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Evaluation of antidiabetic activity of plants used in Western Sudan

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PEER REVIEW

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Comments

This research obtained interesting and valuable results. The authors have demostrated the effect of aqueous extracts from some indigenous plants in supporting the treatment of diabetes.

Details on Page 401

ABSTRACT

Objective: To investigate the traditional antidiabetic uses of some indigenous Sudanese plants on streptozotocin-induced diabetes rats.

Methods: Diabetic rats were treated with a 400 mg/kg dose of aqueous extracts of five plant species orally for 2 h (acute) or 14 days (chronic). In acute model blood glucose levels were monitored at specific intervals. In the chronic model blood samples were collected from overnight fasted diabetic rats on day 15 to estimate blood glucose level. And the body weight, serum lipid profile and activities of liver and kidney enzymes were measured. Histopathological observations of liver sections were also studied.

Results: In the case of acute treatment, aqueous extracts of *Tinospora bakis* (*T. bakis*), *Nauclea latifolia* (*N. latifolia*) and *Randia nilotica* (*R. nilotica*) at 400 mg/kg significantly lowered ($P < 0.05$) blood glucose levels in diabetic rats whereas, chronic treatment of diabetic rats with 400 mg/kg of *T. bakis, N. latifolia, R. nilotica* and *Mitragyna inremis* proved to have significant ($P < 0.05$) antihyperglycemic effect and have the capacity to correct the metabolic disturbances associated with diabetes. Histopathological studies showed that the aqueous extracts of these four plants reinforced the healing of liver. However, *Striga hermonthica* aqueous extract did not exert any antihyperglycemic effect to diabetic rats.

Conclusions: This study demonstrated that *T. bakis, N. latifolia, R. nilotica* and *Mitragyna inremis* have therapeutic value in diabetes and related complications and thus supporting the traditional uses of these plants in Sudanese traditional medicine.

KEYWORDS

Tinospora bakis, *Mitragyna inremis*, *Nauclea latifolia*, *Randia nilotica*, *Striga hermonthica*, Antihyperglycemic effect

1. Introduction

Diabetes mellitus worldwide has shown an alarming upsurge, ranking it as the fourth or fifth leading cause of death in the world. It is associated with serious complications including coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness[1].

The current rapid global rise in diabetes rate is attributed to rapid rise in unhealthy life styles, urbanization and aging[2]. Global estimate of the number of diabetics within the past three decades showed an increase from 153 million in 1980 to 347 million in 2008. Currently the prevalence of diabetes is estimated at 382 million people in 2013 and is expected to rise to 592 million by the year 2035. With this high prevalence, the highest mortality due to diabetis mellitus occurs in low and middle income countries[1].

Management of diabetes using traditional remedies is

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widespread in Africa in rural as well as urban communities. An increasing number of patients opt for traditional remedies driven by a combination of factors including financial constraints and/or geographical accessibility of the population to orthodox antidiabetics, inadequacy of the healthcare systems, ease of accessibility of traditional medicine, indigenous knowledge of community members as well as the role of traditional healers[3,4].

Sudan is characterized by a rich floral diversity and an equally rich ethnomedicinal tradition. Several herbal preparations have been used in folklore practice for the management of diabetes with claims asserting their hypoglycemic effect, which heightened the interest in further research to ascertain the basis of their antidiabetic properties. The hypoglycemic activity of many plants like *Geigeria alata* (*G. alata*) roots[5], *Acacia nilotica* pods[6], *Balanites aegyptiaca* fruits, *Guiera senegalensis* leaves, *Hyphaene thebaica* epicarp and *Trigonella foenum-graecum* seeds[7], used in Sudanese folk medicine has been reported during the last few years. However, the mainstream use of herbal medicines in Sudan is popularized by empirical lore of special groups' experiences and personal contacts as in the case of some tribes in the Nuba Mountains (Western Sudan) who used some plants for the treatment of diabetes, yet these species are not commonly known for their hypoglycemic effect in other regions of the country. Such plant species are exemplified by *Mitragyna inremis* (Wild) O. Kundze (*M. inremis*), *Nauclea latifolia* Smith (*N. latifolia*), *Randia nilotica* Stapf. (*R. nilotica*), *Tinospora bakis* (A.Rich.) Miers (*T. bakis*) and *Striga hermonthica* (Del.) Benth (*S. hermonthica*) which are used in The Nuba Mountains region for the management of diabetes (personal communication). However, some of these species are used for other ailments according to the experience in other parts of the country where *S. hermonthica* is used in Central Sudan against leukoderma[8] and *T. bakis* for the treatment of sleeping sickness in South Eastern Sudan[9]. A similar experience to that of Nuba Mountains groups is evident in India and Turkey where another *Tinospora* species, *Tinospora cordifolia*, is known for its antidiabetic effect[10,11]. The use of *M. inremis* and *N. latifolia* as antidiabetic in the Nuba Mountains is similar to the practice of Hausa/Fulani tribes of Northern Nigeria, with differences in plant organ used, where the stem bark of the former[12] and leaves of the latter[13] were found to have hypoglycemic effect in alloxan induced diabetic Wistar rats. Therefore, the objective of this study was to investigate the hypoglycaemic effect of aqueous extracts from *M. inremis*, *N. latifolia*, *R. nilotica*, *T. bakis* and *S. hermonthica*.

