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Research Article

Antihyperglycemic Potential of Methanolic Extracts from the Medicinal Plants: *Marrubium deserti* De Noé and *Marrubium vulgare* L.

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Abstract

Background and Objective: Plants of the genus *Marrubium* are used to cure several infectious diseases like asthma, rheumatoid and diabetes. This study investigated *in vivo* antihyperglycemic capacity of the methanolic fraction (MeOHE) of two *Marrubium* species via animal model. **Materials and Methods:** The plant extracts were investigated both qualitatively and quantitatively to reveal their phytochemical composition. Pharmacologically speaking, Wistar albino rats of either sex (150-180 g) were used to investigate the antidiabetic effect using 25 rats in each of the two used approaches; respectively Oral Glucose Tolerance Test (OGTT) and streptozotocin-induced diabetes approaches. In addition, various biochemical parameters were investigated to confirm the pharmacological quality of our plants. Evaluated for their possible antidiabetic effect during two consecutive weeks for glycemia levels (2 hrs period). **Results:** Data revealed that MeOHE induced a significant antihyperglycemic effect, in which *M. deserti*, a slight decrease in the glucose level at (143±0.5 mg/dL) was obtained while a reduction was recorded for *M. vulgare* (71.5±0.75 mg/dL) with a same observation for triglycerides, cholesterol, glutamic-oxaloacetic transaminase (TGO), glutamic pyruvic transaminase (TGP), creatinine comparing to standard. Moreover, the result showed high levels of phenolic and flavonoïds content. **Conclusion:** This modest work confirmed once again the real pharmacological capacity of vegetals and their possible implication to cure diabetes and complications related to it underlying the antihyperglycemic activity of these plants.

Key words: Antihyperglycemic, *Marrubium deserti*, *Marrubium vulgare*, phenolic content, flavonoids content

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Abnormal elevation of glycemia is usually a consequence of insulin deficiency, but also an increasing peripheral tissue resistance to this hormone and a dysfunction in carbohydrates and/or lipids metabolism[1]. This illness may dramatically rise in the next decade, in particular in third-world countries[2], generating long-term repercussions on various organs, blurring of vision, excessive urination, infections that may not heal correctly excessive sensation of thirst and feeding. In rare cases, ketoacidosis may evolve into coma, even to death if adequate treatments are not taken in the appropriate time[3]. Western medical approaches to treat diabetes include the injection of hypoglycemic agents called insulin analogs like biguanides derivatives[4]. However, drugs possess side effects and long-term use may decrease receptor reactivity to this hormone, leading to abnormal insulin resistance and the development of prediabetes. Currently, new drugs have been developed like SGLT-2 inhibitors[5]. However, the expensive cost of these drugs has limited their possible clinical usage. Recently, several studies investigated the hypoglycemic effects of natural compounds of plants which represent a real source for new drugs[6,7]. More than 97 species compose the genus *Marrubium* (Labiatae) and are widely distributed over the warm-temperate regions and considered native species in Asia and in the Mediterranean areas of Europe[8]. *Marrubium vulgare* and *Marrubium deserti* De Noé, which are endemic Algerian species[9]. This plant is used to treat fever and respiratory diseases[10], intestinal parasitosis, dysmenorrhoea and cough[11]. Recent works indicated that this species has potent antinociceptive, cytotoxic, antipyretic and antiviral activitiy[9,12,13]. Regarding *M. vulgare*, this popular plant is traditionally used in many countries as an antidiabetic, cardiotonic and antihypertensive agent[14]. This herb contains plenty of terpenes, flavonoïds, tannins and phenols[15]. Marrubenol and marrubiin are two key diterpenes previously isolated from *M. vulgare*, which have shown a variety of activities[16,17].

The purpose of the current work was to investigate the antidiabetic capacity of the methanolic extract from *M. vulgare* and *M. deserti* in animals using streptozotocin-induced diabetic rats test.

MATERIALS AND METHODS

Study area: The study was carried out in collaboration between the Laboratory of Biotechnology of Bioactive Molecules and Cellular Physiopathology (LBMBPC), University Batna 2, Algeria and the Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia from October 2022 to November 2023.

Chemical and reagents: Gallic acid, quercetin, glucose, glibenclamide and streptozotocin (STZ), ($AlCl_3$) were provided from "Fluka Chemie". Methanol used as the solvent for the preparation of plant extracts was purchased from "PROLAB, MERCK EUROLAB".

