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Evaluation of hypoglycemic and hypolipidemic activities of aqueous extract of *Cistus ladaniferus* in streptozotocin-induced diabetic rats

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ABSTRACT

Objective: To evaluate the effect of aqueous leaf extract of *Cistus ladaniferus* (*C. ladaniferus*) on glycemic and lipidemic status in diabetic rats.
Methods: Overnight fasted rats were injected intraperitoneally with streptozotocin (45 mg/kg) to induce experimental diabetes. The aqueous extract of the leaves of *C. ladaniferus* was administered orally at the dose of 500 mg/kg body weight to diabetic rats for a period of 28 days. Hypoglycemic effect, body weight, oral glucose tolerance, change in lipid parameters, urea, creatinine, aspartate aminotransferase and alanine aminotransferase levels of diabetic rats treated with aqueous extract were evaluated in experimental animals.
Results: Administration of 500 mg/kg of *C. ladaniferus* extract to diabetic rats for 28 days resulted in a significant reduction in the levels of blood glucose, alanine aminotransferase, aspartate aminotransferase, urea and creatinine. Furthermore, the extract of *C. ladaniferus* improved glucose tolerance in diabetic rats, and its antidiabetic effect was similar to the one obtained with glibenclamide. The hypolipidemic effect was demonstrated by an important decrease in plasma total cholesterol, triglycerides and low density lipoprotein-cholesterol levels.
Conclusions: It is concluded that *C. ladaniferus* leaf extract showed an antidiabetic activity in experimental diabetes which was similar to the one obtained with glibenclamide.

1. Introduction

Diabetes mellitus is a metabolic disease characterized by a chronic hyperglycemia, resulting from a deficiency in insulin production by β cells or insulin cellular resistance [1]. The International Diabetes Federation estimated that 415 million people worldwide have diabetes in 2015; by 2040, this will rise to 642 million [2]. Diabetes mellitus is a complicated metabolic disease which results from the interaction of multiple factors such as behavioral, genetic predisposition and environmental risk factors.

In Morocco, many medicinal plants are used for the treatment of diabetes in folk medicine systems as well as in traditional healing practices. Furthermore, it is reported that about 1 200 plants were used in traditional medicine for the treatment of diabetes and/or studied for potential antidiabetic activity worldwide [3]. Scientific studies on medicinal plants used in folk medicine to treat diabetes can contribute to the discovery of novel antidiabetic drugs, which can lead to the development of alternative therapeutic strategies [3]. In order to control all pathological complications of diabetes, there is a need of the development of new alternatives treatments derived from medicinal plant resources [3].

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Cistus ladaniferus (*C. ladaniferus*) (gum rockrose) is a shrub from the Cistaceae family, originated from the Mediterranean region, known locally as "Touzal" in the north of Morocco or "Argale" in other regions of Morocco [4]. This plant is widely used in traditional medicine in Northern Morocco as antidiarrhoeal and antispasmodic [5]. Previous studies reported that *C. ladaniferus* exerts different pharmacological effects such as antiaggregant effects [6], antihypertensive [7], antispasmodic [8], antioxidant [9] and cytotoxic activity against human cancer cells [10]. Essential oil and various organic extracts of this plant have been shown to exhibit antifungal and antibacterial effects [11].

According to ethnobotanical data collected in the north of Morocco, the decoction of *C. ladaniferus* leaves is traditionally used in diabetes control and treatment [12]. However, this ethnobotanical information has never been evaluated experimentally. Thus, the objective of the present investigation was to explore the hypoglycemic and hypolipidemic activities of aqueous leaves extract of *C. ladaniferus* in diabetic rats.

2. Materials and methods

2.1. Plants material

Fresh leaves of *C. ladaniferus* (Cistaceae) were collected from Taounate region (north of Morocco) in March 2015. The plant material was taxonomically identified and authenticated and voucher specimens (No. MA-FSTF 16) were deposited in the herbarium of the Department of Biology, Faculty of Sciences and Techniques, Fes, Morocco.

2.2. Preparation of aqueous extract

The leaves of *C. ladaniferus* were washed with water, air-dried for 7 days and reduced to a coarse powder. The powder was extracted by infusion as described in Moroccan folk medicine. About 100 g of leaves powder were mixed with 1 000 mL of distilled water for 1 h. The extract was filtered and evaporated under vacuum at 50 °C using a rotary evaporator. The residue (yield = 26%) was stored at −20 °C until use.

