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RESEARCH ARTICLE

Antihyperglycemic and antidyslipidemic effects of the aqueous extract of *Withania adpressa* in rats

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Abstract

The current study was conducted to evaluate the antihyperglycemic and antidyslipidemic effects of *Withania adpressa* aqueous extract (WAAE) in 2 experimental animal models. In streptozotocin-induced diabetic rats, daily oral administration of WAAE at a dose of 20 mg/kg for 15 days significantly reduced fasting blood glucose, improved plasma lipid profile, increased hepatic, muscle glycogen content and enhanced liver histological architecture. In another model of tyloxapol-induced hyperlipidemia, a single oral dose of WAAE at 400 mg/kg significantly reduced plasma concentrations of total cholesterol, triglycerides and LDL-c without affecting HDL-c levels. These results highlight the promising therapeutic potential of *Withania adpressa* in managing metabolic disorders such as diabetes and dyslipidemia.

Keywords: diabetes; dyslipidemia; streptozotocin; tyloxapol; *withania adpressa*

Introduction

Diabetes mellitus is a prevalent metabolic disorder presenting an increasingly concerning global health problem (1). Over time, it can lead to serious complications in the body, such as nephropathy, neuropathy, retinopathy, cardiovascular, cerebrovascular and peripheral vascular diseases, as well as dyslipidemia (2, 3).

Recently, a growing trend has been observed towards using medicinal plants to treat various health problems, as they are derived from nature and have fewer side effects (4). Indeed, since antiquity, plants have been the primary source of medicines and many drugs have been derived from plants (5). It is estimated that at least 1200 plant species worldwide are utilized in traditional medicine to treat diabetes (6).

The *Withania* genus, belonging to the Solanaceae family, consists of 8 species predominantly distributed in North Africa and from the Mediterranean region to the South-west of Asia (7, 8). Among the diversity of the *Withania* genus, Morocco is represented by 3 distinct species: *Withania somnifera* (L.) Dunal, *Withania frutescens* Pauquy and *Withania adpressa* Cos. The latter, locally known as "aglim", is an endemic plant to the Moroccan Sahara and it is used in traditional medicine to treat food poisoning (9).

Withania species are known for their rich content of withanolides, which have shown multiple biological properties. Previous studies have revealed that *Wi. adpressa* leaves are high in wadpressin, nicotiflorin, withanolide F, coagulin L and withaferin A (7). Among these bioactive compounds, Withaferin A has been the subject of a preceding study demonstrating its potent antidiabetic and antihyperlipidemic effects (10, 11).

To date, minimal scientific data are available on the phytochemical composition and therapeutic potential of *W. adpressa*. Therefore, the current study aimed to assess, for the first time, its antihyperglycemic and antihyperlipidemic effects. Additionally, the antioxidant power, acute toxicity study of this plant and its effect on the histology of the liver were also assessed.

Materials and Methods

Plant material collection and preparation of the extract

Fresh leaves of *W. adpressa* were collected from the Tinejdad region (GPS: 31.375436, -4.640949) in May 2022, washed and air-dried at room temperature. A voucher specimen (WA04) was deposited at the herbarium of the Faculty of Sciences and Techniques of Errachidia. One g of powdered leaves of *W. adpressa* was mixed with 100 mL of distilled water, boiled for 10 min and then allowed to cool for 15 min. Subsequently, the aqueous extract was filtered using a Millipore filter to eliminate fine particles. Finally, the filtrate was lyophilized and stored at 2 - 8 °C until use (12). The doses of WAAE (20 mg/kg and 400 mg/kg) were selected based on preliminary screening studies. In the STZ-induced diabetes model, 20 mg/kg was identified as the minimum effective dose inducing a significant antihyperglycemic effect. In the tyloxapol-induced dyslipidemia model, 400

mg/kg was selected as the optimal dose producing the most significant antihyperlipidemic activity.

Quantification of total content of polyphenols, flavonoids and tannins

The phenolic compound concentration of *W. adpressa* aqueous extract (WAAE) was assessed according to previously established procedures (13). At the same time, total flavonoid content was measured as previously described in a report (14). Additionally, tannin content was quantified as described by an earlier report (15).

DPPH free radical scavenging assay

The anti-DPPH radical activity was conducted following previously reported methods (16).

Animals

Wistar adult albino rats weighing 120 - 200 g were maintained under appropriate environmental conditions throughout the study. All animals were fed a standard laboratory diet ad libitum and had unlimited access to drinking water. All experiments were carried out strictly following the local ethical guidelines for the use of laboratory animals established by the Pharmacological Research Committee of FSTE, Moulay Ismail University (FSTE/2015).

Acute oral toxicity assay

Acute oral toxicity of WAAE was evaluated in rats following the OECD guidelines 423, which requires 2 steps, in which only 3 animals (females) were used in each step (17). The initial dose of 2000 mg/kg was used as the limit dose according to the OECD Acute Toxic Class Method All animals were fasted overnight, weighted before administering the extract and then randomly divided into 2 groups, each consisting of 5 animals: The 1st group (vehicle control) received physiological water, whereas the 2nd group (treated group) was orally administered a single dose of WAAE (2000 mg/kg). All rats were closely monitored individually for the first 4 hr following extract administration and then every day 2 weeks, looking for any clinical signs of toxicity or mortality (18).

Evaluation of the antihyperglycemic potential of WAAE

Induction of diabetes

After an overnight fast, experimental diabetes was induced in male Wistar rats by intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO, USA) at a dose of 65 mg/kg, dissolved in cold 0.1 M citrate buffer (pH 4.5). The control group received distilled water as a vehicle. Seventy-two hours after STZ injection, rats with blood glucose levels above 200 mg/dL were considered diabetic and included in the study (19).

