 782-787.

[2] Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* 2001; **414**(6865): 821-827.

[3] Adaramoye OA, Adeyemi EO. Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia Kola* in streptozotocin-induced diabetes mellitus rats. *J Pharm Pharmacol* 2006; **58**(1): 121-128.

[4] Adisa RA, Choudhary MI, Olorunsogo OO. Hypoglycemic activity of *Buchholzia coriacea* (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. *Exp Toxicol Pathol* 2011; **63**(7-8): 619-625.

[5] Chaulya NC, Haldar PK, Mukherjee A. Antidiabetic activity of methanol extract of rhizomes of *Cyperus tegetum* Roxb. (Cyperaceae). *Acta Pol Pharm* 2011; **68**(6): 989-992.

[6] Troncoso AJ, Cabezas NJ, Faúndez EH, Urzúa A, Niemeyer HM. Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. *Oecologia* 2010; **162**(2): 413-425.

[7] Ojewole JA, Adewole SO. Hypoglycaemic and hypotensive effects of *Globimetula cupulata* (DC) Van Tieghem (Loranthaceae) aqueous leaf extract in rats. *Cardiovasc J S Afr* 2007; **18**(1): 9-15.

[8] Onay-Ucar E, Karagoz A, Arda N. Antioxidant activity of *Viscum album* ssp. album. *Fitoterapia* 2006; **77**(7-8): 556-560.

[9] Sharma SR, Dwivedi SK, Swarup D. Hypoglycemic,

antihyperglycemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. *J Ethnopharmacol* 1997; **58**(1): 39–44.

[10]Nakhoda A, Wong HA. The induction of diabetes in rats by intramuscular administration of streptozotocin. *Experientia* 1979; **35**(12): 1679–1680.

[11]Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**(1): 265–275.

[12]Talke H, Schubert GE. Enzymatische Harnstoff bestimmung in Blut and serum in Optischen Test nach Warburg. *Klin Wochschr* 1965; **43**(1): 174.

[13]Jaffe M. Ueber den Neiderschlag, welchen Pikrinsäure im normalen harn Erzeught und über eine neue Reaction des Kreatinins. *Z Physiol Chem* 1886; **10**(1): 391–400.

[14]Mohun AF, Cook LJ. Simple method for measuring serum level of glutamate-oxaloacetate and glutamate-pyruvate transaminases in laboratories. *J Clin Pathol* 1957; **10**(2): 394–399.

[15]Reitman S, Frankel S. A colorimetric method for the determination of serum level of glutamate-oxaloacetate and pyruvate transaminases. *Am J Clin Pathol* 1957; **28**(1): 56–63.

[16]Fossati R, Melzid'Eril GV, Turenghi G, Precipe L, Berti G. A kinetic colorimetric assay of gamma-glutamyltransferase. *Clin Chem* 1986; **32**(8): 1581–1584.

[17]Gella FJ, Gubern G, Vidal R, Canalias F. Determination of total and pancreatic alpha-amylase in human serum with 2-chloro-4-nitrophenyl-alpha-D-maltotrioside as substrate. *Clin Chim Acta* 1997; **259**(1–2): 147–160.

[18]Zimmerman HJ, Weinstein BS. Lactic dehydrogenase activity in human serum. *J Lab Clin Med* 1956; **48**(2): 607–609.

[19]Hirokawa K, Shimoji K, Kajiyama N. An enzymatic method for the determination of hemoglobin A_{1C}. *Biotechnol Lett* 2005; **27**(14): 963–968.

[20]Williamson T. A comparison between the phosphastrate and phenyl phosphate methods of alkaline phosphatase assay. *Med Lab Technol* 1972; **29**(2): 182–187.

[21]Rutkowski RB, Debaare L. An ultra-micro colorimetric method for determination of total and direct serum bilirubin. *Clin Chem* 1966; **12**(7): 432–438.

[22]Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultra-centrifuge. *Clin Chem* 1972; **18**(6): 499–502.

[23]Akhtar N, Khan BA, Majid A, Khan HM, Mahmood T, Gulfishan, et al. Pharmaceutical and biopharmaceutical evaluation of extracts from different plant parts of indigenous origin for their hypoglycemic responses in rabbits. *Acta Pol Pharm* 2011; **68**(6): 919–925.

[24]Trojan-Rodrigues M, Alves TL, Soares GL, Ritter MR. Plants used as antidiabetics in popular medicine in Rio Grande do Sul, southern Brazil. *J Ethnopharmacol* 2012; **139**(1): 155–163.