2.3. Animals

Healthy adult female and male Wistar rats weighing 180–230 g were used in the experiments. Rats were housed in polypropylene cages at the animal house of the Faculty of Sciences and Techniques (Fes, Morocco). The animals were maintained in standard environmental conditions and fed standard diet and water *ad libitum*. Recommendations of international guidelines were followed in animal experiments [13]. All experimental procedures involving animals were conducted in accordance to the guidelines on the use and care of experimental animals, published by the US National Institutes of Health (NIH) and approved by Institutional Ethical Committee of Sidi Mohamed Ben Abdellah University, Morocco.

2.4. Acute toxicity

Aqueous leaf extract of *C. ladaniferus* in 500 mg/kg doses was administered to the rats orally. All animals were observed for 24 h after treatment.

2.5. Evaluation of antidiabetic activity

2.5.1. Induction of experimental diabetes

To induce experimental diabetes, a single intraperitoneal dose of 45 mg/kg body weight of streptozotocin (STZ) (Sigma–Aldrich, St. Louis, MO, USA) was injected to rats after overnight fasting. STZ was freshly prepared in cold sodium citrate buffer (0.1 mol/L, pH 4.5). The diabetic state was confirmed by the measurement of fasted blood glucose in rats after 3 days following the injection of STZ. Only rats with glycemia higher than 2 g/L were included in experiments.

2.5.2. Experimental design

The animals were divided into four groups and each group consisted of six animals. Groups 1 and 2 served as normal and diabetic control groups, respectively and treated with distilled water (10 mL/kg). Group 3 was treated with the aqueous extract of *C. ladaniferus* at a dose of 500 mg/kg/day. Group 4 was treated with 2 mg/kg/day of a standard care drug, glibenclamide. The 500 mg/kg extract dose used in the treatment was chosen from a preliminary short-term study in our laboratory. Distilled water, glibenclamide, and plant extract were given orally by gavage as single daily treatments. The antidiabetic activity of *C. ladaniferus* extract was evaluated in treated animals in comparison to the control groups, by measuring fasting blood glucose levels and body weight on Days 1, 7, 14, 21 and 28 of the study.

2.5.3. Oral glucose tolerance test (OGTT)

In order to assess the effect of orally administered *C. ladaniferus* extract on systemic glucose homeostasis, an oral glucose tolerance test was carried out in fasted rats after 4 weeks administration of the extract. In the final day of the experiment, overnight fasted rats were loaded with glucose (2 g/kg) orally 60 min after the last dose of distilled water, glibenclamide or the extract administration. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min after glucose loading. Blood glucose levels (g/L) were plotted against time intervals (min), and the respective areas under the curve (AUC) were calculated. The AUCs of the curves of each group were compared and tested for statistical significance against the control diabetic group to evaluate glucose utilization by the tissues.

2.5.4. Determination of the blood glucose levels

Blood samples were collected from the tip of the tail for glycemia measurement. In this study, blood glucose concentrations (g/L) were determined at the defined time points, by means of On Call® Plus glucometer and blood glucose reactive test strips based on the glucose oxidase method.

2.5.5. Estimation of biochemical parameters

At the end of the 28 days experiment, the animals were sacrificed and blood samples were collected from all the four groups in tubes containing heparin. Plasma was separated for the estimation of total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglycerides, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), urea and creatinine. All biochemical parameters were studied by an auto-analyzer "Architect C8000" (Abbott Laboratories, Abbott Park, IL, USA).

2.6. Statistical analysis

All data are presented as mean ± SD of the indicated number of experiments. Results were analyzed by One-way ANOVA followed by Tukey's multiple comparison post-test performed using Graph Pad Prism version 6.0 for Windows. Values of P less than 0.05 were considered statistically significant.

3. Results

3.1. Acute toxicity

After oral administration of the aqueous extracts of leaves of *C. ladaniferus* 500 mg/kg doses, no mortality was recorded for all animals observed 24 h after the leaf extract administration.