Collection and identification of plant materials: *Marrubium vulgare* was collected from (Gabel Amran, Batna, Algeria). This plant was identified by Mr. Hamchi (University Hadj Lakhdar Batna, Algeria). The second plant used in this study named *M. deserti* was collected from Bechar. The principal approaches used in this study were summarized in Fig. 1.

Preparation of plant extracts: To obtain the methanol crude extracts (MeOHE), 500 g of plant leaves powder were macerated in water/methanol (20-80%; v/v) respectively with a final volume 3 L for 72 hrs at 37°C. The obtained filtrate was concentrated and dissolved by Rotavapor "Buchi type" at a temperature of 40°C and then the water was separated by lyophilization. Finally, MeOH extract was conserved at the temperature of 4°C.

Experimental animals and ethical consideration: Wistar albino rats of either sex (150-180 g) were provided by the Agricultural Sciences and Veterinary Research Institute and housed separately in cages. The paper is exempt from ethical committee approval since all the experiments applied to the animals were performed in strict conditions within the limits of the laws and rules taken from the World Medical Association guidelines[18].

Phytochemical screening test: The phytochemical qualitative tests were performed according to standard approaches[19], to detect flavonoïds, saponins, phenolic compounds, terpenoids, steroids, alkaloids and tannins.

Quantitative content: The quantitative phenolic content of *M. deserti* and *M. vulgare* was estimated by Folin-Ciocalteu reagent previously reported by Siddhuraju and Becker[20]. About 25 µg of leaf extracts were taken separately and completed with 1 mL of distilled water. Then 1.25 mL of Folin's-phenol reagent and $NaHCO_3$ were added, vortexed then incubated for 30 min (absorbance 765 nm, calibration 50-200 µg/mL). The flavonoid content of *M. deserti* and *M. vulgare* extracts was revealed by Dewanto *et al.*[21] approach.