Assessment of glycaemia

Normal and diabetic conditions were randomly divided into 3 groups, each consisting of 5 rats (n = 5). The first group, serving as control, received distilled water, a second treated group received *W adpressa* aqueous extract (20 mg/kg) and the third group received glibenclamide (reference drug) at a dose of 5 mg/kg. For single oral administration (acute study), distilled water (control), glibenclamide, or WAAE was administered and blood glucose levels were monitored over a 6 hr period. In the subacute treatment, animals were administered WAAE once daily for 15 consecutive days and glycemia was monitored throughout this period. For the oral glucose tolerance test (OGTT), both normal and diabetic rats were fasted for 12 hr before oral administration of glucose at a dose of 2 g/kg. Blood glucose levels were measured from the tail vein at 30, 60, 90 and 120 min after glucose administration. All blood glucose measurements were performed under fasting conditions, using a glucometer (Contour™ TS, Bayer Diabetes Care), operating based on the glucose oxidase enzymatic method. In parallel, the body weight of rats was measured at the baseline and monitored throughout the experimental period (19).

Plasma lipid profile

To evaluate how WAAE impacts lipid profile changes in both normal and diabetic rats, blood samples were collected by the orbital sinus puncture method under light ether anesthesia before extract administration (day 0) and at the end of the treatment period (day 15). Plasma samples were obtained by centrifuging whole blood samples for 10 min at 5000 rpm and were then used to estimate the lipid profile parameters, including total cholesterol (TC), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c) and triglycerides (TGs). Lipid analysis was performed on an automated biochemistry analyzer (Erba XL-600, Germany).

Estimation of liver and muscle glycogen content

Upon completion of the experiment, the liver, soleus (SOL) and extensor digitorum longus (EDL) muscles of the rats were carefully removed to assess their glycogen content. Glycogen quantification was performed using the direct method described in a study (20). The formula reported in a previous study was used to calculate the glycogen content (21).

Histopathological examinations

The animals were sacrificed and their livers were quickly removed, washed with phosphate buffer solution and immediately fixed in 10 % buffered formalin. The fixed liver tissues were dehydrated and embedded in paraffin wax before being cut into approximately 5 µm-thick sections using a rotary microtome (MICROM, HM 310, Germany). Finally, the sectioned tissues were stained with hematoxylin and eosin. Changes in the morphology of hepatocytes were observed under the Motic BA 210 microscope.

Effect of WAAE on Tyloxapol-induced hyperlipidemic rats

To assess the antihyperlipidemic activity of WAAE, the following experimental procedure was carried out as described in previous studies, with some minor modifications (22). Adult female rats were used in this experiment. All animals were maintained under standard laboratory conditions with unrestricted access to food and drinking water. Tyloxapol (Triton WR-1339) was dissolved in standard saline solution (pH 7.4) under constant stirring. After 12 hr of fasting, the animals were randomly assigned to four groups. Group 1, control (n = 5): Rats were gavaged

with distilled water (10 mL/kg) 30 min before an intraperitoneal injection of normal saline (pH 7.4). Group 2, hyperlipidemic control (n = 5): rats were given distilled water (10 mL/kg) 30 min before intraperitoneal injection with Tyloxapol (200 mg/kg). Group 3, WAAE treated group (n = 5): rats were administered with WAAE (400 mg/kg) 30 min before Tyloxapol (200 mg/kg, ip). Group 4, standard group (n = 5): rats received simvastatin (10 mg/kg, po) 30 min before Tyloxapol (200 mg/kg, ip). After 24 hr of treatment, all animals were anesthetized and blood samples were collected directly by retro-orbital sinus puncture. Blood samples were centrifuged at 5000 rpm for 10 min and the plasma collected was used to determine lipid profile (Using Erba XL-600 automatic biochemistry analyzer).

Statistical data analysis

Quantitative data are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism version 7. One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test was employed to compare means of hyperlipidemic group with treated and normal control groups. Two-way ANOVA with Bonferroni's correction was used to assess the effects of treatment and time on glycemia, body weight and lipid parameters in the context of the antidiabetic study. For the acute toxicity test, comparisons between the 2 groups were carried out using the acute toxicity test and comparisons between the 2 groups were carried out using an unpaired Student's t-test. A p-value <0.05 was considered statistically significant.

Results

Quantification of total phenolic, flavonoids and tannins

Quantification of total phenolic compounds in the extract revealed an estimated value of 207.46 ± 8.73 mg of gallic acid equivalent per gram of extract (207.46 ± 8.73 mg GAE/1 g WAAE). A gallic acid standard was used to perform the calibration curve. The total content of flavonoids was estimated to be 804.48 ± 7.98 mg of rutin equivalent per gram of extract; the calibration curve was plotted using rutin standard. Besides, tannin contents were estimated to be 30.62 ± 3.16 mg of catechin equivalent per gram of the extract, using catechin as a standard reference to perform the calibration curve.

Acute oral toxicity

Oral administration of a single dose of WAAE (2 g/kg) was safe and did not cause deaths during the treatment period (14 days). Moreover, all animals looked healthy and did not reveal any signs of toxicity. Consequently, the estimated LD_{50} is more than 2000 mg/kg.

DPPH radical scavenging effect

The antioxidant activity of WAAE against DPPH free radicals showed a concentration- dependent response. The radical scavenging potential of WAAE was 83.15 ± 1.28, 80.52 ± 0.96, 54.38 ± 0.89, 31.40 ± 1.05 and 16.14 ± 0.78 at concentrations of 250, 125, 62.5, 31.25 and 15.62 µg/mL, respectively, with an IC_{50} of 85.27 µg/mL. In turn, the IC_{50} of the synthetic antioxidant BHT was 13.63 µg/mL.

Effect of WAAE on diabetes condition induced by streptozotocin

Effect of WAAE on Body weight

Fig. 1 illustrates the impact of WAAE on body weight variation. Over a 2 week treatment period, no noticeable changes were detected in the body weight of normoglycemic and STZ-induced diabetic rats compared with the control group. The same result was observed in the glibenclamide-treated group after 15 days of treatment.

Single oral dose administration

Fig. 2 shows the evolution of blood glucose levels in normoglycemic and diabetic rats after a single oral dose administration of WAAE over a 6 hr period (acute test). Results showed that WAAE did not affect fasting blood glucose levels in both normal and diabetic rats after 6 hr of single oral administration. However, a significant drop in blood glucose levels was observed in normal rats treated with glibenclamide as early as the 1st hr ($p < 0.05$), with a more pronounced reduction at the 6th hr ($p < 0.0001$). Similarly, in diabetic rats, glibenclamide significantly decreased blood glucose levels starting from the 2nd hr and continued to decline until the 6th hr ($p < 0.0001$).

