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Research Article

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Preliminary phytochemical screening of *Arbutus unedo* L. and anti-hyperglycemic effect of the root aqueous extract on streptozotocin-induced diabetic Wistar rats

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ABSTRACT

The present study focused on the preliminary phytochemical screening and anti-hyperglycemic effect of the roots of Arbutus unedoL. belonging to family Ericaceae. Preliminary qualitative chemical tests for aqueous, acetone and etheric extracts of the roots revealed the presence of various classes of compounds such as tannins, flavonoids, saponins, amino acids and alkaloids. The effect of aqueous extract was evaluated by using in vivo methods on normal and streptozotocin (STZ) induced-diabetic rats (STZ: 50 mg/kg, iv). Animals were treated orally by the aqueous extractat a dose of 100 mg/kg body weight for four weeks. Glycaemia and body weight were measured at specific intervals.Our results indicated that the aqueous extract produced a significant fall (more than 51%.) in blood glucose in diabetic rats Nevertheless, on normal rats the aqueous extract has no effect and glycaemia is being normal without significant variation. These findings demonstrate that the aqueous extract of Arbutus unedoL. roots have a good anti-hyperglycemic activity in streptozotocin-induced diabetic rats and this effect might be at least due to the presence of active components. Significant results obtained in the estimated parameters confirmed the use of the plant in the traditional medicine.

Keywords: *Arbutus unedo*L., phytochemical, rootextracts, anti-hyperglycemic, streptozotocin

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INTRODUCTION

Diabetes mellitus is characterized by disorder in carbohydrate, protein and fat metabolism caused by the insufficiency of insulin secretion and/or insulin action [1].Chronic hyperglycemia causes secondary complications affecting eyes, kidney, nerves and arteries [2].Control of hyperglycemia involves exercise, diet and current therapeutic agents including sulfonylureas and related compounds, biguanides, thiazolidinediones, α-glucosidase inhibitors and insulin [3].However, the therapeutic agents are either too expensive or have undesirable side effects and contraindications [4]. Plants used in traditional medicines to treat diabetes mellitus represent a valuable alternative for the control of this disease in many countries [3-5-6]. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are prescribed widely [7]. This leads to increasing search for improved antidiabetic drugs, when the World Health Organisation has recommended that this area warrants attention and a novel strategy is in the aim to maximise the possibility of plants medicine [8]. Since ancient time, plants extracts have been used in traditional medicine for the treatment of diabetes mellitus. In many countries, *Arbutus unedo* L. (Ericacea) is known to have medicinal proprieties [9]. For example, the fruits are well known in folk medicine as antiseptic, diuretic, and laxative [10], while the leaves are used as astringent, diuretic, urinary anti-septic, antidiarrheal, depurative and more recently in the therapy of hypertension, diabetes and in the treatment of inflammatory diseases [11]. It has been also reported that *Arbutusunedo* L. leaves are a potential

source of natural compounds with valuable bioactive properties that could be explored by the pharmaceutical industries [12].

The present study was carried to analyze the phytochemical constituents of various root extracts of *Arbutus unedo* L. and to evaluate the glucose-lowering effect of the aqueous extract of the roots in normal and streptozotocin-induced diabetic rats.

EXPERIMENTAL SECTION

Plant material and extraction

Roots of *A. unedo*were collected in March from Mascara (West of Algeria). The plant material was dried in the laboratory at room temperature and powdered in a mixer grinder. The root extracts were obtained as follows. In brief, 5 g of the sample was extracted by refluxing with distilled water, acetone and diethyl ether separately for 30 min. Thereafter, the extracts were decanted, cooled and filtered. The filtrate was used to screen for the presence of phytochemicals.

To study the anti-hyperglycemic effect of *A. unedo*, the filtrate of the aqueous extract was dried in the oven at 50°C to make a powder. The solid residue was dissolved in Tween 80 at 5% (w/v) for subsequent experiments.