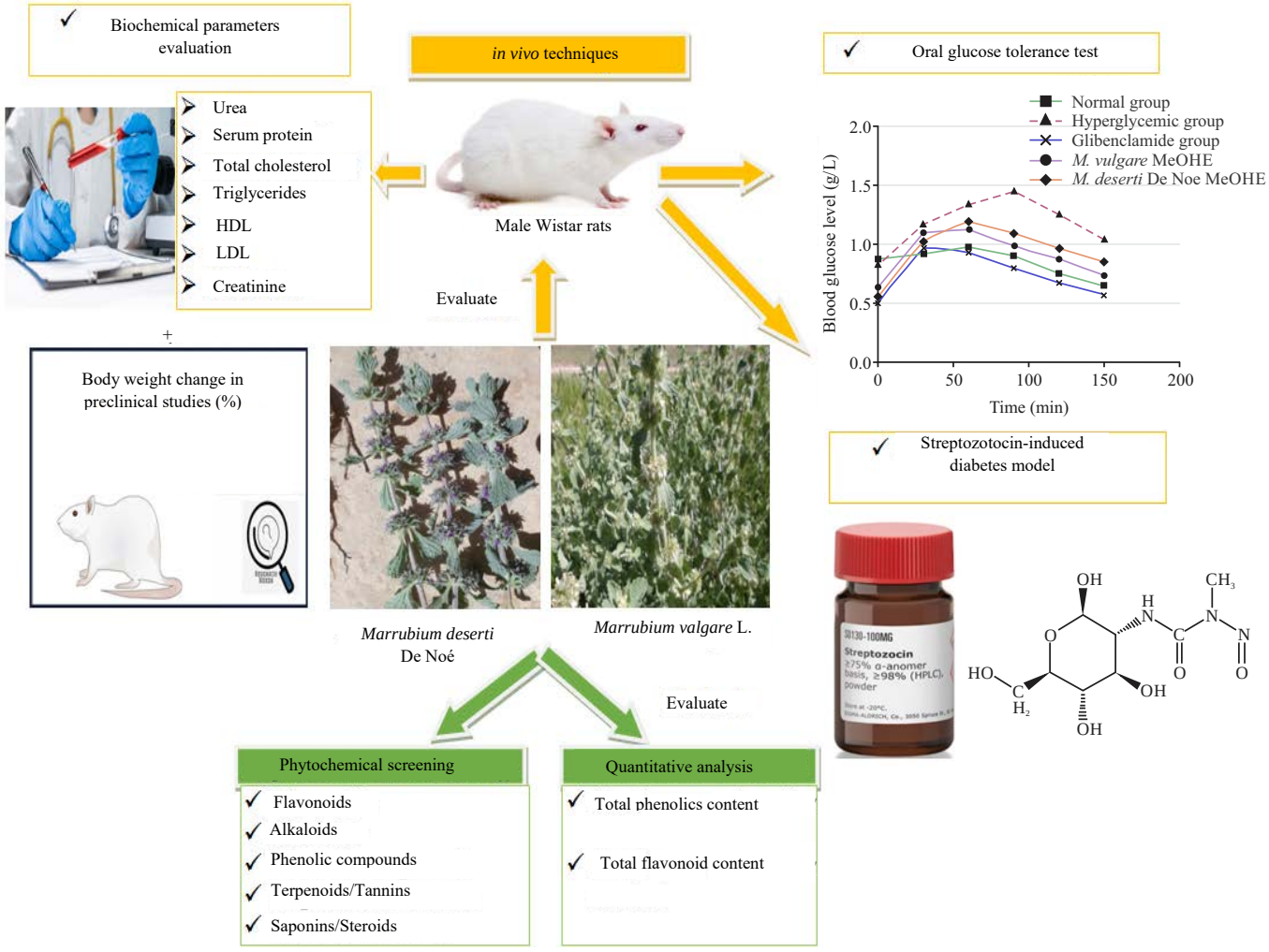

Fig. 1: Summary of the experimental work

Antidiabetic activity test

OGTT test: The possible hypoglycemic activity was assessed using the Oral Glucose Tolerance Test (OGTT) model. The extracts were given orally to rats (600 mg/10 mL/kg) one hour before the gastric gavage of a glucose solution. The choice can be explained by the fact that *Marrubium* species are pharmacologically active between the doses of 300-800 mg/kg in *in vivo* models[8]. Rats were divided respectively; the control group received a physiological solution (0.9% NaCl), a hyperglycemic group received only a glucose solution (4 g/kg), a standard group and finally, two groups received (MeOHE) extracts of *Marrubium deserti* and *Marrubium vulgare*[22]. About 1 hr before oral glucose loading. Glycemia was measured before and after half an hour over 2 hrs of treatment. Note that blood samples were collected from rats' veins by "ACCU-CHEK® Active" Glucometer.

STZ induced diabetes: Diabetes was induced by streptozotocin (STZ) Intraperitoneal injection as a single dose, according to Marchetti *et al.*[23], this chemical drug destroys selectively β-pancreatic cells of islets of langerhans. After 72 hrs, the hyperglycemia was confirmed with "ACCU-CHEK® Active" Glucometer and only animals with glycemia levels above 200-300 mg/dL were chosen. Streptozotocin can induce fatal hypoglycemia resulting from the massive pancreatic secretion of insulin. That's why, after the STZ loading and to prevent its fatal effect[24], the rats were given a 5% glucose solution for 48 hrs. Usually, the diabetes stabilizes in rats after 3 days of the injection. After 72 hrs of the STZ loading.

Study design and biochemical assays: Twenty-five female Wistar albinos rats (130-180 g) were divided into: Group I (NC or placebo); received an equivalent volume of 0.9% of

physiological water (10 mL/kg) and were not treated with any other substance. Group II (DC: Diabetic Control); diabetic rats were injected with STZ then 0.9% physiological water, daily and once a day for two weeks as a vehicle for the tested extracts. In group III (positive control); animals were given reference drugs for two weeks. In group IV and V, animals received respectively *M. vulgare* and *M. deserti* at a dose of 600 mg/kg, for 16 consecutive days.

The rats were kept at room temperature and each cage contained 3 to 4 rats the fasting blood sugar and weight evolution of rats of different groups was monitored weekly until the final day. It is imperative to adjust the treatment doses for the batches each week to the weight variation[25]. The tail vein was used to properly collect blood. Various biochemical parameters were investigated such as total protein, ASAT and ALAT urea. All the parameters were assayed according to the "Spinreact technical sheet".

