Evaluation of antidiabetic activity of aqueous extract of *Origanum floribundum* Munby leaves in alloxan induced diabetic rats wistar

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Abstract

The present study aimed to evaluate the phytochemicals and the antidiabetic properties of *Origanum floribundum* aqueous extract. Phytochemical screening was conducted by using various standard procedures. Acute toxicity of the extract was determined by OECD guidelines 423. The antidiabetic activity of the plant was determined by alloxan-induced diabetes in Wistar albino rats. Data obtained showed the detection of various secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids in the extract. No mortality was observed during acute toxicity studies up to the dose of 2000 mg/kg. The aqueous extract presented the ability of reducing blood glucose levels. These findings indicated that *Origanum floribundum* aqueous extract had various phytopharmaceutical activities and thus it would be useful to isolate and characterize the compounds responsible for these bioactivities in the future.

Introduction

Diabetes mellitus is a metabolic disorder characterized by a chronic elevation of blood glucose levels due to disturbances of carbohydrate, fat and protein metabolism. This may result from defects in production, secretion and action of insulin. In long-term, it can cause serious diseases, such as retinopathy, nephropathy, neuropathy, and/or cardio-vascular illnesses. These complications can lead to death. An average of 425 million adults had diabetes worldwide in 2017, and this number is predicted to rise to 629 million by 2045. Interestingly, the WHO also reported that diabetes will be the seventh cause of death in 2030. The incidence and prevalence of diabetes have continued to increase globally, despite a great deal of research with the resulting burden resting more heavily on tropical developing countries. Type 2 diabetes, once considered a disease of industrialized nations, is now becoming increasingly prevalent in Algeria and other emerging countries, ranking as the fourth most prevalent disease. Despite the significant progress made in the treatments of diabetes over the years in modern therapeutics, the use of herbal medicines for the treatment of diabetes mellitus has gained importance worldwide. Besides, the demand to use natural products with antidiabetic activity is becoming increasingly important, because of several disadvantages that have been reported related to the use of the oral hypoglycemic agents including reduction of efficiency, side effects, and even toxicity. This influences researchers to focus towards natural products for less or no side effects and low-cost future drug development strategies. The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control. In Algeria, *Origanum genus* includes two species: *glandulosum* Desf, which is common over northern Algeria and endemic to the Algerian and Tunisian areas and *floribundum* Munby, which is rare, endemic to the north-central part of Algeria. Traditionally, the whole plant is used in the treatment of bronchial pulmonary, digestive and urinary infections and possesses spasmyloytic, antitussive and expectorant properties but to our knowledge, there is no scientific study available that reports the anti-hyperglycemic activity of *Origanum floribundum*. Therefore, the current study was performed with the objective to evaluate the antidiabetic potential of *Origanum floribundum* aqueous extract in rats’ model.
Materials and Methods

Plant collection and identification

The aerial parts of *Origanum floribundum* were collected in May 2019 in the El Hamdania locality, Chrea National Park of Blida region (Altitude of 800 m; Latitude of 36°21'0N; Longitude of 2°450'E) at 50 km south of Algiers, Algeria. Identification of the plant was confirmed by Higher National Agronomic School, Botany Department (Algiers, Algeria). A voucher specimen was deposited at the Giffen Herbarium in the Laboratory of Ethnobotany and Naturel Substances, Department of Natural Science, High Normal School, Vieux Kouba, 16308, Algiers, Algeria for future reference.

Aqueous extraction preparation

In this work, 100 g of *Origanum floribundum* powder leaves were macerated for 24 hours in 1L of distilled water. The macerate has been filtered twice through cotton wool, then through the Whatman filter paper (Number 1). The filtrate was evaporated to dryness using under vacuum in a rotary evaporator at 40°C. After that, it was lyophilized using the lyophylizer for 12 hours and the collected product was preserved in a refrigerator at 4°C for further use.

Experimental animals

Healthy young adult male and female with an average weight of 200±20g Wistar albino rats were obtained from the Pasteur Institute of Algiers. Control and treated animals were housed separately in sterile polypropylene cages under standard environmental conditions (temperature of 22 ± 3°C, relative humidity: 55-65% and 12h light/dark cycle). They were fed with basic food and purified water, with free access to drinking water and standard pelleted diet. All rats were allowed to adapt to the new environment for one week before the study. The work was conducted in accordance with Guide for the Care and Use of Laboratory Animals and approved by the Ethno botany, Department of Natural Sciences, High Normal School of Kouba, Algiers. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

Preliminary phytochemical screening

Phytochemical screening was performed to identify phytochemicals, such as alkaloids, carbohydrates, coumarins, cardiotonic glycosides, steroids and triterpenes, flavonoids, phenols, naphtho- and anthraquinones, saponins, sesquiterpene lactones, tannins, and terpenes in the aqueous extract of *Origanum floribundum* leaves, with the standard procedures as previously described by Harborne,9 Tease and Evans.10 The results were evaluated by visual inspection for change in color or precipitation.