Phytochemical screening of the root extracts

The root extracts were tested for the presence of different families of compounds according to methods previously described [13-14].

Toxicity evaluation of the aqueous root

The aqueous extract was tested for its acute toxicity in rats. Various doses of the drug (0.5, 1, and 1.5 g/kg) were administrated orally to different groups of rats (4 rats/group). Control groups received Tween 80 at 5%.

Animals

Male Wistar rats were purchased from the animal house of the faculty of Science, University of Mascara at a weight of 215 to 240 g. The animals were fed with standard laboratory diet and given water *ad libitum*. Prior to the experiment, the animals were subjected to fast for 18 hours with free access to water.

Streptozotocin-induced diabetic rats

Diabetes was induced in fasted rats by tail vein injection of streptozotocin (STZ: 50 mg/kg, *iv*) dissolved in 0.1 M citrate buffer (pH 4.5). Fasted blood glucose level were assessed 2 weeks after STZ injection to confirm the diabetic state. Only rats with a fasting blood glucose level at least 2.0 g/l were used in the experiments [15]. Such experiments were conducted three days after streptozotocin administration.

Antidiabetic effect evaluation

Animals were divided into four groups of five rats:

- Normal control rats received Tween 80 at 5%,
- Normal treated rats by 100 mg/kg of crude aqueous extract
- Diabetic control rats received Tween 80 at 5%,
- Diabetic treated rats by 100 mg/kg crude aqueous extract

Animals were treated for 28 days. Glycaemia and body weight were measured.

Oral glucose tolerance test

At end of the experiment (28 days), an Oral Glucose Tolerance Test (OGTT) was carried out. All groups were fasted overnight (18h) and were loaded with glucose (3 g/kg). Serum glucose levels were measured at 0, 60 and 120 min after glucose loading.

5. Statistical analysis

Results were expressed as mean values ± SEM. Differences between groups were considered to be significant at $P <$ 0,05 using unpaired Student's `t´ test.

RESULTS AND DISCUSSION

Phytochemical screening of the root extracts

The qualitative analysis for the presence of phytochemical constituents of the various extracts (Table 1) showed the presence of phytoconstituents such as tannins, flavonoids, saponins, amino acids and alkaloids. However, the root extracts tested negative for the presence of mucilages and cardiac glycosides classes.

Table 1: Phytochemical tests of root extracts of Arbutus unedo L.

Family of Compounds	Aqueous extract	Acetone extract	Etheric extract
Tanins	+	+	-
Mucilages	-	-	-
Flavonoids	+	+	-
Cardiac Glycosides	-	-	-
Saponins	+	+	-
Aminoacids	+	-	+
Alkaloïds	+	-	+

+ : *Positive test* - : *Negative test*

The preliminary phytochemical investigation revealed the absence of the most phytoconstituentsin the etheric extract followed by the acetone extract while the aqueous extract is rich with phenolic compounds such as tannins, flavonoids and alkaloids. These phytochemical constituents have been reported to be associated with different pharmacological activities of plants.In the other hand, it was noticed [16] that alkaloids, saponins and coumarins were not detected in the water extract prepared by cold maceration.

Toxicity

The rats were followed for 15 days. No mortality was observed with normal behavior.

Antidiabetic effect

Effect of the aqueous extract on glycaemia:

The effect of the aqueous extract on glycaemia in normal and diabetic rats is shown in table 2. We observe a significant ($p < 0.01$) glycaemia-lowering effect in diabetic treated rats at the 28^{th} day. This variation is more than 51%. On normal rats the aqueous extract has no effect. Glycaemia is being normal without significant variation.