2. Materials and methods

2.1. Plant materials

Plant materials from the five plant species were collected from the Nuba Mountains in Western Sudan on December 2012. Botanical identification and authentication were performed in the Botany Department Herbarium, Faculty of Science, University of Khartoum, Sudan. The identities, parts used and voucher specimen numbers of the investigated plants are shown in Table 1.

Table 1

Name, family, voucher number and part studied of the investigated plants.

Plant Latin name	Family	Vernacular name	Voucher No.	Part used
M. inremis	Rubiaceae	Hikmat	2013-12/HBD	Fruits
N. latifolia	Rubiaceae	Karmadoda	2013-13/HBD	Fruits
R. nilotica	Rubiaceae	Kir Kir	2013-14/HBD	Fruits
T. bakis	Menispermaceae	Irg alhager	2013-15/HBD	Seeds
S. hermonthica	Scrophulariaceae	Boda	2013-16/HBD	Whole plant

2.2. Preparation of aqueous crude extracts

The studied parts of the plant materials were shade-dried and then ground to powder. Aqueous extract was prepared by simple maceration of 500 g of powdered sample in 1 500 mL of distilled water maintained at ambient temperature for 4 h. Extract was first filtered on filter paper and then freeze-dried. The extract yields were 33.5 g of *M. inremis*, 38.5 g of *N. latifolia*, 33.4 g of *R. nilotica*, 42.3 g of *T. bakis* and 30.5 g of *S. hermonthica*. Each extract was dissolved in distilled water before its administration to the diabetic rats. For treatments, the concentration of aqueous extract was 400 mg/kg body weight. This was prepared by dissolving 2 g of plant extract in 25 mL water in a falcon tube in a hot water bath with vigorous shaking. The dose of each extract was calculated according to body weight before administration to the diabetic rats.

2.3. Animals and experimental design

Wistar rats of either sex, aged 60 days and weighing 200–250 g, were obtained from the Medicinal and Aromatic Plants Research Institute, Khartoum, reared in the premises of the Institute under illumination at night and early morning, with feed and drinking water provided *ad libitum*. This work was carried out according to the international regulations for the use of laboratory animals. Prior to experimental treatment, animals were fasted an overnight, but were allowed free access to water.

2.4. Acute oral toxicity study

The acute oral toxicity study was conducted using test guidelines on acute oral toxicity test 423 according to Organisation for Economic Cooperation and Development[13]. A limit dose of 2000 mg/kg body weight /oral was used. The signs of toxic effects and/or mortality were observed 3 h after administration then for the next 48 h. The body weight was recorded for consecutive 14 days. Since the extracts were found safe up to the dose level of 2000 mg/kg body weight, a dose of 400 mg/kg body weight of the different plants extracts was selected for screening of the antidiabetic activity.

2.5. Induction of diabetes mellitus

Diabetes was induced by intraperitoneal injection of 55 mg/kg body weight of streptozotocin (STZ) (Sigma, St Louis, MO, USA) dissolved in freshly prepared citrate buffer (0.1 mol/L, pH 4 5). Fasting blood sugar for the animals was measured after 72 h using

Medisafe Mini Blood Glucose Reader (TERUMO Corporation Ltd., Hatagaya, Tokyo, Japan). Rats with fasting blood sugar level 200 mg/dL were considered diabetic[14].

2.6. Oral glucose tolerance test with extracts in diabetic rats

Fourty eight rats (42 diabetic surviving rats and 6 normal rats) were divided into eight groups of six rats each. In Group 1, normal rats were treated with distilled water and used as the negative control. In Group 2, diabetic control rats were treated with distilled water. In Group 3, diabetic rats were given standard drug glibenclamide (5 mg/kg body weight). Groups 4, 5, 6, 7 and 8 served as diabetic rats given *M. inremis*, *N. latifolia*, *R. nilotica*, *T. bakis* and *S. hermonthica* aqueous extracts at dose of 400 mg/kg respectively. All the diabetic rats were fasted overnight (14 h) before the oral glucose tolerance test was done. Thirty minutes following the extracts or glibenclamide treatment, each rat was given an oral glucose load of 2 g/kg body weight. Blood samples were withdrawn from retro-orbital site at intervals of 60, 120 and 180 min of glucose administration.

2.7. Chronic treatment with extracts

The experimental rats were randomly divided into seven groups of six rats each: Group I, normal control rats; Group II, untreated diabetic rats; Groups III, IV, V,VI and VII are *M. inremis*, *N. latifolia*, *R. nilotica*, *T. bakis* and *S. hermonthica* treated diabetic rats respectively. In the case of extracts-treated diabetic rats, the extracts were administered orally at a dose of 400 mg/kg per 5 mL water once daily for 14 days. Normal control rats and untreated diabetic rats received equal volumes of water in place of the extract. The body weight was measured every day and the dose was calculated accordingly.