3.2. Changes in body weight

Effect of *C. ladaniferus* extract on body weight is shown in Table 1. Diabetic control rats showed a progressive fall in body weight during the study period in comparison to normal control rats ($P < 0.001$). The treatment of diabetic rats with *C. ladaniferus* extract at a dose of 500 mg/kg for 28 days showed marked improvement in body weight in comparison to diabetic control group ($P < 0.01$). The effect of *C. ladaniferus* extract on body weight was comparable to that of glibenclamide.

3.3. Hypoglycemic effect of aqueous extract

The effect of *C. ladaniferus* extract administration on blood glucose level is presented in Figure 1. The fasting blood glucose level (FBG) was measured in all groups of rats on Days 1, 7, 14, 21 and 28 of the treatment. STZ-induced diabetic rats had significantly higher level of FBG compared to that of normal control rats ($P < 0.001$). Oral daily administration of *C. ladaniferus* extract produced a significant decrease in FBG level, observed from the 7th day after the treatment compared to diabetic control ($P < 0.001$). Furthermore, treatment with *C. ladaniferus* extract produced 34% fall in FBG level compared to the FBG level of diabetic control at the end of the experiment.

3.4. Oral glucose tolerance test

Figure 2 presents the changes in blood glucose levels of rats following the OGTT. Glycemia with a maximum level in normal and diabetic groups occurred 30 min after the oral glucose challenge. Administration of *C. ladaniferus* extract 60 min before glucose loading produced a significant decrease in the rise in blood glucose level at 30 min ($P < 0.001$) and 60 min ($P < 0.01$) compared to diabetic control. Treatment of diabetic rats with glibenclamide and *C. ladaniferus* extract induced a significant reduction in glucose AUC relative to the diabetic control group by 15% and 12.5%, respectively (Figure 3).

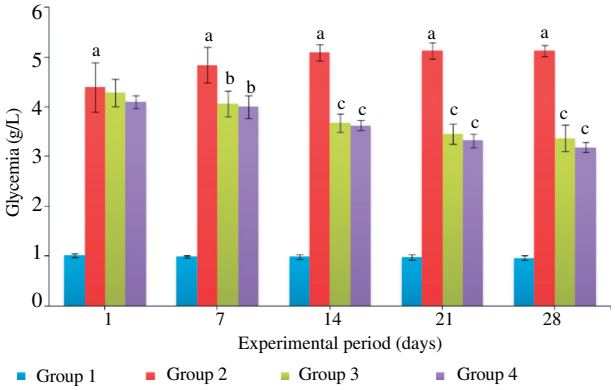

Figure 1. Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on blood glucose in STZ-induced diabetic rats.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.001$ compared to normal control; [b]: $P < 0.01$, [c]: $P < 0.001$ compared to diabetic control.

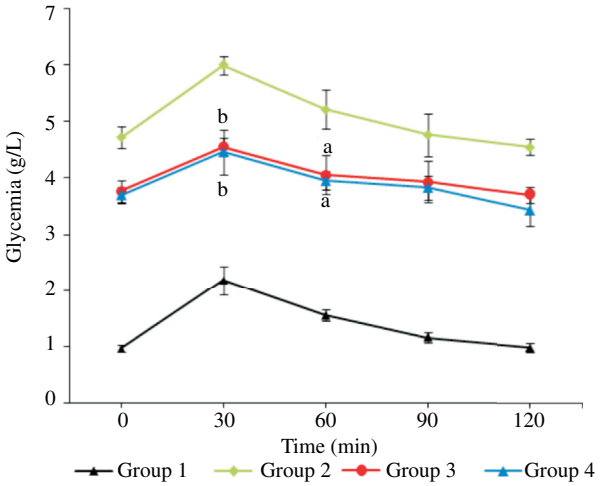

Figure 2. Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on blood glucose in STZ-induced diabetic rats during OGTT.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.01$, [b]: $P < 0.001$ compared to diabetic control.

3.5. Changes in lipid profile

Figures 4 and 5 show the levels of plasma total cholesterol and triglycerides of experimental animals. The plasma levels of total cholesterol and triglycerides were found to be significantly higher ($P < 0.05$) in the diabetic control animals in comparison

Table 1
Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on body weight in STZ-induced diabetic rats.