Table 2: Effect of the aqueous extract on glycaemia (g/L) during 28 days

	0 day	14 days	28 days
Diabetic control	2.95 ± 0.34	2.84 ± 0.68	2.60±1.10
Diabetic treated	3.00 ± 0.35	2.80 ± 0.27	1.46±0.24 **
Normal control	0.92 ± 0.05	0.83 ± 0.01	1.04±0.06
Normal treated	1.03 ± 0.09	0.87 ± 0.04	1.33±0.21

*** Significant effect between 0 and 28 days.*

Effect of aqueous extract on body weight:

Normal rats have a standard variation of body weight and normal treated rats have a better one. Body weight increased considerably starting from the first weeks. We observe a normal variation (Table 3).

Table 3: Effect of aqueous extract on body weight (g) during 28 days

	0 day	14 days	28 days
Diabetic control	226±24	224±30	268±63
Diabetic treated	218±04	226±17	224±12
Normal control	214±10	245±06	260±06
Normal treated	239±10	259±12	237±08

Table 4: Oral Glucose Tolerance Test

	0 min	60 min	120 min
Diabetic control	2.60±0.24	3.57±0.13	3.08±0.60
Diabetic treated	1.46±0.24	3.90±0.13	3.11±0.60
Normal control	0.97±0.01	1.67±0.19	1.00±0.04
Normal treated	1.33±0.21	1.28±0.06	1.21±0.10

Effect of aqueous extract on the OGTT:
Oral glucose administration of 3g/kg increases glucose level (Table 4). The aqueous extract at 100mg/kg has no effect on glucose tolerance. Hyperglycemia induced in diabetic treated rats cannot be corrected. However, on normal treated rats glycaemia rate increases a little and is rapidly restored.

The treatment of diabetes involves exercise, diet and current therapeutic agents including sulfonylureas and related compounds, biguanides, thiazolidinediones and α-glucosidase inhibitors. For these classes of drugs, the discussion is mainly about their effects on the pancreas (insulin), liver (glucose metabolism) and intestine (absorption of sugars). Two mechanisms summarize this: fasting and post-prandial [17-18]. Currently, several therapeutic strategies have focused on: 1) reducing the excessive production of glucose by liver, 2) increasing insulin secretion stimulated by glucose, 3) improving the sensitivity of cells to insulin [19].

The aqueous extract of *Arbutus unedo* roots is endowed with a notable anti-hyperglycemic activity without toxicity. This positive effect on fasting hyperglycemia could be explained by mechanisms on different levels. Extract improves fasting hyperglycemia resulting from the toxic effects of STZ. This could be the result increasing insulin or insulin-mimetic effects. Biguanides class represented by metformin that allows to normalize excessive glucose in presence of insulin [20]. It inhibits gluconeogenesis [17] and glycogenolysis [18]. We can add the example of thiazolidinediones, a new class of antidiabetic agents that are strong of insulin potentiators. They act on adipocytes where they contribute to three effects: 1) potentiating insulin effect on free fatty acids storage and metabolism, 2) inducing adipocytokines production (Adiponectin, leptin) by increasing cells sensitivity (muscle and liver) to insulin [21], 3) reducing production of factors inducing insulin resistance [19]. Antagonism of glucagon to its receptor is another mechanism leading to inhibit biological effects of this hormone hyperglycemic.

The aqueous extract is rich with compounds that can be active on diabetes such as aminoacids, flavonoids and alkaloids.

CONCLUSION

In the light of the results shown in this study, it was concluded that the aqueous root extract of *Arbutus unedo*is rich in phytochemicals such as tannins, flavonoids, saponins, amino acids and alkaloids. These phytochemicals have been reported to have pharmaceutical potential.

In the other hand, this study proves that aqueous extract of *Arbutus unedo* roots is endowed with a remarkable antidiabetic activity especially on fasting hyperglycemia. Tested extract isn't toxic and is rich in substances potentially causing the effects found. Then, it would be interesting to investigate about the molecule responsible of the active antidiabetic effect and to understand its mechanism. The current work remains a contribution and requires further research with *in vitro* and in vivo test to isolate the active compound and for a better understanding of the action.

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