The enzymatic assays were carried out at a private laboratory of "GOUAREF" using "COBAS" INTEGRA 400 plus auto-analyzer (automaton).

Statistical analysis: Statistics were performed via one-way ANOVA along with the Dunnett's test using GraphPad (GraphPad Software, Inc., La Jolla, California, USA) software. For *in vivo* tests, results were expressed as Mean±SEM and significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

Phyto-screening test: Data revealed that both *M. vulgare* L. and *M. deserti* De Noé methanol fractions contained flavonoids, tannins, terpenoids and phenolic compounds, while the tests for saponins, steroids and alkaloids yielded negative results (Table 1). Another study of Karioti *et al.*[26] investigated the phytochemical profile of two *Marrubium* species named *Marrubium velutinum* and *Marrubium cylleneum* and showed the richness of these species in various secondary metabolites classes comparable to the findings of this study.

Understanding the phytochemical constituents of plants is crucial to predict their pharmacological effect. Several studies[27-30] showed that flavonoïds may effectively reduce glycemia, reducing α-glucosidase activity and saving pancreas structure. It was also revealed that high consumption of flavonoids may considerably reduce the incidence of diabetes.

Quantitative content: The total phenolic content of *M. deserti* and *M. vulgare* fractions varied between (184±0.78 to 195±0.36 mg GAE/g extract). While flavonoïds was high in *M. vulgare* (33.10±0.60 mg QE/g extract) against (28.48±0.40 mg QE/g extract) in *M. deserti*. Several studies[31,32] made on two species of the genius *Marrubium* named *Marrubium persicum and Marrubium friwaldskyanum*, respectively reported the important amount of flavonoids, tannins and phenolics in these species which is by the results of this study. In this context, another spectrophotometric approach made by Karioti *et al.*[26] revealed that *Marrubium velutinum* contains high proportions of flavonoids and phenylethanoid glycosides compounds.

Antidiabetic activity: Data of hyperglycemic animals treated with methanolic extracts of *M. vulgare* and *M. deserti* as well as the standard were shown in Fig. 2.

Both extracts and glibenclamide have a considerable anti-hyperglycemic effect in rats that were given serum glucose. Thus, data indicated that glycemia was considerably reduced ($p<0.001$) in the pre-treated groups but not in untreated rats. After a period of 90 min, a considerable elevation ($p<0.001$) in glycemia levels was noticed in hyper-glycemic rats group, which reached a threshold of 145 mg/dL compared to *M. vulgare* and *M. deserti* groups. The recorded values are of the order of 99 and 110 mg/dL, respectively. An equivalent effect is observed with glibenclamide (80 mg/dL).

The results of this study were in agreement with Bustos-Brito *et al.*[32], who revealed the antidiabetic capacity of aqueous fraction of *M. vulgare* in diabetic rabbits. Hyperglycemia was induced by oral administration of 50% dextrose solution. After 60 min, blood glucose was determined during a period of 5 hrs. These researchers found that tolbutamide (reference drug) and extract significantly reduced hyperglycemia compared to the control group. Another study showed the hypoglycemic effectiveness of *C. tougourensis* which could be linked to various polyphenol classes, especially in flavonoids and tannins content[33]. Note that an important proportion of these two classes were also estimated in the two *Marrubium* species investigated in this study, which may explain this pharmacological effect.

It is also interesting to note that tannins; a well-known bioactive compound, can exert a significant antihyperglycemic and antihyperlipidemic effect but also to preserve liver histopathological integrity and this was demonstrated using diabetic rats[29]. This may partially explain the growing interest of the scientific community to study in depth the pharmacological aspect of these classes of secondary metabolites.

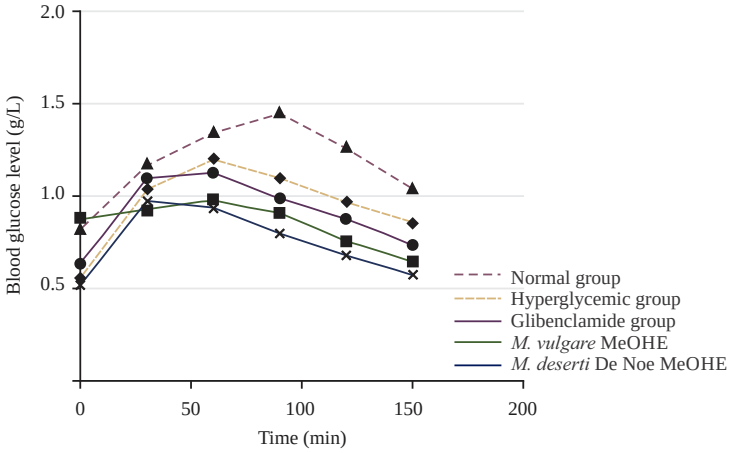

Fig. 2: Evolution of glucose tolerance in hyperglycemic rats treated with methanolic extracts from the studied plants