2.8. Collection of blood samples and estimation of biochemical parameters

At the end of the experimental period, day 14, the animals were fasted an overnight and the rats were sacrificed by cervical decapitation and fasting blood samples were collected in tubes with heparin. For serum samples, blood was allowed to coagulate, followed by centrifugation at 3 000 r/min for 15 min at 4 ˚C to separate serum. Sera were divided into aliquots and stored at -80 ˚C for biochemical assay.

2.9. Biochemical analysis

For biochemical analysis we used standard commercial kits according to the manufacturer's protocol. Fasting serum glucose level was determined on day 15 by glucose oxidase-peroxidase method using the kit of RANDOX Laboratories Ltd, UK. Serum total cholesterol (TC), serum triglycerides (TG), and high density lipoprotein-cholesterol (HDL-C)] were also measured using kits from Randox Laboratory Ltd. Low density lipoprotein-cholesterol (HDL-C) was calculated as follows: HDL-C = TC - TG / 5 - HDL. Serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured using kits from Randox Laboratory Ltd., UK. Serum urea was determined using a commercial kit from QUIMICA Clinica Aplicada, Amposta, Spain. Blood urea nitrogen was calculated by the following formula: Blood urea nitrogen (mg/dL) = urea (mg/dL) × 0.467.

2.10. Histopathology

Small pieces (5–8 mm) of tissue from the liver in all groups were excised and were immediately fixed in 10% neutral buffered formalin. Fixed samples were trimmed and processed for paraffin embedding. Sections (5–7 μm) were cut and picked up on clean silane-coated glass slides. After de-waxing and rehydration through descending concentrations of ethanol, the sections were stained with haematoxylin and eosin and examined microscopically.

2.11. Statistical analysis

All values are expressed as mean ± SE. Statistical analysis was performed by One-way analysis of variance followed by Tukey's multiple comparison tests. The results were considered statistically significant if P is < 0.05.

3. Results

3.1. Acute toxicity study

The different plants aqueous extracts were safe up to a dose of 2000 mg/kg body weight. Behavior of the animals was clearly observed for the first 8 h then at an interval of every 4 h during the next 48 h, the extracts did not produce significant changes in the behavior of the animals or mortality.

3.2. Effect of plants aqueous extracts on oral glucose tolerance in diabetic rats

Blood glucose level of the normal control rats, STZ induced diabetic control rats and diabetic rats treated with glibenclamide and different aqueous extracts dosed at 400 mg/kg at different time points (0, 60, 120 and 180 min) after oral administration of glucose (2 g/kg) is shown in Figure 1. In the diabetic control rats, the peak increase in blood glucose level was observed after 60 min and remained high over the next 120 min. Aqueous extracts of *T. bakis*, *N. latifolia* and *R. nilotica* significantly reduced (P < 0.05) the blood glucose level at 60 min and remained low over the next 120 min when compared with the diabetic control rats. However, the aqueous extract of *S. hermonthica* induced an insignificant reduction in the blood glucose level at 120 min followed by a significant increase (P < 0.05) at 180 min to a level approaching that of diabetic control rats. *M. inremis* did not exert any change in

blood glucose level of the diabetic rats.

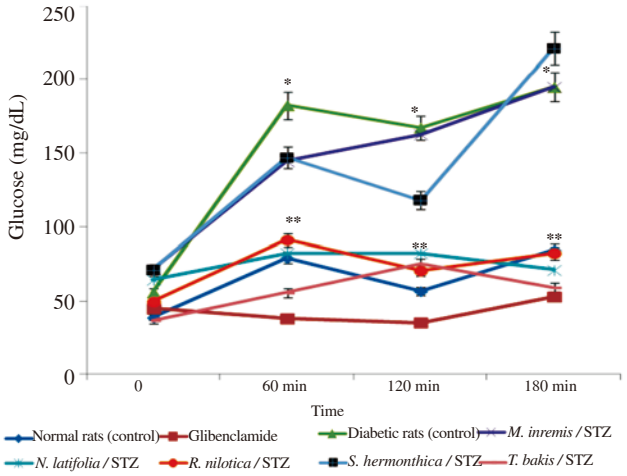

Figure 1. Effect of plants aqueous extracts on oral glucose tolerance in STZ induced diabetic rats.

Values are expressed as mean ± SEM (n = 6); *: $P < 0.05$ by comparison with normal rats; **: $P < 0.05$ by comparison with diabetic control rats.

3.3. Effect of plants aqueous extracts on body weight

Results of the effect of different plants aqueous extracts at 400 mg/kg/day dose on body weight of STZ induced diabetic rats after 2 weeks of treatment are presented in Figure 2. By the end of second week, diabetic rats gained less body weight with significant reduction (25%) ($P < 0.05$) to the normal control rats. Only aqueous extract of *T. bakis* improved the body weight of diabetic rats with significant increase (21%) ($P < 0.05$) compared to the diabetic control rats. Aqueous extract of *S. hermonthica* significantly reduced ($P < 0.05$) the body weight of diabetic rats (26%) compared to diabetic control rats. The differences in body weight of diabetic rats treated with other plant aqueous extracts were not significant.

