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Assessment of possible efficacy of aqueous leaves extract of *Psoralea bituminosa* L. for anti-hyperglycaemic activity

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ABSTRACT

Objective: To evaluate for the first time the anti-hyperglycaemic potential of *Psoralea bituminosa* L. leaves in normal and streptozotocin-induced diabetic rats.
Methods: The aqueous extract was screened for its phytochemicals and tested for acute toxicity. Diabetes was induced in male Wistar rats by the administration of streptozotocin (50 mg/kg, *i.v.*). The aqueous extract was administered orally once a day for a period of 21 days. Body weight and blood glucose lowering capacity were determined in different experimental days.
Results: The results of acute toxicity showed that the rats had a good tolerance to high doses of extract (up to 1.5 g/kg) and that no mortality was observed. The extract had shown a good blood glucose lowering effect in the oral glucose tolerance test. After 21 days of daily oral administration of the extract to streptozotocin-induced diabetic rats, the aqueous extract can reduce hyperglycemia by reaching more than 31%.
Conclusions: Aqueous extract possesses a good anti-hyperglycaemic effect and is showing a bright future in the therapy of diabetes mellitus.

1. Introduction

Diabetes mellitus is one of the most common chronic diseases in the world and its prevalence is increasing rapidly. The most recent and highest quality data on diabetes prevalence for 219 countries and territories reported 382 million people with diabetes in 2013, and this number is expected to reach 592 million by 2035[1]. Diabetes mellitus represents a syndrome with disordered metabolism of carbohydrate and fat and has a significant impact on the health and quality of life of patients; hyperglycemia can cause retinopathy, nephropathy, and cardiovascular damage[2]. In the world, diabetes mellitus associated with long-term complications is known to be a major cause of mortality[3]. The pharmacological treatment of diabetes mellitus is managed by oral administration of hypoglycemic drugs such as sulfonylureas and related compounds as biguanides[4]. However, many of the currently available treatment choices mentioned undesirable side effects[5].

Moreover, during the past few years, considerable attention has been focused on the identification of plants with antidiabetic ability that may be used either alone or in combination with other forms of treatments[6]. Some of the new bioactive compounds isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents[7].

Psoralea bituminosa L. (C.H. Stirton, Fabaceae, Psoraleeae; Stirton 1981) (*P. bituminosa*) (syn. *Bituminaria bituminosa* L.), commonly known as the Arabian pea or pitch trefoil, is a perennial herb species widely distributed in the Mediterranean region.

P. bituminosa has been used as animal feed especially for dairy goats[8]. Moreover, the plant is a rich source of secondary metabolites with considerable pharmacologic properties. *P. bituminosa* is known for producing furanocoumarins such as psoralen and angelicin used in the treatment of skin diseases[9,10],

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pterocarpans such as bitucarpin A and B with antitumor activity against colon cancer[11,12].

P. bituminosa has been reported to possess a high cytotoxic efficacy[13]. A recent work showed that *P. bituminosa* extracts exhibited effective antibacterial and *in vitro* antioxidant activities[14].

In the north-west region of Algeria (Tlemcen), some population is found to use leaf decoction of *P. bituminosa* to treat diabetes, but up to now, there are no scientific data available for its antidiabetic effect.

The aim of the present study was to assess for a possible anti-hyperglycaemic activity of aqueous leaves extract of *P. bituminosa* both in normal and streptozotocin (STZ)-induced diabetic rats.

2. Materials and methods

2.1. Plant material collection and authentication

The leaves of *P. bituminosa* were collected in March 2013 from Ouchba-Tlemcen, north-west of Algeria.

The species was authenticated by Dr. Kada Righi at the Department of Biology of Mascara University and a voucher specimen was deposited in the laboratory.

The plant material was shade dried at room temperature and powdered in a mixer grinder.

2.2. Aqueous extract preparation

Aqueous extract was obtained as follows: 10 g of the dried powdered material was extracted by refluxing with 100 mL distilled water for 1 h. It was decanted, filtered and followed by centrifugation for 30 min at 5 000 r/min. To eliminate any residues, the supernatant was filtered and the filtrate was dried at 50 ℃ to make a powder yielding 5.3% (w/w). The solid residue was preserved in desiccator for further use.