Table 1: Qualitative phytochemical analysis of methanolic leaf extracts of *M. vulgare* and *M. deserti*

Phytochemical classes/plants	Test name	*Marrubium vulgare*	*Marrubium deserti*
Flavonoids	Shinoda test	+	+
Saponins	Foam test	-	-
Tannins	Ferric chloride test	+	+
Steroids	Liebermann Burchard test	-	-
Terpenoïds	Salkowski test	+	+
Phenolic compounds	Ferric chloride test	+	+

+: Present and -: Absent

Table 2: Effect of *M. vulgare* and *M. deserti* MeOHE on blood glucose level

	Processing period (Days)					
Group	1	3	5	7	9	11
Normal control	82.2±0.23	84.2±0.75	81.6±0.45	61.2±1.25	82.5±0.36	82.5±0.78
Diabetic control	525.5±0.70	400.75±0.50	304.5±1.00	373.25±0.7	383±0.5	340±1.7
M. vulgare (600 mg/kg)	537±1.14^{ns}	322.3±0.8^{b}	206±0.6^{b}	191.6±1.30^{c}	96±0.38^{c}	71.5±0.75^{c}
M. deserti (600 mg/kg)	521±1.10^{ns}	399.5±0.7^{ns}	283±2.30^{ns}	202±1.10^{c}	175±0.75^{c}	143±0.5^{c}
Glibenclamide (5 mg/kg)	442±0.70^{b}	373.4±1.50^{a}	208.2±0.70^{b}	144.8±1.92^{c}	91.8±1.03^{c}	90.4±0.47^{c}

Data±SEM (n = 5), Significance level: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 vs DC group and ^{ns}No significant

Streptozotocin-induced diabetic rats

Effect on blood sugar level: The effect of methanolic extracts of *M. vulgare* and *M. deserti* on animal glycemia was presented in Table 2 and suggests that STZ caused after its injection (72 hrs) a very significant increase (p<0.001) in glycemia level in treated and untreated groups comparing to the group of healthy control rats and the obtained values varied between (442-537 mg/dL).

Streptozotocin (STZ) is classified as an anti-tumour and antibiotic (chemotherapy)[34]. Intravenous injection of 50 to 60 mg/kg of streptozotocin in rats, makes the pancreas swell, causing degeneration in Langerhans cells and an induction of diabetes in 2-4 days[35]. The STZ has been reported to alter the antioxidant defense system by significantly decreasing antioxidant enzyme performance[36,37], causing an acceleration of the diabetogenicity process[38]. On the other hand, a decrease in glycemia was noticed 3rd day after treatment of diabetic rats with (MeOHE) fraction with a corresponding value of 322.3±0.8 mg/dL, while (MeOHE) fraction from *M. deserti* reached 399.5±0.7 the same day. Under the same conditions, the glibenclamide group exhibited a considerable effect. From 7 days of investigation, all groups that were treated with standard or plant extract expressed a highly hypoglycemic effect. Noting that *M. vulgare* fraction (600 mg/kg) showed a more effective decrease (p 0.001) in glycemia (71.5±0.75 mg/dL) when compared to *M. deserti* (143±0.5 mg/dL) in the same period which does not represent any difference (vs DC group (p 0.05)).