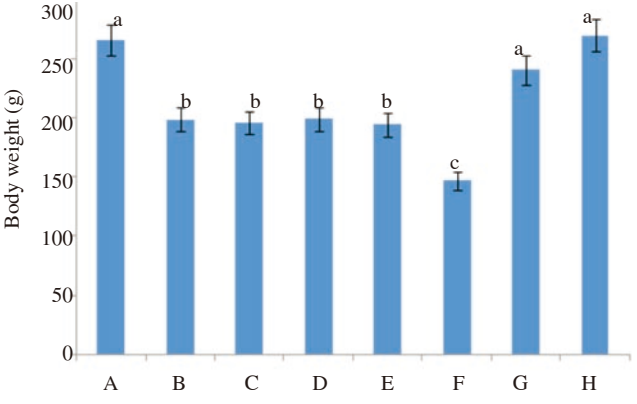

Figure 2. Effect of plants aqueous extracts on body weight of STZ induced diabetic rats after 2 weeks treatment.

Values are expressed as mean ± SEM (n = 6); A: Normal control; B: Diabetic (STZ); C: *M. inremis*; D: *N. latifolia*; E: *R. nilotica*; F: *S. hermonthica*; G: *T. bakis*; H: Glibenclamide. Different letters indicate differences among the groups ($P < 0.05$).

3.4. Effect of plants aqueous extracts on fasting blood glucose

Results of the effect of different plants aqueous extracts at 400 mg/kg/day dose on fasting blood glucose of STZ induced diabetic rats after 2 weeks of treatment are presented in Figure 3. The blood glucose concentration of diabetic rats increased significantly ($P < 0.05$) (by 500%) compared to the normal control rats. However, treatment of diabetic rats with aqueous extracts of *R. nilotica*, *M. inremis*, *N. latifolia* and *T. bakis* significantly reduced ($P < 0.05$) the blood glucose concentration (70%, 42%, 26% and 16% reduction, respectively). *S. hermonthica* aqueous extract exerted negative effect where the blood glucose increased significantly ($P < 0.05$) (12% rise) compared to the diabetic control rats.

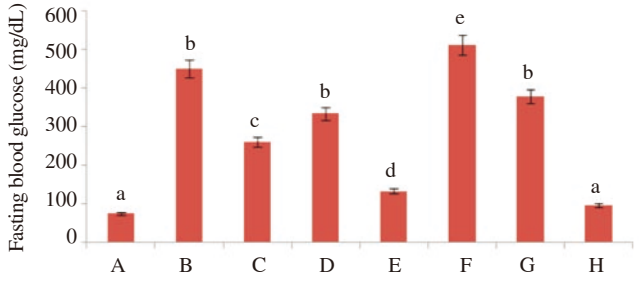

Figure 3. Effect of plants aqueous extracts on fasting blood glucose in STZ induced diabetic rats after 2 weeks treatment.

Values are expressed as mean ± SEM (n = 6); A: Normal control; B: Diabetic (STZ); C: *M. inremis*; D: *N. latifolia*; E: *R. nilotica*; F: *S. hermonthica*; G: *T. bakis*; H: Glibenclamide. Different letters indicate differences among the groups ($P < 0.05$).

3.5. Effect of plants aqueous extracts in serum lipid profiles

Results of the effect of different plants aqueous extracts at 400 mg/kg/day dose on serum lipid profiles of STZ induced diabetic rats after 2 weeks of treatment are presented in Table 2.

Table 2

Effect of plants aqueous extracts on total lipids profile in STZ induced diabetic rats after 2 weeks treatment.

Group	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	28.41 ± 1.90^{a}	22.05 ± 3.00^{a}	11.04 ± 0.10^{a}	12.96 ± 6.70^{a}
Diabetic (STZ)	63.65 ± 3.50^{b}	43.81 ± 1.40^{b}	9.13 ± 0.20^{b}	45.76 ± 9.90^{b}
M. inremis + STZ	35.68 ± 9.70^{c}	27.18 ± 3.80^{a}	12.47 ± 0.90^{ac}	17.77 ± 6.70^{ac}
N. latifolia + STZ	42.04 ± 8.70^{b}	21.04 ± 1.90^{a}	11.82 ± 0.10^{b}	26.01 ± 7.30^{c}
R. nilotica + STZ	43.05 ± 7.90^{b}	17.91 ± 3.60^{a}	15.44 ± 0.20^{c}	24.03 ± 6.40^{c}
S. hermonthica + STZ	70.50 ± 7.10^{b}	19.42 ± 7.90^{a}	12.17 ± 0.20^{b}	53.84 ± 5.70^{b}
T. bakis + STZ	37.03 ± 4.70^{c}	29.93 ± 2.00^{a}	14.58 ± 0.10^{c}	16.46 ± 5.90^{ac}
Glibenclamide + STZ	30.46 ± 1.90^{a}	26.11 ± 1.40^{a}	12.00 ± 0.10^{a}	15.66 ± 8.70^{a}

Values are expressed as mean ± SEM (n = 6); Values with different superscript letters indicate differences among the groups ($P < 0.05$).