Repeated oral dose administration

Fig. 3 shows the changes in blood glucose levels in both normoglycemic and diabetic rats treated daily for 15 days with WAAE (20 mg/kg). Concerning normal rats, no significant reduction in blood glucose was observed. However, in diabetic rats, a substantial decrease in fasting glycemia was recorded from the 2nd day of treatment with WAAE (20 mg/kg) and this reduction was maintained until the end of the treatment ($p < 0.0001$). Furthermore, in normal rats treated with glibenclamide, a decrease in blood glucose levels was observed from the 4th day ($p < 0.001$), with a more pronounced decrease on the 7th and 15th days of treatment ($p < 0.0001$). Whereas in glibenclamide-treated diabetic rats, a marked drop in blood glucose level was evident from day 2 ($p < 0.001$), with a progressive decrease observed until the end of the treatment ($p < 0.0001$).

Effect of WAAE on oral glucose tolerance test

Fig. 4 shows the effect of WAAE on glucose tolerance in normal and streptozotocin-diabetic rats. In normal rats, oral treatment with WAAE (20 mg/kg) did not affect glycemia for 120 min. However, glibenclamide decreased blood glucose levels at 60 min ($p < 0.05$) and this decrease was more significant at 90 and 120 min ($p < 0.01$). Moreover, a substantial drop in blood glucose level at 120 min ($p < 0.001$) was observed in WAAE-treated diabetic rats. At the same time, those treated with glibenclamide showed no significant change in blood glucose.

Effect on lipid profile

Tables 1 and 2 showed that the daily oral administration of diabetic rats with WAAE (20 mg/kg) for 15 days significantly decreased plasma concentrations of total cholesterol

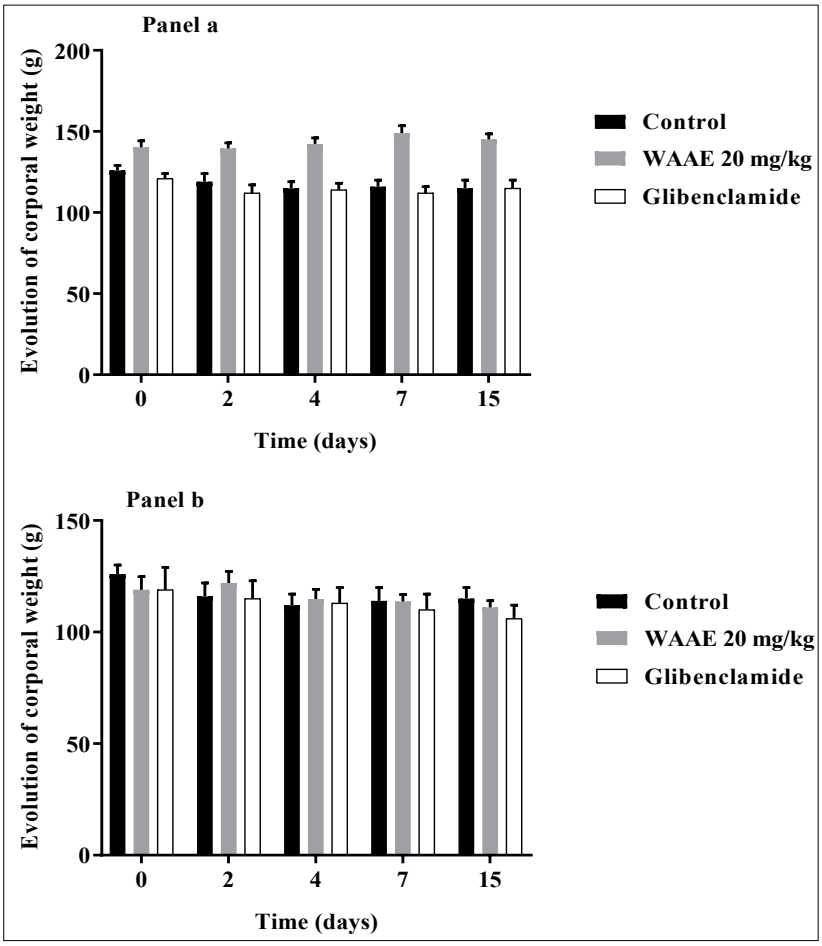

Fig. 1. Body weight variation in normal (Panel a) and diabetic (Panel b) rats following repeated oral administration of WAAE (20 mg/kg) for 15 days. All data were expressed as mean ± SEM, n = 5.