For subsequent experiments, the resulting extract was dissolved in 0.9% NaCl solution.

2.3. Phytochemical screening of the aqueous extract

The preliminary phytochemical screening was performed using standard methods previously described[15,16]. The freshly prepared aqueous extract was analysed for the presence of different families of compounds.

2.4. Acute toxicity study

To evaluate acute toxicity, healthy Wistar rats were divided into 4 groups of five each and were orally fed with increasing doses (0.5, 1.0, and 1.5 g/kg) of the aqueous extract. The control group received 0.9% NaCl solution. The animals were observed continuously during the following hours to notice any behavioral changes such as writhing and respiratory dysfunction or mortality and were kept under close observation for a period of 15 days.

2.5. Animals

Male Wistar rats weighing 215-240 g were used for the experiments. Animals were kept in the animal house of the Faculty of Science, University of Mascara under controlled conditions of temperature, humidity and light-dark cycle. Animals were fed with standard laboratory diet and a free access to water was allowed.

Before the experiment, all the animals were acclimatized with standard laboratory conditions and were fasted for 18 h. In addition, careful measures were taken to prevent any stress.

2.6. Induction of diabetes by STZ

Diabetes was induced in fasted rats by single intravenous (*i.v.*) injection of a freshly buffered (0.1 mol/L citrate, pH 4.5) solution of STZ at a dose of 50 mg/kg body weight. Fasted blood glucose level was assessed two weeks after STZ injection to confirm the diabetic state. Only rats with a fasting blood glucose level at least 200 mg/dL were considered diabetic and included in the experiments[17].

2.7. Antidiabetic effect evaluation

Selected rats were divided into four groups comprising five rats each as follows:

Group 1: Normal control rats received 0.9% NaCl solution.

Group 2: Normal treated rats by 200 mg/kg of aqueous extract.

Group 3: Diabetic control rats received 0.9% NaCl solution.

Group 4: Diabetic treated rats by 200 mg/kg of aqueous extract.

Animals were treated with different doses once daily for 21 consecutive days. The rats were fasted overnight then glycaemia and body weight were measured in the morning.

2.8. Oral glucose tolerance test (OGTT)

On the 21st day of multiple dose experiment, an OGTT was estimated. Glucose (3 g/kg) was administered orally to 18-hour fasted overnight rats and blood samples were collected from the retro-orbital plexus at 0, 60 and 120 min after glucose loading. Serum glucose levels were measured by the enzymatic glucose oxidase method[18].

2.9. Statistical analysis

Results were expressed as mean ± SEM. A difference in the mean P value < 0.05 was considered as statistically significant using unpaired student's t-test.

3. Results

3.1. Phytochemical screening of the aqueous extract

Various tests conducted for presence of phytochemicals in aqueous extract indicated the presence of tannins, alkaloids, flavonoids, anthocyans, terpenes and sterols. However, the leaves extracts tested negative for the presence of mucilages, reducing sugar and saponins classes.

3.2. Toxicity

The toxicity study revealed the non-toxic nature of the crude extract. The rats treated with different doses of *P. bituminosa* did not show any changes in behavioral pattern in the following 15 days, and no mortality was observed in rats even at the highest dose tested.

3.3. Antidiabetic effect

3.3.1. Effect of the aqueous extract on glycaemia

The mean blood glucose concentration of controlled and aqueous extract treated animals was shown in Table 1. The results from the repeated daily administration of the aqueous extract up to 21 days exhibited good anti-hyperglycaemic activity in STZ-induced diabetic rats, whereas there was no significant effect observed on normoglycaemic rats. However, at the end of 21 days of treatment, a moderate glycaemia-lowering effect in diabetic treated rats reached more than 31%.

Table 1
Effect of the aqueous extract on glycaemia (mg/dL).

Groups	Day 0	Day 21	Change (%)
Normal control rats	95 ± 14	92 ± 8	-3.16
Diabetic control rats	326 ± 45	321 ± 104	-1.53
Normal treated rats	90 ± 59	89 ± 1	-1.11
Diabetic treated rats	382 ± 58	263 ± 63	-31.15

3.3.2. Effect of aqueous extract on body weight

Body weights of rats in the four groups were monitored during the experimental period. As shown in Table 2, at the end of 21 days treatment, the body weight of normal rats and treated group increased moderately starting from the first week; a normal variation was observed.