3.5.1. Changes on total cholesterol levels

Diabetic rats showed significantly high level ($P < 0.05$) of total cholesterol (124% compared to the normal control rats). A significant decrease ($P < 0.05$) (44% and 42%) was observed in total cholesterol of diabetic rats given *M. inremis* and *T. bakis* respectively, whereas an insignificant decrease was observed for diabetic rats given *N. latifolia* and *R. nilotica* aqueous extracts compared to the diabetic control rats. Diabetic rats given *S. hermonthica* aqueous extracts showed a slight but insignificance increase in total cholesterol level.

3.5.2. Changes on total triglycerides levels

Diabetic rats showed significantly high level ($P < 0.05$) of total trigycerides (99% compared to the normal control rats). Administration of aqueous extracts from all plants under study for two weeks significantly ($P < 0.05$) decreased total triglycerides in all diabetic rats compared to their corresponding control. Percentage reduction in total triglycerides after treatments ranged from 59% in case of *R. nilotica* to 56% in *S. hermonthica*, 52% in *N. latifolia*, 38% in *M. inremis* and 32% in *T. bakis*. Thus, treatment of diabetic rats with different plants extracts enabled them to reduce the total triglycerides to levels comparable to that of normal controls.

3.5.3. Changes on HDL-C level

Diabetic rats showed insignificantly low level of HDL-C. Treatment of diabetic rats with different extracts induced a significant increase ($P < 0.05$) in HDL-C compared to the diabetic controls (69% in *R. nilotica*, 60% in *T. bakis*, 37% in *M. inremis*). However, an insignificant increase was observed in case of diabetic rats treated with aqueous extracts from *S. hermonthica* and *N. latifolia*. Interestingly, values of HDL-C in diabetic rats treated with *R. nilotica* and *T. bakis* were significantly ($P < 0.05$) higher than those of normal control rats.

3.5.4. Changes on LDL-C level

Diabetic rats showed highly significant ($P < 0.05$) HDL-C level (253% compared to the normal control rats). Treatment of these rats with *T. bakis*, *M. inremis*, *R. nilotica* and *N. latifolia* aqueous extracts resulted in significant reduction in the level of HDL-C ($P < 0.05$), corresponding to percentage reduction of 64%, 61%, 47% and 43% respectively. However, in diabetic rats treated with *S. hermonthica* aqueous extract there was no significant change in the level of HDL-C. Yet, treatment of diabetic rats with *T. bakis* and *M. inremis* aqueous extracts resulted in lowering HDL-C to a level closer to that of normal control rats.

3.6. Effect of plants aqueous extracts on diabetic liver function markers

Liver function markers (AST, ALT and LDH) in diabetic rats treated for 2 weeks with plants aqueous extracts at 400 mg/kg/day are presented in Table 3.

Table 3

The effect of plants aqueous extracts on liver function markers in STZ induced diabetic rats after 2 weeks of treatment.

Group	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
Normal control	14.55 ± 0.63^{a}	18.91 ± 0.56^{a}	12.54 ± 0.40^{a}
Diabetic (STZ)	16.65 ± 0.15^{b}	33.60 ± 7.75^{b}	15.03 ± 1.25^{b}
M. inremis + STZ	19.23 ± 3.74^{c}	19.95 ± 1.42^{a}	12.28 ± 0.60^{a}
N. latifolia + STZ	11.54 ± 0.25^{a}	23.68 ± 0.66^{a}	17.88 ± 0.35^{c}
R. nilotica + STZ	12.70 ± 1.38^{a}	27.10 ± 3.35^{b}	14.41 ± 1.62^{b}
S. hermonthica + STZ	15.67 ± 0.16^{ab}	21.31 ± 0.73^{a}	14.45 ± 0.65^{b}
T. bakis + STZ	13.36 ± 1.35^{a}	27.42 ± 2.45^{b}	14.06 ± 0.70^{b}

Values are expressed as mean ± SEM ($n = 6$); Values with different superscript letters indicate differences among the groups ($P < 0.05$).

3.6.1. Changes in activity of plasma AST

In diabetic control rats the activity of plasma AST was significantly ($P < 0.05$) increased (14% relative to normal levels). After treatment of diabetic rats with *N. latifolia*, *R. nilotica* and *T. bakis* aqueous extracts, the level of AST was significantly ($P < 0.05$) decreased by 31%, 24% and 20% respectively to reach values closer to normal level, though treatment of diabetic rats with *M. inremis* aqueous extracts increased it significantly ($P < 0.05$) by 15% as compared to the diabetic control rats. However, treatment with *S. hermonthica* aqueous extracts did not significantly affect the AST level.

3.6.2. Changes in activity of plasma ALT

In diabetic control rats the activities of plasma ALT was significantly ($P < 0.05$) increased by 78% relative to normal level. The level of ALT was back closer to normal value after treatment with *M. inremis* aqueous extracts, whereas treatment of diabetic rats with *S. hermonthica* and *N. latifolia* aqueous extracts significantly reduced ($P < 0.05$) the level of ALT by 37% and 30% respectively compared to the diabetic control rats. An insignificant decrease was observed for the diabetic rats treated with *R. nilotica* and *T. bakis* aqueous extracts.