[25]Gandhi GR, Ignacimuthu S, Paulraj MG, Sasikumar P. Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from *Solanum torvum* Swartz. fruit in streptozotocin induced diabetic rats. *Eur J Pharmacol* 2011; **670**(2–3): 623–631.

[26]Khan BA, Abraham A, Leelamma S. Hypoglycemic action of *Murraya Koenigii* (curry leaf) and *Brassica juncea* (mustard): mechanism of action. *Indian J Biochem Biophys* 1995; **32**(2): 106–108.

[27]Vats V, Grover JK, Rathi SS. Evaluation of antihyperglycemic and hypoglycemic effect of *Trigonella foemumgraceum*, *Ocimum sanctum* and *Pterocarpus marsupium* in normal and alloxanized diabetic rats. *J Ethnopharmacol* 2002; **79**(1): 95–100.

[28]Usuki S, Tsai YY, Morikawa K, Nonaka S, Okuhara Y, Kise M, et al. IGF-1 Induction by acylated steryl β-glucosides found in a pre-germinated brown rice diet reduces oxidative stress in streptozotocin-induced diabetes. *PLoS One* 2011; **6**(12): e28693.

[29]Maric-Bilkan C, Flynn ER, Chade AR. Microvascular disease precedes the decline in renal function in the streptozotocin-induced diabetic rat. *Am J Physiol Renal Physiol* 2011; doi: 10.1152/ajprenal.00421.2011.

[30]Gezginci-Oktayoglu S, Basaraner H, Yanardag R, Bolkent S. The effects of combined treatment of antioxidants on the liver injury in STZ diabetic rats. *Dig Dis Sci* 2009; **54**(3): 538–546.

[31]Iwu MM, Igboko OA, Okunji CO, Tempesta MS. Anti-diabetic and aldose reductase activities of biflavanones of *Garcinia kola*. *J Pharm Pharmacol* 1990; **42**(4): 290–292.

[32]Barth JH, Marshall SM, Watson ID. Consensus meeting on reporting glycated haemoglobin and estimated average glucose in the UK: report to the National Director for Diabetes, Department of Health. *Ann Clin Biochem* 2008; **45**(Pt 4): 343–344.

[33]Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement Altern Med* 2011; **20**(11): 5–14.

[34]Alderson NL, Chachich ME, Frizzell N, Canning P, Metz TO, Januszewski AS. Effect of antioxidants and ACE inhibition on chemical modification of proteins and progression of nephropathy in streptozotocin diabetic rat. *Diabetologia* 2004; **47**(5): 1385–1395.

[35]Asvadi I, Hajipour B, Asvadi A, Asl NA, Roshangar L, Khodadadi A. Protective effect of pentoxyfilline in renal toxicity after methotrexate administration. *Eur Rev Med Pharmacol Sci* 2011; **15**(9): 1003–1009.

[36]Jaramillo-Juarez F, Rodriquez-Vazquez ML, Rincon-Sanchez AR, Consolación Martínez M, Ortiz GG, Llamas J, et al. Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis. *Ann Hepatol* 2008; **7**(4): 331–338.

[37]Breitling LP, Arndt V, Drath C, Brenner H. Liver enzymes: interaction analysis of smoking with alcohol consumption or BMI, comparing AST and ALT to γ-GT. *PLoS One* 2011; **6**(11): e27951.

[38]Adjroud O. The toxic effects of nickel chloride on liver, erythropoiesis, and development in Wistar albino preimplanted rats can be reversed with selenium pretreatment. *Environ Toxicol* 2011; doi: 10.1002/tox.20719.

[39]Ota Y, Imai T, Onose J, Takami S, Cho YM, Hirose M, et al. A 55-week chronic toxicity study of dietary administered kojic acid (KA) in male F344 rats. *J Toxicol Sci* 2009; **34**(3): 305–313.

[40]Mir SH, Baqui A, Bhagat RC, Darzi MM, Shah AW. Biochemical and histological study of streptozotocin-induced diabetes mellitus in rabbits. *Pak J Nutr* 2008; **7**(2): 359–364.

[41]Marinou KA, Georgopoulou K, Agrogiannis G, Karatzas T, Iliopoulos D, Papalois A, et al. Differential effect of *Pistacia vera* extracts on experimental atherosclerosis in the rabbit animal model: an experimental study. *Lipids Health Dis* 2010; **9**: 73.