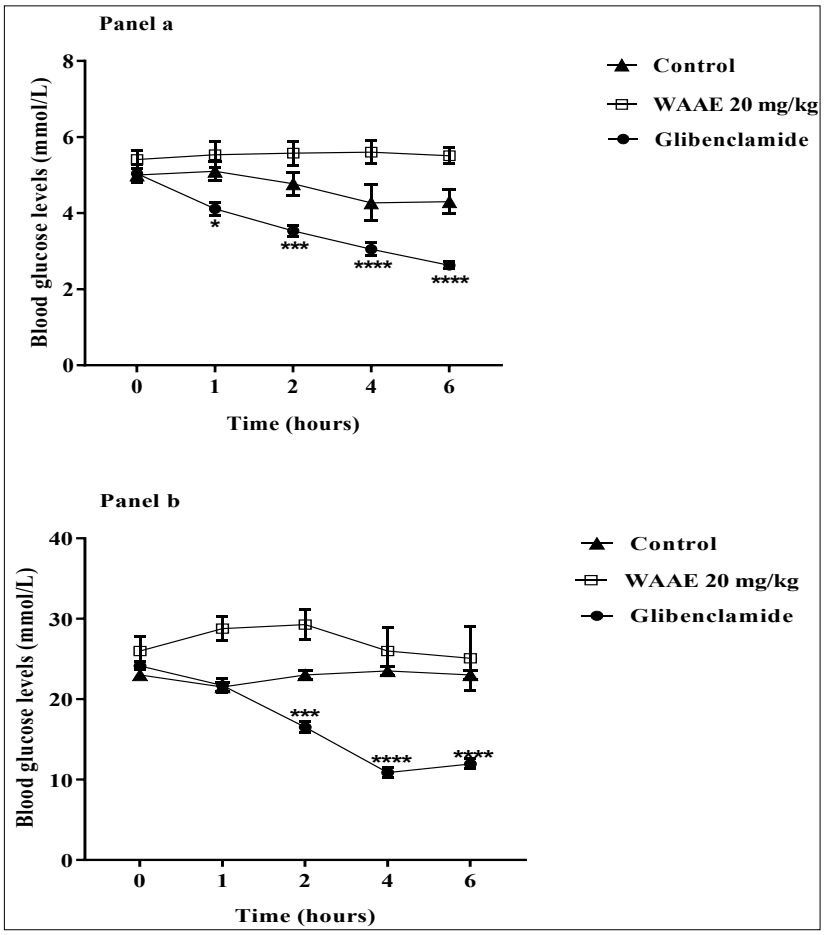

Fig. 2. Effect of a single oral administration of WAAE (20 mg/kg) on fasting blood glucose over 6 h in normal (Panel a) and diabetic (Panel b) rats. Values are expressed as mean ± SEM, n = 5. *p<0.05, ***p<0.001 and ****p<0.0001 vs baseline (t0).

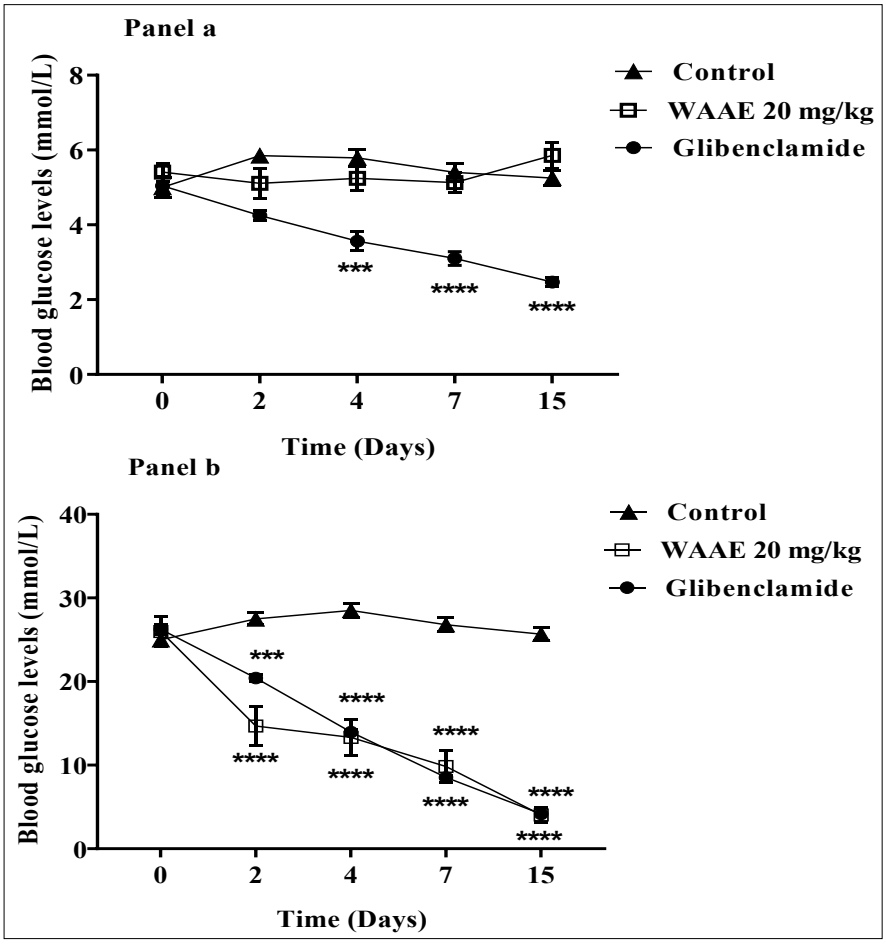

Fig. 3. Effect of repeated oral administration of WAAE (20 mg/kg) for 15 days on fasting blood glucose levels in normal (Panel a) and diabetic (Panel b) rats. Data are expressed as mean ± SEM, n = 5. ***p<0.001 and ****p<0.0001 vs baseline (Day 0).

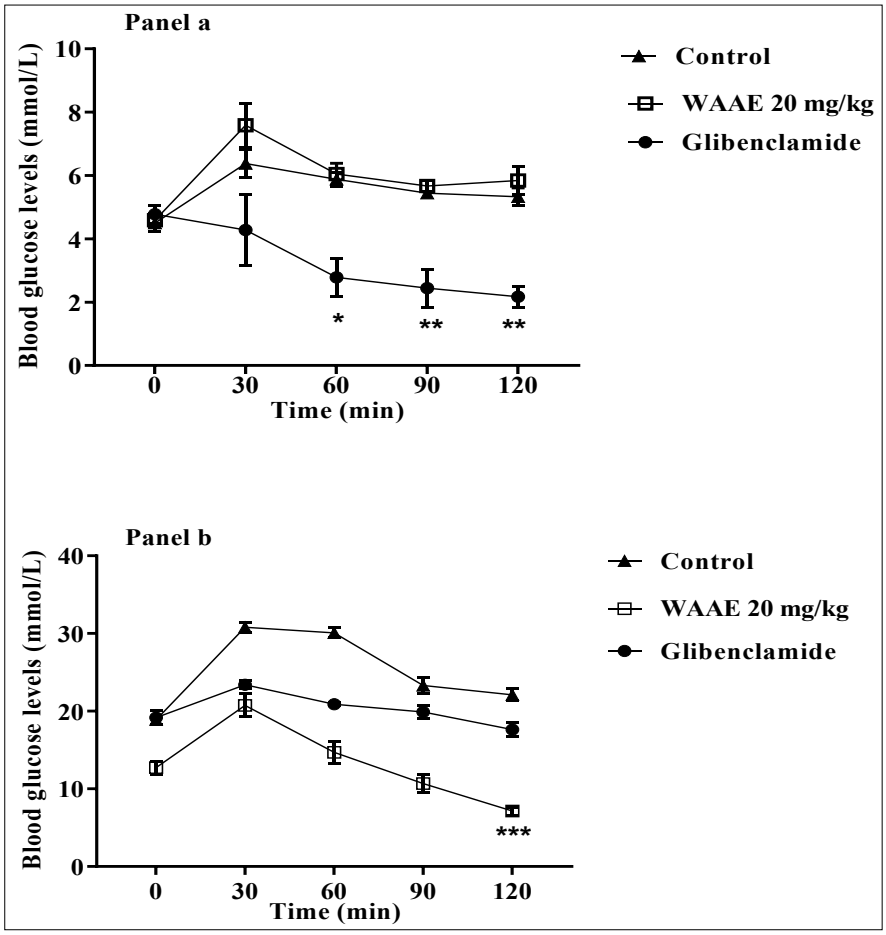

Fig. 4. Effect of WAAE on OGTT results in normal (Panel a) and diabetic (Panel b) rats. Values are expressed as mean ± SEM, n = 5. *p<0.05, **p<0.01 and ***p<0.001 vs baseline values.