Treatment	Body weight (g)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Group 1	200.00 ± 20.95	197.67 ± 20.53	202.17 ± 21.31	198.00 ± 30.38	200.83 ± 31.28
Group 2	207.67 ± 19.76	187.67 ± 26.91	150.00 ± 17.34[a]	128.67 ± 23.68[b]	116.00 ± 21.43[b]
Group 3	214.00 ± 11.33	200.33 ± 10.11	184.33 ± 9.07[d]	166.33 ± 6.38[c]	160.83 ± 5.15[d]
Group 4	208.17 ± 6.94	202.34 ± 9.04	190.67 ± 7.45[d]	170.17 ± 5.42[c]	164.10 ± 5.83[d]

Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.01$, [b]: $P < 0.001$ compared to normal control; [c]: $P < 0.05$, [d]: $P < 0.01$ compared to diabetic control.

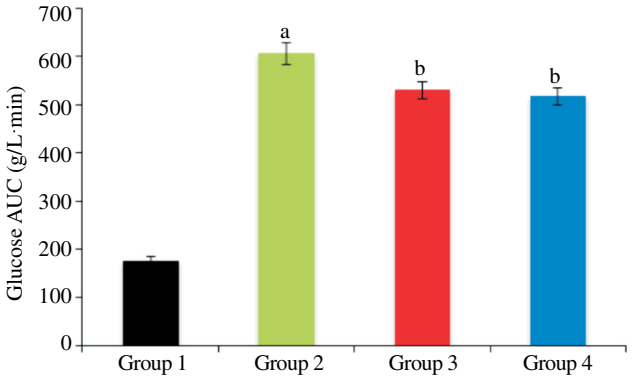

Figure 3. Effect of *C. ladaniferus* extract on total area AUCs of blood glucose.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.001$ compared to normal control; [b]: $P < 0.01$ compared to diabetic control.

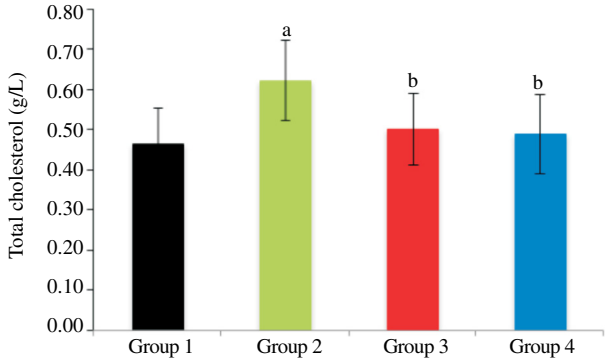

Figure 4. Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on plasma total cholesterol in STZ-induced diabetic rats.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.05$ compared to normal control; [b]: $P < 0.05$ compared to diabetic control.

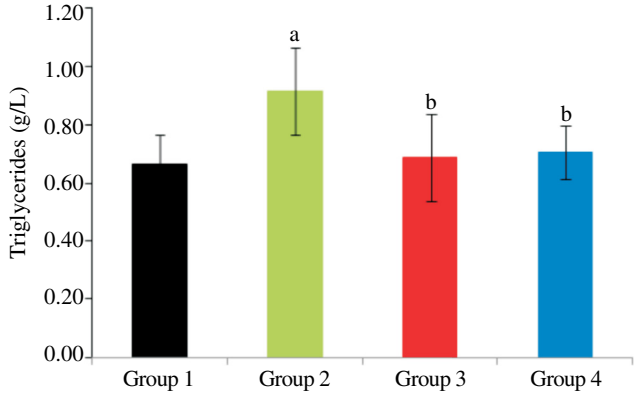

Figure 5. Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on plasma triglycerides in STZ-induced diabetic rats.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.05$ compared to normal control; [b]: $P < 0.05$ compared to diabetic control.

with normal control rats. The rats treated with *C. ladaniferus* extract showed a significant decrease in plasma triglycerides and total cholesterol when compared with control diabetic rats ($P < 0.05$). The leaf extract and glibenclamide showed similar effects in reducing plasma total cholesterol and triglycerides levels.