3.6.3. Changes in activity of LDH

A significant increase ($P < 0.05$) (20% relative to normal level) in LDH was observed in the diabetic control rats. As observed for ALT activity, the level of LDH was back closer to normal value after treatment with *M. inremis* aqueous extract. However, *N. latifolia* aqueous extract increased significantly ($P < 0.05$) the level of LDH by 19% as compared to the diabetic control rats. *R. nilotica*, *S. hermonthica* and *T. bakis* aqueous extracts did not exert a significant effect.

3.7. Effect of plants aqueous extracts on diabetic kidney function markers

Results of the effect of plant aqueous extracts at 400 mg/kg/day on kidney function markers (creatinine, urea and blood urea nitrogen) in STZ induced diabetic rats after 2 weeks are presented in Table 4.

Table 4

The effect of plants aqueous extracts on kidney function markers in STZ induced diabetic rats after 2 weeks of treatment.

Group	Creatinine (mg/dL)	Urea (mg/dL)	Blood urea nitrogen (mg/dL)
Normal control	0.49 ± 0.08^{a}	42.92 ± 2.06^{a}	0.27 ± 0.01^{a}
Diabetic (STZ)	0.99 ± 0.12^{b}	60.22 ± 2.54^{b}	0.38 ± 0.01^{b}
M. inremis + STZ	0.73 ± 0.07^{b}	27.27 ± 0.49^{c}	0.19 ± 0.01^{c}
N. latifolia + STZ	0.77 ± 0.08^{b}	54.59 ± 0.98^{a}	0.31 ± 0.02^{a}
R. nilotica + STZ	0.58 ± 0.07^{a}	57.67 ± 3.06^{b}	0.37 ± 0.01^{b}
S. hermonthica + STZ	0.53 ± 0.07^{a}	80.78 ± 3.46^{d}	0.56 ± 0.00^{d}
T. bakis + STZ	0.60 ± 0.03^{a}	51.96 ± 1.12^{a}	0.29 ± 0.02^{a}

Values are expressed as mean ± SEM ($n = 6$); Values with different superscript letters indicate differences among the groups ($P < 0.05$).

3.7.1. Changes on level of creatinine

In diabetic control rats, a significant increase in the level of creatinine was observed ($P < 0.05$) (102% compared to level of