Table 1. Effects of repeated oral administration of WAAE (20 mg/kg) on plasma levels of total cholesterol and triglycerides in normal and diabetic rats. Data are expressed as mean ± SEM, n = 5. **p<0.01 vs baseline value. d: day

Experimental groups		Total cholesterol (TC) mmol/L		Triglycerides (TGs) mmol/L	
		0d	15d	0d	15d
Normal rats	Control	2.740 ± 0.043	2.855 ± 0.051	2.357 ± 0.064	2.415 ± 0.057
	W. adpressa (20 mg/kg)	2.64 ± 0.145	2.28 ± 0.139	2.06 ± 0.089	1.89 ± 0.105
	Glibenclamide (5 mg/kg)	2.390 ± 0.075	2.061 ± 0.119	2.376 ± 0.048	1.998 ± 0.038**
Diabetic rats	Control	3.604 ± 0.120	2.969 ± 0.161	1.775 ± 0.200	1.403 ± 0.054
	W. adpressa (20 mg/kg)	2.88 ± 0.066	2.040 ± 0.031*	1.720 ± 0.056	1.050 ± 0.040**
	Glibenclamide (5 mg/kg)	3.440 ± 0.493	2.252 ± 0.092**	1.98 ± 0.134	1.26 ± 0.121**

Table 2. Effects of repeated oral administration of WAAE (20 mg/kg) on plasma levels of HDL-c and LDL-c in normal and diabetic rats. Data are expressed as mean ± SEM, n = 5. *p<0.05 vs baseline values. d: day

Experimental groups		HDL-c (mmol/L)		LDL-c (mmol/L)	
		0d	15d	0d	15d
Normal rats	Control	1.833 ± 0.031	1.898 ± 0.031	0.869 ± 0.054	0.823 ± 0.052
	W. adpressa (20mg/kg)	1.54 ± 0.11	1.73 ± 0.099	1,084 ± 0,134	0,956 ± 0,099
	Glibenclamide (5 mg/kg)	1.324 ± 0.283	1.891 ± 0.232	0.798 ± 0.268	0.782 ± 0.190
Diabetic rats	Control	1.952 ± 0.029	1.607 ± 0.043	0.920 ± 0.150	1.060 ± 0.190
	W. adpressa (20 mg/kg)	1.780 ± 0,086	1.890 ± 0,069	1.670 ± 0,045	1.400 ± 0,033
	Glibenclamide (5 mg/kg)	1.508 ± 0.134	1.906 ± 0.121*	1.020 ± 0.125	0.890 ± 0.135

(p<0.05) and triglycerides (p <0.01) without altering plasma levels of HDL-c and LDL-c. While glibenclamide administration at a dose of 5 mg/kg to diabetic rats led to a notable reduction in plasma levels of total cholesterol and triglycerides (p <0.01), accompanied by a modest elevation in HDL-concentration (p <0.05). In contrast, treatment with WAAE (20 mg/kg) had no impact on plasma levels of total cholesterol, HDL-c, LDL-c and triglycerides in the normal group. On the other hand, oral administration of glibenclamide to normal rats resulted in a significant drop in plasma triglyceride levels (p <0.01), without affecting other lipid profile parameters.

Effect of WAAE on liver and muscle glycogen

Fig. 5 illustrates data on changes in liver and muscle glycogen content in normal and diabetic rats treated with WAAE. As expected, a significant decrease in liver and muscle glycogen levels was detected in diabetic rats compared to the normal control group. The findings reveal that diabetic rats, supplemented with WAAE daily for 15 days, exhibited a significant increase in glycogen levels in the liver (p <0.01) and skeletal muscles (p <0.05) when compared to untreated diabetic rats.

Histopathological examinations

Fig. 6 illustrates the histopathological changes observed in the liver of diabetic rats daily treated over 15 days with WAAE (20 mg/kg) and glibenclamide (5 mg/kg). Analysis of liver sections from normal control rats revealed standard hepatic histological structure with a lobular hepatic structure, hepatic sinusoids and a central vein (Fig. 6a). In untreated diabetic rats, significant alterations in liver morphology were recorded, characterized by disorganized hepatic cell arrangement, noticeable hepatocellular lesions, dilatation of sinusoidal spaces and thickening of vein walls (Fig. 6b). However, diabetic rats treated with the extract (Fig. 6c) or with glibenclamide (Fig. 6d) showed an improvement in the histological structure of their liver, manifested by attenuation of hepatic lesions and better organization of hepatocytes.

Effect of WAAE on Triton WR-1339-induced dyslipidemia

The plasma concentrations of TC, TGs, HDL-c and LDL-c of WAAE (400 mg/kg)-treated groups are illustrated in Fig. 7 and 8. Compared with the normolipidemic control group, tyloxapol significantly increased levels of plasma TC (p <0.01), TGs (p <0.0001) and LDL-c (p <0.001). However, no significant difference was observed between the control and hyperlipidemic groups concerning HDL-c level. Compared with the hyperlipidemic control group, treating hyperlipidemic rats with WAAE significantly lowered plasma levels of total cholesterol and LDL-c (p <0.05) and triglycerides (p <0.01), without impacting plasma HDL-c level. Similarly, simvastatin effectively reduced plasma TC and TG levels in hyperlipidemic rats. These reductions were statistically significant, with p-values below 0.05 for TC and below 0.001 for TGs. Besides, treating rats with simvastatin induced a substantial reduction in LDL-c levels (p <0.01), with no notable effect on plasma HDL-c levels.