Oral acute toxicity test

The oral acute toxicity study was performed as per the guidelines of Organization for Economic Cooperation and Development (OECD; guideline 423).11 Nulliparous healthy female rats were used for this study. Animals were kept under standard conditions for five days. Limit test was performed at 2000 mg/kg per os as single dose. The rats were divided into 2 groups, with 3 animals in each group. The control group received normal saline at 1 mL/kg by gavage while the treated group received 2000 mg/kg of *Origanum floribundum aqueous* extract. A total of 3 nulliparous and nonpregnant female rats (aged 9-10 weeks) were used, and each rat received a plant extract. Both groups were observed closely for any toxic effect within first 6h and then at regular intervals for a total period of 14 days to note any toxicity signs (changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems) and to record any mortalities.

Antidiabetic activity

Oral glucose tolerance test

Glucose tolerance test was used to determine the rate at which glucose was cleared from the blood after the administration of a massive dose of glucose. The acclimatized animals were fasted for 24 hours with water ad libitum, normal rats were divided into four groups and each group consisted of six animals. Group I served as control, received 5% Tween 80 in distilled water at 5mL/kg b.w.o. Group II received a reference drug, 5mg /kg b.w. of Metformin p.o., whereas other Groups III and IV received the plant extract (200 and 400 mg/kg b.w., respectively). Then, 30 min after the administration of test samples, the rats of all the groups were loaded with Glucose (2g/kg) after half an hour of drug administration. Blood samples were taken via the tip of the tail vein. The blood was collected at times 0, 30, 60, 90 and 120 min. The Accu-check Glucometer was used to determine the level of glucose in blood by Jain et al.,12 and Balamurugan et al.13

Effects of *Origanum floribundum* aqueous extract on blood glucose levels in induced diabetic rats

Induction of experimental diabetes

The albino rats weighing 150-200 g of either sex were allowed to fast for 24 hours prior to experimentation. The animals were weighed and hyperglycemia was induced by a single dose of alloxa monohydrate dissolved in sterile normal saline and administered by a single dose of intraperitoneal injection of 150 mg/kg body weight. Glucose solution (1%, w/v) 10 mL/kg was immediately administered orally to alloxa-treated rats in order to prevent transient hypoglycemia. Fasting serum glucose levels were estimated using Accu-check Glucometer. The rats exhibiting serum glucose more than 300 mg/dl, were considered hyperglycemic and included in the study by Vogel et al.,14 and Nagappa et al.15

Experimental design

The diabetic rats were divided into five groups, each containing six animals:

i) Group I- Normal control rats received 5% Tween 80 in distilled water p.o. at 5 mL/kg b.w; ii) Group II - Diabetic control rats received 5% Tween 80 in distilled water p.o.; iii) Group III - Diabetic received Metformin at the dose of 5mg/kg b.w., p.o.; iv) Group IV - Diabetic rats received *Origanum floribundum aqueous* extract (200mg/kg b.w., p.o.); v) Group V - Diabetic rats received *Origanum floribundum aqueous* extract (400mg/kg b.w., p.o.).

The effects of *Origanum floribundum* aqueous extract in normal and diabetic rats were observed by measuring fasting blood glucose and changes in body weight. The administrations of extracts were continued for 21 days, once daily. Blood samples were collected through the tail vein on days 1, 7, 14 and 21 after drug administration and the blood glucose levels and changes in body weight were estimated.

Statistical analysis

All data were expressed as mean ± Standard Error of Mean (SEM). Data normality was checked using the Shapiro-Wilk test
and variance homogeneity was verified with Levene’s test. Then, data with evidenced normality were analyzed with parametric tests. The statistical significance between homogenous groups was estimated using one-way ANOVA and post hoc Tukey tests post hoc test applied for multiple comparisons of data. The differences were considered significant, by fixing the P value as <0.05. Analysis was performed using SPSS software package Version 21.

Results

Phytochemical Screening

Data of the qualitative phytochemical screening (secondary metabolites) of *Origanum floribundum* aqueous extract are showed in (Table 1). The latter revealed the presence of various bioactive constituents such as polyphenol, tannins, saponins, terpenoids, flavonoids, steroids, coumarins and alkaloids. However, Anthocyanin, antraquinones and Starches were absent in the Leaf extract.