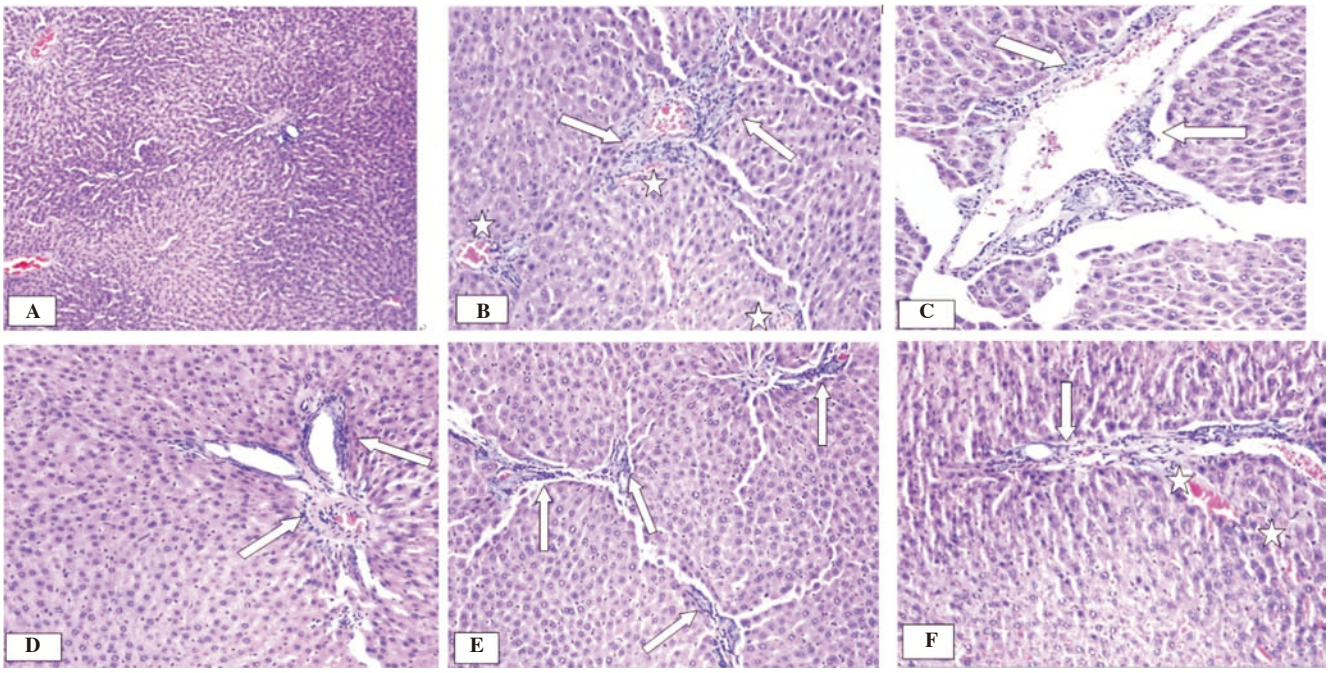

Figure 4. Light micrograph of liver sections representative for rats given aqueous plant extracts at 400 mg/kg/day oral dose (H & E).
A: Normal control rats; visible is normal hepatic parenchyma with more or less normal portal triads and sinusoids; B: Diabetic control rats showing hemorrhagic foci replacing necrotic hepatocytes (asterisks). Arrows indicate infiltration of leucocytes in portal triads. Visible also are hypetrophied Kupffer cells along hepatic sinusoids, and hepatocytes at various stages of degeneration; C: Diabetic rats treated with *M. inremis* showing leucocytic infiltration proliferating bile canaliculi (arrows); also seen are hypertrophied Kupffer cells along sinusoids; D: Diabetic rats treated with *N. latifolia*; the image shows leucocytic infiltration in portal triad (arrows) and thickening of the portal artery wall. Hypertrophied Kupffer cells are also seen along the sinusoids; E: Diabetic rats treated with *R. nilotica*; the image shows leucocytic infiltration in portal triads (arrows); F: Diabetic rats treated with *T. bakis*; the image shows leucocyic infiltration in portal triad (arrow) and hemorrhage (asterisks). Seen also are hypertrophied Kupffer cells along sinusoids.

normal control rats). However, treatment of diabetic rats with aqueous extracts of *S. hermonthica*, *R. nilotica* and *T. bakis* significantly ($P < 0.05$) reduced their creatinine levels by 46%, 41% and 39% respectively compared to the diabetic group, bringing the level closer to normal value. An insignificant reduction was observed for diabetic rats treated with aqueous extracts of *M. inremis* and *N. latifolia*.

3.7.2. Changes on level of urea and blood urea nitrogen

In diabetic control rats, the levels of urea and blood urea nitrogen were significantly increased ($P < 0.05$) by 40% and 41% respectively compared to normal control rats. When diabetic rats were treated with aqueous extracts of *M. inremis*, the urea level was reduced by 55% compared to diabetic control rats and 36% below the normal level. A similar result was observed for the blood urea nitrogen where a significant ($P < 0.05$) reduction by 50% compared to diabetic controls rats and 30% below the normal controls ones was occurred. Treatment of diabetic rats with *T. bakis* and *N. latifolia* aqueous extracts was able to bring the blood urea nitrogen closer to normal level. *R. nilotica* aqueous extract did not have a significant effect on both urea and blood urea nitrogen levels; however, *S. hermonthica* aqueous extract increased them significantly ($P < 0.05$) by 34% and 47% respectively compared to the diabetic control rats.

3.8. Histopathology

Images of sections of different rat groups are shown in Figure 4. Normal control rats (Figure 4A) showed more or less normal hepatic parenchymal organization, with normal portal tracks and sinusoids. Unlike other rats, the sinusoids of normal controls did not show hypertrophied Kupffer cells. Sections of the diabetic control rats (Figure 4B) seemed to reflect friable parynchemal architecture with intense infiltration of polymorph leucocytes in portal triads zones. Hypertrophied Kupffer cells ran along the sinusoids, and hepatocytes at different stages of necrosis could be detected; in addition, there was hemorrhage into foci of lysed hepatocytes. Histopathological features in sections of rats treated with different extracts (except *S. hermonthica*) (Figures 4C–4F) were not markedly different from each other. The main features in liver sections from these groups were infiltration of portal tracks with polymorph leucocytes and hypertrophied Kupffer cells. Hemorrhage was less frequently detected compared to diabetic control, indicating a degree of healing process. However, in sections from rats treated with *S. hermonthica* extract (section not shown), there were more intense infiltration with polymorph leucocytes and more hemorrhagic foci compared to others; moreover, more hepatocytes with pyknotic nuclei and more necrosis were observed.

4. Discussion

The rising cost of medical care in Sudan is increasingly driving patients to herbal medicine and in case of diabetes, emerging experience with certain plants drew attention to some communities rarely known for such experience in the country. In the Nuba Mountains, Western Sudan, it emerged that plant species including *M. inremis*, *N. latifolia*, *R. nilotica*, *T. bakis* and *S. hermonthica* have

been used for the management of diabetes. However, hypoglycemic herbs are used by many communities in other countries to treat hyperglycemia and hyperlipidemia precipitated by disturbed glucose and lipid metabolism in diabetes mellitus. The current results confirm the potential of a number of herbs in the control of glucose level as observed for rats given aqueous extracts of *T. bakis*, *N. latifolia* and *R. nilotica* at 400 mg/kg. The extracts of the three plants significantly improved glucose tolerance in diabetic rats suggesting that they enhanced insulin secretion as reported in similar results for the hypoglycemic effect of *G. alata*[5]. Moreover, the two-week treatment of diabetic rats with *R. nilotica*, *M. inremis*, *N. latifolia* and *T. bakis* aqueous extracts resulted in significant ($P < 0.05$) antihyperglycemic effect. Interestingly, *M. inremis* did not exert an effect upon testing oral glucose tolerance of diabetic rats but a significant reduction in glucose level was observed after two-week treatment. Relevant to our findings is the demonstration by Gidado *et al*.[15] that the aqueous and ethanolic extracts of *N. latifolia* leaves had a significant antihperglycemic effect on fasting blood glucose levels in streptozotocin-induced diabetic rats in a dose-dependent manner.