Table 2
Effect of aqueous extract on body weight (g).

Groups	Day 0	Day 21
Normal control rats	222.00 ± 14.97	269.00 ± 15.46
Diabetic control rats	256.00 ± 18.61	271.00 ± 39.37
Normal treated rats	223.00 ± 5.46	276.00 ± 11.62
Diabetic treated rats	224.00 ± 24.77	268.00 ± 41.71

3.3.3. Effect of aqueous extract on the OGTT

The effect of *P. bituminosa* aqueous extract on plasma glucose level after glucose loading at 3 g/kg orally to the normal and diabetic rats was expressed in Table 3. The aqueous extract at 200 mg/kg had no effect on glucose tolerance. Hyperglycaemia induced in diabetic treated rats cannot be corrected. However, on normal treated rats, glycaemia rate increased a little.

Table 3
Oral glucose tolerance test (mg/dL).

Groups	0 min	60 min	120 min
Normal control rats	92.0 ± 8.5	115.0 ± 20.0	88.0 ± 8.0
Diabetic control rats	319.0 ± 230.0	307.0 ± 117.0	263.0 ± 86.0
Normal treated rats	89.0 ± 0.9	119.0 ± 14.0	116.0 ± 34.0
Diabetic treated rats	264.0 ± 118.0	280.0 ± 110.0	359.0 ± 169.0

4. Discussion

Diabetes mellitus is a serious chronic disease characterized by high blood glucose levels. The number of the diabetics around the globe is dramatically increasing and the disease has rapidly reached epidemic proportions. Several oral anti-hyperglycaemic agents used have many side effects. However, management of diabetes with safety and less side effects is still a challenge to health care professionals. As an alternative approach, medicinal herbs with anti-hyperglycaemic activities are explored. It has been recommended that traditional medicinal herbs should be further investigated[19].

The current study was carried out to assess for a possible effect of *P. bituminosa* aqueous extract on the blood glucose level in STZ-induced diabetic rats.

Phytochemical analysis of the aqueous extract revealed the presence of tannins, alkaloids, flavonoids, anthocyans, terpenes and sterols in the extract. Diabetes was induced in experimental animals by administration of STZ which causes destruction of pancreatic β-cells accompanying with hyperglycemia. To evaluate the toxicity of the extract, our study is limited to acute toxicity test using several high doses where we can dispense with the determination of the traditional lethal dose (LD_{50})[20].

Acute toxicity was tested up to a single oral dose of 1.5 g/kg and at this dose, the extract did not produce any symptoms of toxicity.

After treatment for 21 days, in the effect of *P. bituminosa* not only the fasting blood glucose level reduced more than 31% but also the glucose tolerance in STZ-induced diabetic rats was improved. As far as the changes in body weight, a gain was observed both in normal and STZ-induced diabetic rats.

Here, the glucose lowering activity of *P. bituminosa* may be attributed to reduce the liver release in fasting animals. Several therapeutic strategies have focused on: a) reducing the excessive production of glucose by liver, b) increasing insulin secretion stimulated by glucose, c) improving the sensitivity of cells to insulin[21].

It has been reported that the antidiabetic activity of medicinal plants is attributed to the presence of their active principles such as polyphenols, flavonoids, terpenoids, coumarins and other constituents which show reduction in blood glucose levels[7,22]. This is the case of the *P. bituminosa* aqueous extract which is rich with compounds that can be active on diabetes mellitus such as tannins, alkaloids, flavonoids, anthocyans, terpenes and sterols.

Hence, experimental results demonstrate that *P. bituminosa* could be beneficial to treat diabetes mellitus owing to its anti-hyperglycaemic activity. Further studies are necessary to investigate about the active compounds and to understand exact mechanism of action involved in anti-hyperglycaemic activity of this plant.

These findings suggest that aqueous extract possesses anti-hyperglycaemic potential and is showing a bright future in the therapy of diabetes mellitus.

Conflict of interest statement

We declare that we have no conflict of interest.

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