Acute toxicity study

In this study, single oral administration of *Origanum floribundum* aqueous extracts at a dose of 2000 mg/kg as per OECD guideline 423 for 14 days did not produce any mortality in the tested animals. No observable signs of toxicity were detected during the experimental period.

Effect of *Origanum floribundum* aqueous extracts on oral glucose tolerance test

The plasma glucose levels of the normal rats reached a peak at 30 minutes after the oral administration of glucose and gradually decreased to the pre-prandial level (Table 2). The Metformine (5 mg/kg) and *Origanum floribundum* aqueous extracts at the dose of 200 and 400 mg/kg produced significant reduction (p<0.05) in plasma glucose levels compared with those of the controls at 30, 60 and 120 min after glucose load. The significant reduction of the peak levels of blood glucose within 120 min manifested the anti-diabetogenic potential of *Origanum floribundum* extract in the glucose tolerance test models.

Effect of *Origanum floribundum* aqueous extract on blood glucose levels in induced diabetic rats

In this study, daily oral administration of *Origanum floribundum* aqueous extract for 28 days was evaluated for its anti-diabetic activity using alloxan as a diabetic inducing agent. The blood glucose level was increased significantly in untreated alloxan-induced diabetic rats (Table 3) as compared to untreated normal rats (p≤0.05). Indeed, the *aqueous* leaf extract at a dose of 200 and 400 mg/kg exhibited significant anti-hyperglycemic activity on 7th, 14th, 21th and 28th day post treatment and caused reduction in blood glucose levels. The antidiabetic activity was not found to be dose dependent as there was no significant difference between the treated groups. Anti-hyperglycemic effect of aqueous extract was similar to the standard drug, Metformin.

Effect of *Origanum floribundum* aqueous Leaf extracts on body weight in Alloxan- induced diabetic rats

In the present study, the body weight was slightly increased in normal control rats compared to initial body (Table 4). The extract treatment groups at the dose of 200 and 400 mg/kg b.w. showed an improvement (p<0.05) in the body weight in comparison with to initial body. However, body weight of diabetic rats was not restored by treating with metformin.

Discussion

Diabetes Mellitus (DM) is a metabolic syndrome that has become more and more prevalent and rampant throughout the world. Diabetes incorporates various disorders considered by the raised blood glucose levels hyperglycemia.16 Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. Many of the animal models described apparently share similar characteristic features of type 2 diabetes and have allowed experimentation that would be impossible in humans. None of the known single species is exactly equivalent to human diabetes, but each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying aetiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Hence, care must be taken in interpretation and extrapolation of the results obtained from these animal models to humans.17

The phytochemical screening shows the presence of flavonoids, terpenoids, tannins and saponins. The presence of these secondary metabolites suggests potentials for the plant as a source of important phytomedicines. Previous studies have indicated that the species of the genus *Origanum* were well known as medicinal and culinary herbs traditionally used as an antidiabetic agent.4 The presence of flavonoids, terpenoids, tannins and saponins explains why the species of the genus *Origanum* is used for diabetes treatment. Furthermore, the natural constituents could have act separately or synergistically to induce the hypoglycemic effect.19

Acute toxicity evaluation is necessary for the authentication of herbal medications safety and for the determination of the safer dose in order to manage the clinical signs and symptoms of the drugs.20 The limit test method was not intended for determining a precise LD50 value, but it served as a suggestion for classifying the crude extracts based on the expectation at which dose level the animals were expected to survive.21 According to globally harmonized classification system, chemicals are divided into five groups

![Table 1. Phytochemical screening of *Origanum floribundum* leaf extracts.](Image)

| Flavonoids       | ++  |
|------------------|-----|
| Gallic Tannins   | +   |
| Cathelic tannins | +   |
| Saponins         | +   |
| Alkaloids        | ++  |
| Anthocyanes      | -   |
| coumarins        | ++  |
| Starches         | -   |
| Alkaloids        | +   |
| Anthraquinetic derivatives Free Anthraquinones | - |
| Anthraquinones combinées= Combined anthraquinones | +++ |
| O-Heterosides    | ++  |
| C-Heterosides    | -   |
| Sterols and Terpenes | +++ |

+++ Very abundant; ++, Abundant; +, Not abundant; −, Not detected.
on their LD50 basis. *Origanum floribundum* aqueous extract can be put in group 5 (LD50>2000 mg/kg), in lower toxicity class.

The hyperglycemic peak was decreased to near normal indicating a normal glucose metabolism. This property made it potentially useful in human type 2 diabetes with insulin resistance prone to high postprandial glucose surge. This effect may be due to the potentiation of insulin from existing β-cells of the islets of Langerhans. Various plants with similar hypoglycemic activity have been reported by Zanatta.