Diabetic rats manifested significant ($P < 0.05$) reduction in body weight concomitant with hyperglycemia. Protein-energy wasting accompanying hyperglycemia has been attributed to altered glucose metabolism as indicated by Rajasekar *et al*.[16] in their experiments with diabetic rats where failure of body cells to utilize glucose as energy source seems to have ushered proteins as an alternative energy source leading to metabolic imbalance in protein metabolism with consequent loss of body weight or continuous excretion of glucose from the body[16-18]. Oral administration of *T. bakis* aqueous extract for 14 consecutive days to diabetic rats improved their body weight, which could be due to better control of the hyperglycemic state in these rats. However, aqueous extracts of *N. latifolia*, *R. nilotica* and *M. inremis* did not improve the body weight of diabetic rats.

Diabetic rats exhibited abnormalities in lipid metabolism as evidenced from the elevated levels of cholesterol, triglycerides and high levels of low density lipoprotein cholesterol and low levels of HDL-C[19,20]. Two-week treatment of diabetic rats with *T. bakis*, *M. inremis*, *R. nilotica* and *N. latifolia* aqueous extracts significantly ($P < 0.05$) ameliorated the serum lipid levels in diabetic rats and thus could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics[21]. A similar hypolipidemic effect was reported in diabetic rats given ethanol extract of *N. latifolia* leaves where significant reduction of TG, HDL-C, VHDL-C and total cholesterol were observed as well as significant elevation of HDL-C[22].

The increment in the activities of plasma AST, ALT and LDH indicated that diabetes may induce hepatic parenchymal injury and hepatic dysfunction[23,24] and leakage of these enzymes from the liver cytosol into the blood stream[24,25]. On the other hand, treatment of the diabetic rats with either *N. latifolia*, *R. nilotica* or *T. bakis* brought back the activity of AST enzyme to normal level whereas *N. latifolia* and *M. inremis* caused reduction in the activity of ALT closer to normal level. The LDH level of diabetic rats was only improved by *M. inremis* aqueous extract suggesting proper regulation of NAD^+/NADH ratio[26].

The elevation in the levels of plasma renal function parameters in diabetic rats is associated to renal dysfunction and metabolic disturbance[27,28]. In this study the effect of plants aqueous extracts on diabetic kidney function markers was variable; *M. inremis* induced significant ($P < 0.05$) reduction on levels of urea and blood urea nitrogen whereas *T. bakis* and *R. nilotica* reduced significantly ($P < 0.05$) the creatinine level. *N. latifolia* aqueous extract exerted only significant ($P < 0.05$) effect on blood urea nitrogen level.

Daily oral administration of *S. hermonthica* aqueous extract to diabetic rats for 14 days appeared to increase the blood glucose level and decrease their body weight thus indicating that it has no antihyperglycemic effect. Moreover, although this aqueous extract reduced the triglyceride level and ALT activity of diabetic rats, the levels of cholesterol, low and HDL-C, urea and blood urea nitrogen of these rats were not improved.

Our findings demonstrated that the aqueous extracts of *R. nilotica*, *M. inremis*, *N. latifolia* and *T. bakis* had satisfactory efficacy in glycemic and lipidemic homeostasis in diabetic rats, and that they were apparently targeting more than one metabolic pathway. Their effects on different biochemical parameters were in a variable way, which might reflect the complexity of the phytochemicals and bioactivities in the plants regarding modulation of metabolic disturbances.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The objective of this research was to evaluate antidiabetic activity of aqueous extracts from some plants (*M. inremis*, *N. latifolia*, *R. nilotica*, *T. bakis* and *S. hermonthica*) used in Western Sudan on streptozotocin-induced diabetes rats.

Research frontiers

The research in this paper demonstrated that the extracts from *T. bakis*, *N. latifolia*, *R. nilotica* and *M. inremis* have therapeutic value in diabetes and related complications, and thus supporting the use of these plants in Western Sudan traditional medicine.

Related reports

The researches related to this paper also reported during the last few years as the use of *G. alata* roots, *Acacia nilotica* pods, *Balanites aegyptiaca* fruits, *Guiera senegalensis* leaves, *Hyphaene thebaica*

epicarp and *Trigonella foenum-graecum* seeds to hypoglycemia in Sudanese folk medicine.

Innovations and breakthroughs

In the present work, authors have demonstrated the potential of some indigenous medicinal plants in the treatment of diabetes.

Applications

The results of this work can be applied to support in the treatment of diabetes.

Peer review

This research obtained interesting and valuable results. The authors have demostrated the effect of aqueous extracts from some indigenous plants in supporting the treatment of diabetes.

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