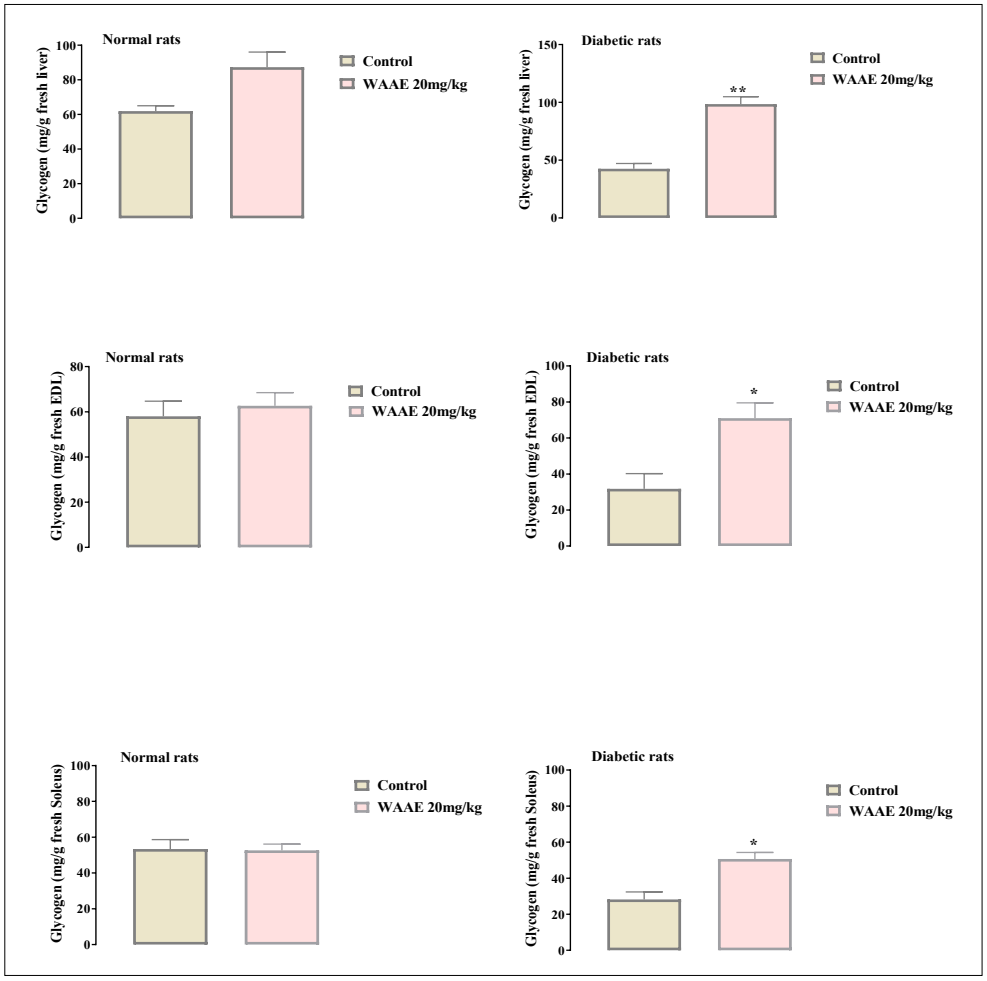

Fig. 5. Effect of repeated oral administration of WAAE (20 mg/kg) on hepatic and muscle glycogen levels. *p<0.05 and ** p<0.01 vs control group.

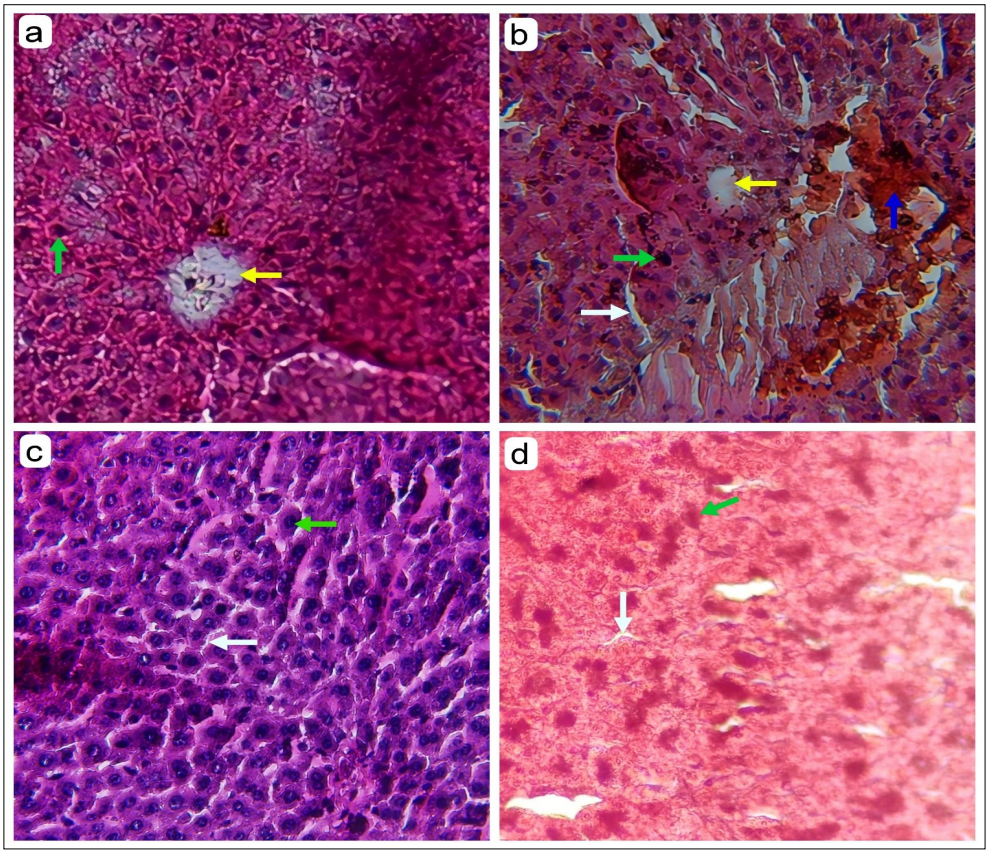

Fig. 6. Histological study of the liver in experimental rats after 15 days of repeated oral administration of WAAE (20 mg/kg). a) Normal control, b) Untreated diabetic rat, c) Diabetic rats treated with Glibenclamide, d) Diabetic rats treated with WAAE. Sections were stained with (H and E); magnification: 400x. Green arrow indicates hepatocytes, white arrow indicates sinusoids, yellow arrow indicates the central vein and blue arrow indicates STZ-induced lesions.