Alloxan-induced hyperglycaemia was considered as a suitable experimental model to study the hypoglycaemic effect of antidiabetic agents in type 2 diabetes mellitus. In alloxan-induced diabetes mellitus, it may lead to the destruction of the beta cells by free radicals produced by alloxan, which results in an impaired pancreatic function and reduced secretion of insulin and consequently hyperglycaemia.

The *aquaous* leaf extract at a dose of 200 and 400 mg/kg exhibited significant anti-hyperglycemic activity on 7th, 14th, 21th and 28th day post treatment and caused reduction in blood glucose levels. The antidiabetic activity was not found to be dose dependent as there was no significant difference between the treated groups. Anti-hyperglycemic effect of aqueous extract was similar to the standard drug, Metformin. The hypoglycaemic activity of the *Origanum floribundum* extract may, therefore, be due to inhibition of hepatic glucose production and/or stimulation of glucose utilisation by peripheral tissues, especially muscle and adipose tissue. Also, *Origanum floribundum* leaf extract may exert its hypoglycemic effect by other mechanisms such as the regeneration of the β-cells of the pancreas and potentiation of insulin secretion from surviving β-cells, causing an anti-hyperglycemic effect. It may also assume that the hypoglycemic effect of the aqueous extract may be attributed to its constituents, out of these secondary metabolites polyphenols, in particular, flavonoids were suggested as the better therapeutic agents in the management of diabetes mellitus and its chronic complications.

### Table 2. Effect of *Origanum floribundum* aqueous extracts on oral glucose tolerance test in rats.

| Groups       | Dose (mg/kg) | 00 min | Plasma glucose concentration (mg/dl) | 30 min | 60 min | 120 min |
|--------------|--------------|--------|-------------------------------------|--------|--------|---------|
| Group I      | 100.20±4.4a  | 156.30±3.54b | 152.30±3.25b | 137.40±5.06c |
| Group II     | 5            | 97.08±4.8b   | 114.80±4.58b | 107.20±3.45b | 110.25±5.28b |
| Group III    | 200          | 102.07±5.2a  | 127.60±4.53b | 123.20±4.02b | 108.40±5.45c |
| Group IV     | 400          | 98.65±5.4a   | 123.40±4.07b | 118.60±3.54c | 102.20±4.80a |

Values expressed as means±SEM (n=6); *P<0.05; **P<0.01; NS, Not Significant within initial and final body weight each group.

### Table 3. Effect of *Origanum floribundum* aqueous leaves extract on blood glucose levels in Alloxan-induced diabetic rats.

| Groups       | Treatment            | 0 day                  | 7th day                | 14th day               | 21st day               | 28th day               |
|--------------|----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Group I      | Normal control       | 199.33±1.86            | 246.25±10.42           | 0.008**                |
| Group II     | Diabetic control     | 202.65±6.38            | 161.08±4.34            | 0.010**                |
| Group III    | Standard Metformin   | 210.07±3.23            | 218.46±4.34            | 0.071NS                |
| Group IV     | Alloxan + Extract    | 208.44±3.23            | 234.47±2.73            | 0.008**                |
| Group V      | Alloxan + Extract    | 204.25±2.57            | 232.17±3.58            | 0.008*                 |

Values are expressed as Mean±SEM. (n=6); Different superscript letters (a–e) in the same line indicate differences (P<0.05).

### Table 4. Effect of *Origanum floribundum* aqueous leaves extract on total body weight of alloxan-induced diabetic rats.

| Groups       | Treatment            | Initial body weight (g) | Final body weight (g) | P  |
|--------------|----------------------|-------------------------|-----------------------|----|
| Group I      | Normal control       | 199.33±1.86             | 246.25±10.42          | 0.008** |
| Group II     | Diabetic control     | 202.65±6.38             | 161.08±4.34           | 0.010** |
| Group III    | Standard Metformin   | 210.07±3.23             | 218.46±4.34           | 0.071NS |
| Group IV     | Alloxan + Extract    | 208.44±3.23             | 234.47±2.73           | 0.008** |
| Group V      | Alloxan + Extract    | 204.25±2.57             | 232.17±3.58           | 0.008*  |

Values expressed as means±SEM. (n=6); *P<0.05; **P<0.01; NS, Not Significant within initial and final body weight each group.

**Conclusions**

At the end, it can be concluded that the *Origanum floribundum* leaves may contain bioactive constituents with antidiabetic activi-
ty, which can be potentially used for the treatment of diabetes mellitus. The extract possesses an antidiabetic effect that is comparable with that of the standard Metformin drug. Acute toxicity test showed that the plant generally regarded as safe. Further study to substantiate the use of the plant as antidiabetic is recommended.

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