The plasma levels of LDL-C were significantly higher in the diabetic control group than in the normal control group (Figure 6). Contrariwise, the level HDL-C was significantly

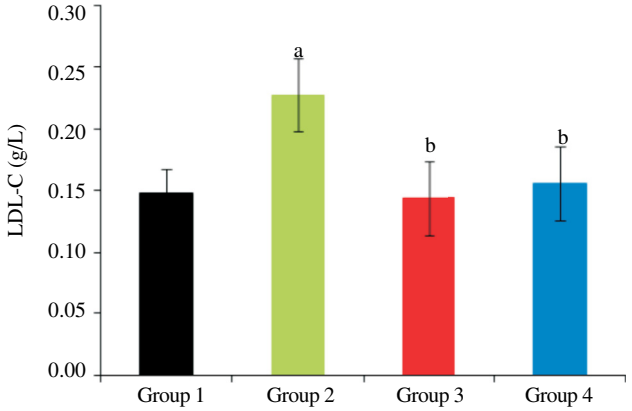

Figure 6. Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on plasma LDL-C in STZ-induced diabetic rats.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.05$ compared to normal control; [b]: $P < 0.05$ compared to diabetic control.

diminished in comparison with the normal control group ($P < 0.05$). A marked decrease in the level of LDL-C was observed in diabetic rats treated with glibenclamide without significant change in HDL-C level (Figure 7). The treatment with *C. ladaniferus* extract showed a significant reduction in plasma level of LDL-C by 34% ($P < 0.05$). However, the plasma level of HDL-C did not show a significant increase in comparison with diabetic control rats.

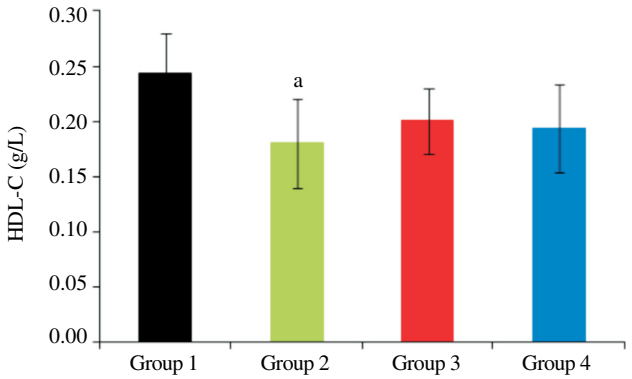

Figure 7. Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on plasma HDL-C in STZ-induced diabetic rats.
Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.05$ compared to normal control.

3.6. Changes in transaminases, urea and creatinine levels

Table 2 shows the levels of hepatic and renal markers in normal and diabetic animals. A significant increase in the level of ASAT and ALAT was observed in diabetic control rats when compared to non-diabetic control group ($P < 0.05$). Diabetic rats treated with *C. ladaniferus* extract (500 mg/kg body weight) and glibenclamide showed a significant decrease in the levels of ASAT and ALAT in comparison to control diabetic rats ($P < 0.05$). Moreover, a significant elevation in urea and creatinine levels was observed in diabetic control rats when compared to non-diabetic control group ($P < 0.05$). Treatment with *C. ladaniferus* extract and glibenclamide for 28 days showed a significant reduction in urea and creatinine levels in the diabetic treated group in comparison with the diabetic untreated group ($P < 0.05$).

Table 2
Effect of daily oral administration of the aqueous extract of *C. ladaniferus* on levels of urea, creatinine, ALAT and ASAT in STZ-induced diabetic rats.

Treatment	Hepatic markers		Renal markers	
	ALAT (IU/L)	ASAT (IU/L)	Urea (g/L)	Creatinine (mg/L)
Group 1	49.67 ± 5.89	110.20 ± 14.53	0.41 ± 0.05	5.51 ± 0.55
Group 2	125.00 ± 18.38[b]	225.50 ± 25.40[c]	0.66 ± 0.09[b]	7.50 ± 1.45[b]
Group 3	94.17 ± 17.53[a]	171.50 ± 24.43[a]	0.54 ± 0.09[a]	7.02 ± 1.10[a]
Group 4	87.51 ± 12.50[a]	171.83 ± 15.21[a]	0.56 ± 0.07[a]	6.38 ± 0.86[a]

Values are expressed as mean ± SD, $n = 6$. [a]: $P < 0.05$ compared to diabetic control; [b]: $P < 0.05$, [c]: $P < 0.01$ compared to normal control.