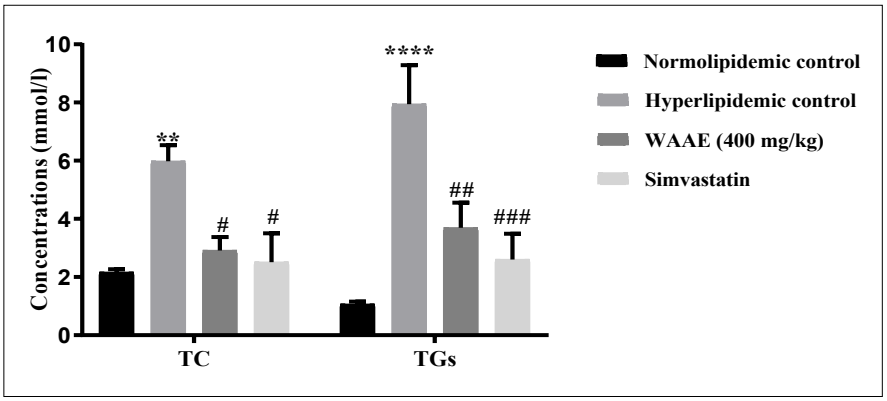

Fig. 7. Effect of WAAE (400 mg/kg) on total cholesterol and triglyceride levels in Triton WR-1339-induced hyperlipidemic rats. Values are expressed as mean ± SEM, n = 5. **p<0.01 and ****p<0.0001 vs normolipidemic group, # p<0.05, ## p<0.01 and ### p<0.001 in comparison to hyperlipidemic group.

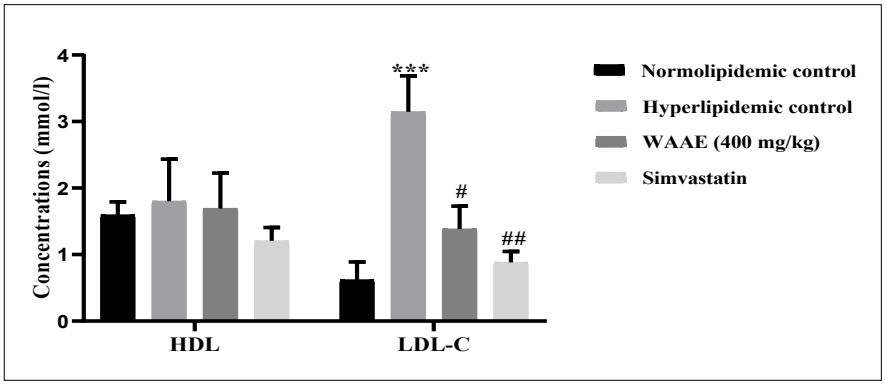

Fig. 8. Effect of WAAE (400 mg/kg) on HDL-c and LDL-c levels in Triton WR-1339-induced hyperlipidemic rats. Values are expressed as mean ± SEM, n = 5. ***p<0.0001 vs normolipidemic group, #p<0.05 and ## p<0.01 vs hyperlipidemic group.

Discussion

Before assessing the antihyperglycemic effect of the aqueous extract of *W. adpressa*, a preliminary toxicological study was conducted to determine the safe doses. The results indicate that the administration of WAAE at a limit dose of 2000 mg/kg produced no mortality, behavioral changes, or clinical signs of toxicity during the 14-day study period. According to the OECD guideline 423, the administered dose corresponds to a relatively low hazard classification, suggesting that the extract possesses a favorable safety profile at the tested concentration and supports its potential for further pharmacological development. However, further toxicity studies, especially sub-chronic and chronic toxicity studies, as well as biochemical and histopathological analyses, are required to establish the long-term toxicological profile. The results demonstrated that aqueous extract of *W. adpressa* exhibited a significant and powerful anti-hyperglycemic activity in STZ-induced diabetic rats. Chronic (15 days) oral administration of a low dose of WAAE (20 mg/kg) resulted in a significant reduction in blood glucose levels in diabetic rats. Streptozotocin is an alkylating agent that can specifically target and damage the insulin-producing beta cells within the pancreatic islets of Langerhans's, leading to a reduction in insulin release and causing diabetes (23). Based on our findings, it is reasonable to hypothesize that the observed antihyperglycemic effect of WAAE may be linked to the regeneration of pancreatic β cells, probably through its ability to prevent STZ-induced free radical formation (24). Furthermore, this antihyperglycemic effect could potentially be associated with a potentiation of insulin activity, probably through an increase in insulin secretion by remaining β cells or an increase in peripheral glucose uptake via the activation of AMPK or PI3K/Akt signaling pathways, which promotes GLUT4 translocation to the cell membrane (25, 26). The oral glucose tolerance test (OGTT) is a diagnostic test used to assess the body's ability to metabolize glucose. It is used clinically to diagnose diabetes and investigate antidiabetic agents by evaluating their impact on glucose metabolism. The results show that WAAE administration had no significant effect on blood glucose levels in normal rats during OGTT. In contrast, treating diabetic rats with WAAE significantly reduced blood glucose levels after 120 min. This could be attributed to the difference in glycemic regulation between these 2 groups, normal rats possess efficient and optimal glycemic regulation characterized by rapid and sufficient insulin secretion, making the additional effect of the extract imperceptible, in contrast, in diabetic rats with impaired glucose regulation, the extract could improve insulin sensitivity, stimulate residual insulin secretion, or inhibit intestinal glucose absorption, thereby contributing to a significant reduction in postprandial glycemia (27, 28). Antioxidants, such as ascorbic acid, N-acetylcysteine and α-lipoic acid, are recognized for their potential in the management of diabetes and their combination with antidiabetic agents is often recommended for optimal glycemic control (25, 29, 30). Another possible explanation for the antihyperglycemic effect of WAAE could be attributed to its abundance of antioxidants, Our study demonstrated that WAAE exhibited significant free radical scavenging capacity, as evidenced by DPPH assay results.