According to the literature, the antidiabetic activity is attributed to flavonoids and verbascosides known for their antidiabetic effect[39]. According to Deng *et al.*[40], the ethanolic fraction of *M. vulgare* roots has a significant hypoglycemic

Table 3: Effect of *M. vulgare* and *M. deserti* MeOHE on body weight

	Body weight (g) Processing period (Days)						
Group	T0	1	3	5	7	9	11
Normal control	150±0.3	154±0.35	158.8±0.2	160±0.65	155±0.90	158.67±2.00	157±3.00
Diabetic control	150±0.6	138.6±1.40	130.1±0.75	128±0.35	128±0.60	124.5±0.45	129.5±0.50
M. vulgare (600 mg/kg)	152.16±0.7^{ns}	146±1.50^{a}	147.25±1.00^{b}	151±0.42^{c}	149±0.75^{b}	153.5±0.10^{c}	150±0.49^{c}
M. deserti (600 mg/kg)	151±0.85^{ns}	145±0.60^{a}	144±0.40^{b}	147.4±0.1^{b}	143±2.10^{b}	144.7±0.30^{b}	145±0.6^{b}
Glibenclamide (5 mg/kg)	150.5±0.50^{ns}	145.8±0.20^{a}	148±2.00^{b}	149.5±0.50^{b}	150.20±0.63^{c}	154.5±0.8^{c}	155.5±0.26^{c}

Data±SEM (n = 5), Significance level: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 vs DC group and ^{ns}No significant

Table 4: Effect of *M. vulgare* and *M. deserti* MeOHE on biochemical parameters

			Diabetic treated groups		
Biochemical parameters/group	Normal group	Diabetic control	Glibenclamide	*Marrubium vulgare*	*Marrubium deserti*
Fasting blood sugar (mg/dL)	85	345	93^{c}	92^{c}	130^{c}
Blood urea nitrogen (g/L)	0.5	1.2	0.8^{b}	0.40^{c}	1.3^{ns}
Cholesterol (g/L)	0.66	1.90	0.85^{c}	0.57^{c}	0.88^{c}
Triglycerides (g/L)	1.08	2.47	0.77^{c}	0.41^{c}	0.66^{c}
TGO (UI/L)	39.3	108	84.73^{b}	74.44^{c}	101^{ns}
TGP (UI/L)	41	80	52^{b}	24.06^{c}	49^{b}
Creatinine (mg/L)	7.3	14.25	4.92^{c}	7^{b}	6.5^{b}
Cholesterol HDL (g/L)	0.43	0.56	0.68^{a}	0.57^{ns}	0.52^{ns}
Cholesterol LDL (g/L)	-0.12	-0.07	-0.17^{b}	-0.05^{ns}	-0.05^{ns}
Serum protein (g/L)	81.9	70.5	81.5^{a}	80.2^{a}	74.2^{ns}

Data±SEM (n = 5), Significance level: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 vs DC group and ^{ns}No significant

effect which may be due to the suppression of carbohydrate absorption in the intestines. According to the same researchers, this effect is linked to the presence of bioactive molecules in the extract that inhibit the α-glucosidase enzyme.

Monoterpenes are mostly considered to have some antioxidant and antimicrobial effects[14,41] and recently, these compounds have been reported to have antidiabetic effects[42-44]. The important decrease of blood glucose levels in the group of rats that received (MeOHE) fraction may be linked to the high proportions of flavonoids recorded in this species. Indeed, several studies revealed that flavonoids can inhibit gluconeogenesis, which will consequently prevent hyperglycemia[45,46]. It is also interesting to note that flavonoids can inhibit the cAMP/PKA pathway, which will contribute to ınsulin sensitivity and thus diminish hyperglycemia[46].

The mechanism of action of *M. vulgare* that played a crucial role in lowering serum glucose concentration may be linked to sirtuin 1 (diabetic gene) activation[47,48]. Phenolic and flavonoïds have been shown to activate sirtuin 1 with relevance to glucose and insulin release from the pancreas[49].

Effect on body weight: Data indicated variation in body weight between groups. Before treatment (T0), the rats in all groups have almost the same weight, since the constitution of these groups is done according to the principle of homogeneity (Table 3). After induction of diabetes, the DC group showed a gradual decrease in body weight and was considered a severe loss, when compared to the healthy group. This decrease is of the order of 14% from the initial body weight. In general, the statistical study showed a high difference ($p<0.01$) in body weight between diabetic control and diabetic treated groups, which represented during the same periods of treatment a regular increase in weight compared to the starting weight.