4. Discussion

The present data indicated that *C. ladaniferus* aqueous extract at the dose of 500 mg/kg significantly decreased blood glucose, triglycerides, total cholesterol, LDL-C, urea, creatinine, ASAT and ALAT levels. Furthermore, the extract improved glucose tolerance in treated diabetic rats as compared with control diabetic rats. The aqueous extract of *C. ladaniferus* showed a comparable antidiabetic effect to glibenclamide, a standard hypoglycemic drug which is used as a standard antidiabetic agent to evaluate the antidiabetic activities in experimental diabetes studies [14,15].

Severe loss in body weight is one of the characteristics observed in STZ-induced diabetes, which was observed in the present study. Administration of *C. ladaniferus* extract and glibenclamide significantly improved the body weight loss in diabetic rats in comparison to diabetic control group. The observed decrease in body weight of diabetic control rats could be attributed to insulin deficiency, which causes degradation of structural proteins and lipids that are known to contribute to body weight [16]. The treatment with *C. ladaniferus* extract, as well as glibenclamide enhanced glucose metabolism and thus improved the body weight in diabetic rats.

The OGTT evaluates the body's capacity to metabolize glucose. A marked increase in the AUC of the glucose was observed in diabetic control rats following the glucose challenge test. This effect may be due to the reduction of glucose tissue utilization and an increased hepatic glucose production, as a result of decreased insulin secretion [17]. The administration of *C. ladaniferus* extract showed a marked diminution in the glucose AUC of diabetic rats. These results revealed that *C. ladaniferus* extract increased glucose utilization which indicates the improvement of glucose homeostasis in treated diabetic animals.

One of the associated metabolic disorders of diabetes is dyslipidemia which contributes to secondary complications of diabetes [18]. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats [19,20]. Under normal conditions, the lipolytic hormones action is activated by insulin allowing hydrolysis of triglycerides and blocks mobilization of free fatty acids [21]. Contrariwise, in the diabetic state, the activity of lipoprotein lipase is reduced due to lack of insulin resulting in hypertriglyceridemia as well as over production of LDL-C by the liver [22]. The observed diminution in plasma total cholesterol, triglycerides and LDL-C status in treated diabetic animals suggests that the extract possesses insulinotropic effect or insulinomimetic activity.

According to our results, diabetic control rats showed a significant elevation of urea and creatinine levels, which are used to evaluate renal dysfunction [23]. Administration of *C. ladaniferus* extract to diabetic rats significantly decreased these parameters which could be attributed to the attenuation of metabolic alterations in protein and nucleic acid metabolisms which are caused by hyperglycemia in diabetic states. This finding was demonstrated in our study by improved glucose homeostasis in diabetic rats.

Previous studies have reported an increase in ASAT and ALAT activities in the diabetic state which indicates active liver damage [24]. Therefore, the increase in the activities of plasma ASAT and ALAT observed in this study could be attributed to the hepatocellular damage caused by diabetes. This finding was also demonstrated by a previous study reporting a necrotic liver in diabetic rats [25]. Oral administration of *C. ladaniferus* extract and glibenclamide for 28 days to diabetic groups decreased the activity of these enzymes in plasma. These results are in agreement with those obtained in a previous study which reported the antidiabetic effect of *Piper longum* root aqueous extract in diabetic rats [26] and indicate the hepatoprotective effect of *C. ladaniferus* against STZ-induced toxicity.

The observed antidiabetic activity of *C. ladaniferus* may be attributed to the presence of bioactive compounds such as flavonoids in the extract. The presence of flavonoids in *C. ladaniferus* has been reported previously [27,28]. It is documented that hypoglycemic activities of many medicinal plants are attributed to the presence of phenolic compounds and flavonoids [29]. Studies also reported that flavonoids have antidiabetic properties because they stimulate glucose uptake in peripheral tissues and attenuate oxidative stress during diabetic conditions [30,31]. They also exert a stimulatory effect on insulin secretion by changing Ca^{2+} concentration [32]. However, the observed antidiabetic activity may be due to synergistic effect of different classes of bioactive compounds present in *C. ladaniferus* extract.

In conclusion, aqueous leaves extract of *C. ladaniferus* was effective in decreasing blood glucose levels in STZ-induced diabetic rats. The hypolipidemic effect was demonstrated by a significant reduction in plasma lipid parameters. The present results confirm the use of this plant to treat diabetes complications. Finally, the mechanism of action, as well as the bioactive compound(s) involved in these pharmacological activities, remains to be determined in addition to toxicological studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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