These findings are consistent with those reported in previous studies showing that the polyphenol-rich fraction of *W. adpressa* leaves possesses a significant antioxidant potency (IC_{50} value of 27.84 µg/mL) (31). The notable antioxidant activity exhibited by WAAE may be essentially attributed to its richness in bioactive compounds such as flavonoids, polyphenols and tannins, which are known to neutralize reactive oxygen species (ROS) (32, 33). In this context, quantitative phytochemical analyses carried out in the present study showed that WAAE is particularly rich in phenolic compounds, flavonoids and tannins. Previous studies on *W. adpressa* have led to isolating the following bioactive compounds: wadpressin, withanolide F, withanolide J, coagulin L, nicotiflorin and Withaferin A. The latter is a powerful steroidal lactone exhibiting a broad spectrum of pharmacological effects, including anti-inflammatory, anticancer and antioxidant activity (34). The antihyperglycemic effect of WAAE may be linked to Withaferin A, since previous studies have reported that this compound possesses a powerful antidiabetic effect, by significantly increasing glucose uptake in skeletal myotubes (35). Furthermore, another study showed that withaferin A contributes to diabetes treatment by attenuating inflammation in pancreatic β-cells and protecting them against cytokine-induced cell damage (36). A previous study revealed the potential of withaferin A as a leptin sensitizer, exhibiting significant antidiabetic effects in mice (10). Besides, in silico studies have reported that the antidiabetic activity of withaferin A is probably due to the inhibition of alpha and beta-glucosidase (37). The liver and skeletal muscles are essential in maintaining blood glucose homeostasis (38). Previous studies have shown reduced glycogen levels in the liver and muscle of diabetic rats (39). Similarly, a significant decrease in hepatic and muscle glycogen levels of diabetic rats was observed in our study, perhaps resulting from reduced availability of the active form of glycogen-synthetase, probably caused by the reduced insulin levels characteristic of the diabetic state (40). Treatment of diabetic rats with WAAE significantly improved glycogen levels compared to the diabetic control group. The plant's ability to restore liver and muscle glycogen levels is probably attributed to increased insulin levels. It is well established that liver injury is recognized as one of the serious complications of diabetes (41). In this context, the histological findings from the present study revealed a significant improvement in the architectural integrity of the liver of diabetic rats treated with WAAE compared with the diabetic control group, suggesting a protective effect of the extract against diabetes-induced liver damage. It is well known that hyperglycaemia and hyperlipidemia are the main features of diabetes mellitus. Persistent hyperglycaemia can lead to various metabolic disorders, including alterations in the lipid profile characterized by increased levels of total cholesterol, triglycerides and LDL-c, as well as decreased levels of HDL-c. Elevated levels of total cholesterol and LDL-c are considered as potential risk factors for cardiovascular disease. In contrast, higher levels of HDL-c are generally associated with lower ischemic cardiovascular risk (42, 43). The present study's findings demonstrate that WAAE significantly lowered levels of total cholesterol and triglycerides in STZ-induced diabetic rats after 15 days of repeated treatment. These initial findings sparked interest in the potential of this extract for managing hyperlipidemia. To further solidify these results, we expanded the investigation using a separate model of acute hyperlipidemia induced by tyloxapol, a non-ionic detergent, that has been widely used in animal models to produce acute hyperlipidemia for screening and studying lipid-lowering agents, its mechanism of action consists of inhibiting lipoprotein lipase involved in the hydrolysis of triglycerides and stimulating 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase), a crucial enzyme in the cholesterol biosynthesis (45). Simvastatin, a standard drug, is a statin that inhibits the HMG-CoA reductase enzyme (44, 46). Statins are an effective choice for the treatment of dyslipidemia. Although they are very effective, they have many undesirable side effects (47). It would therefore be advisable to develop drugs based on natural substances that would have a hypolipidemic effect similar to that of statins, with fewer side effects. The study showed that treatment of hyperlipidemic rats with WAAE (400 mg/kg) significantly reduced total cholesterol, triglycerides and LDL-c levels compared to the untreated hyperlipidemic group. This hypolipidemic effect may be attributed to the extract's ability to inhibit HMG-CoA reductase activity, thus decreasing cholesterol biosynthesis, or to its potential to stimulate lipoprotein lipase activity, responsible for lowering plasma triglycerides. Based on the results obtained in the present study, it can be suggested that *W. adpressa* could be exploited as a potential therapeutic agent in managing diabetes and dyslipidemia. These findings are consistent with previous studies on *W. coagulans*, which have demonstrated significant antidiabetic and antihyperlipidemic effects in various experimental models (48). This similarity suggests a pharmacological consistency within the *Withania* genus and supports the potential relevance of *W. adpressa* in the management of metabolic disorders, Moreover, recent systematic reviews and meta-analyses have highlighted the efficacy of *W. somnifera* in improving glycemic control, lipid metabolism and oxidative stress, providing further scientific rationale for investigating *W. adpressa* and supports further investigations focused on the isolation of its bioactive compounds, elucidation of underlying molecular mechanisms and evaluation of its long-term safety and efficacy in clinical models (49, 50).

Conclusion

The experiments carried out in the present study suggest that the aqueous extract of *W. adpressa* exhibits a considerable antihyperglycemic and antidyslipidemic potential. Further studies are needed to identify the specific bioactive compounds of this plant and clarify the molecular mechanisms involved in these observed effects.

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Authors' contributions

AQ conceived and designed the study, carried out all laboratory experiments and drafted the manuscript. IB contributed to the realization of experimental techniques and laboratory manipulations. AM and AA contributed to the analysis and interpretation of the data. ME supervised this work and contributed to the critical revision and correction of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors' declare no conflict of interest, financial or otherwise.

Ethical issues: All applicable institutional guidelines for the care and use of animals were followed according to the local committee of the Faculty of Sciences & Techniques Errachidia, Moulay Ismail University (FSTE/2015).

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