Likewise, the diabetic groups that received MeOHE fractions of the plant showed signs of recovery in body weight and this effect was comparable to that obtained with animals that received glibenclamide. Results of this study follow Boudjelal *et al.*[50] work which showed that alloxan injection caused a significant decrease in body weight gain in rats, which is probably because plants may regulate lipids metabolism level[51]. The hypoglycemic effect of *M. vulgare* and *M. deserti* may be also the result of a stimulation of the gluconeogenesis process which also contributes to the control of protein loss[52].

Effect of extracts on different biochemical parameters: The results for biochemical data treated in this study were reported in Table 4. A non-negligible decrease in serum glucose concentration in rats treated by MeOHE from *M. vulgare* was reported (92 mg/dL) against (145 mg/dL) in the DC group.

In the untreated diabetic group, an increase in urea and creatinine concentrations was observed, but the total protein level was reduced dramatically while in groups of rats that were treated with *M. vulgare* or glibenclamide maintained physiological serum protein values. According to Yamamoto *et al.*[53], this mechanism can be interpreted by the fact that proteins can be degraded into amino acids, then converted into urea and creatinine considered as principal markers of renal dysfunction. The enzymatic activity of transaminases (TGO and TGP) was increased in the diabetic control group compared to the normal group. According to Cooper and Kuhara[54], this could be explained by the accumulation of amino acids like alanine and glutamate in serum then transformed under the action of serum transaminases into carboxylic compounds such as α-ketoglutarate and pyruvate. On the other hand, the TGO and TGP values of *M. vulgare* were considered better than glibenclamide.

Marrubium vulgare may have played a crucial role in lowering serum glucose concentration, through a possible influence on glucose absorption and its use by the different tissues. These results therefore confirm the initial hypothesis of Elberry *et al.*[52] who found that *M. vulgare* administration at 500 mg/kg considerably diminishes glycemia after two weeks of treatment. The same researchers have shown that this plant's significantly increased plasmatic insulin and the storage capacity of glycogen in the liver and muscles in rats and suggested that the actual increase of serum insulin concentration in plasma is probably due to the presence of plant bioactive molecules causing the closure of the K^+/ATP channels, which leads to membrane depolarization and will automatically stimulate the influx of Ca^{2+} ions into β-pancreatic cells, a primordial first step for insulin release process[55].

Herrera-Arellano *et al.*[56] investigated the clinical capacity of *M. vulgare* aqueous fraction on glycemia and serum lipid levels in type 2 diabetic patients to find more effective solutions and treatment to poor antidiabetic response related to the conventional treatment drugs. During their research, they found that all treated patients showed blood glucose levels $\geq$140 mg/dL. These patients were treated with *M. vulgare* aqueous extract associated with glibenclamide as a co-drug at different doses. After 21 days of treatment, the fasting glycemia was moderately diminished by 0.64%, likewise, the cholesterol and triglyceride levels decreased by 4.16 and 5.78%. This information is very important and suggests *Marrubium* species are very important at that the pharmacological level.

It is also interesting to note that flavonoids can also significantly improve glucose tolerance, which will considerably limit the sensation of thirst, fatigue, blurred vision and frequent urination, considered as well-known signs of developing diabetes[43]. Flavonoids can also contribute to a decrease in the level of caspase-3, which will considerably prevent a reduction in retina thickness by attenuating neurodegeneration, frequently observed in diabetic retinopathy[57].

CONCLUSION

The methanolic extracts of *M. deserti* and *M. vulgare* possess good antidiabetic activity. The qualitative phytochemical screening of these plants exhibits the existence of phenols, terpenoids and tannins which may be linked to this antidiabetic capacity. However, extensive works on both *in vitro* and *in vivo* approaches are mandatory to justify the pharmacological quality of these active compounds linked to the antidiabetic capacity of the methanolic fraction of *M. deserti* and *M. vulgare*.

SIGNIFICANCE STATEMENT

This preliminary study described for the first time the phytochemical and pharmacological aspects of two endemic *Marrubium* species using various approaches. Our findings revealed that the methanolic fraction of our plants possesses a significant anti-hyperglycemic effect but may also exert a considerable regulation in the levels of hematological and biochemical parameters, especially triglycerides, cholesterol, glutamic-oxaloacetic transaminase (TGO), glutamic pyruvic transaminase (TGP) and creatinine, which suggest that our plant species may be a suitable candidate in the elaboration process of anti-diabetic drugs, to cure diabetes and its hazardous related complications.

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