Original Article

Antidiabetic effect of *Anacyclus valentinus* L. aqueous extract in normoglycemic and streptozotocin induced-diabetic rats

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ABSTRACT

In Algeria, number of plant species is used to heal diabetes mellitus. The Saharan population of Algeria use *Anacyclus valentinus* L. called "Gartoufa" against several diseases. The objective of this study is to evaluate the effect of the aerial crude aqueous extract (CAE) part of this plant on diabetes induced in Wistar rats by streptozotocin. The extract was prepared macerating the plant powder in water during 24h, filtrating it and then drying it at 50°C.
CAE is rich with tannins, saponins, flavonoids, cardiac glycosides, coumarines, alkaloids, mucilage, amino acids, sugars (46.4%), ash (24.66%) and proteins (11.37%). At 300mg/kg CAE decreased significantly fast hyperglycemia on diabetic treated rats and restored the loss of body weight caused by streptozotocin.
Anacyclus valentinus L. is endowed with a significant antidiabetic activity. Further studies are needed to determine compounds responsible of discovered effects and to understand its mechanism.

Keywords: *Anacyclus valentinus* L.,aqueous extract, phytochemistry, diabetes, streptozotocin

INTRODUCTION

Since ancient time, mankind is aware of soothing and analgesic plants. Nowadays, two-thirds of pharmacopoeia uses their healing properties. Through centuries, human traditions have developed knowledge and use of medicinal plants[1]. Herbal medicine is the art of healing with plants[2]. A medicinal plant is called so when at least one part has curative or preventive properties against diseases with or without active determined ingredients[3]. Active principles of plants are organic: polysaccharides, amino acids[4], flavonoids, saponins, fatty acids, alkaloids[5,6] or

inorganic nature, such as the organic chromium, an insulin effect potentiator[7,8].

Besides chromium, vanadium was used for glycemic control[5]. It is an insulin-mimetic[9] known before discovering of insulin. Other minerals such as magnesium, copper, selenium and iron also have beneficial effects[9, 10].

Several plants are known to have antidiabetic, hypoglycemic, hypolipidemic and antihyperglycemic activity. More than 1,123 plants are traditionally used to treat diabetes mellitus[6].

In Algeria, a large number of plant species are used to heal diabetes mellitus, even though majority of them have not been scientifically evaluated. Fenugreek, ginseng, oregano, lavender and other plants are examples of recognized antidiabetic plants. *Anacyclus valentinus* L. called "Gartoufa" is a herb used by the Saharan population of Algeria against several diseases. The objective of this study was to evaluate the effect of the aerial crude aqueous extract part of this plant on diabetes induced in Wistar rats by streptozotocin.

MATERIALS AND METHODS

Chemicals

The streptozotocin (STZ; S-0130) was obtained from Sigma-Aldrich.

Plant Material & Extraction

Aerial parts of *A. valentinus* (figure 1) were collected in March 2009 from El-Bayadh, South of Algeria. The species was identified by Mr Kada RIGHI, from SNV faculty, University of Mascara and the voucher specimen of the plant has been retained in the Department of Biology.

The plant material was dried at room temperature and powdered in a mixer grinder. One hundred grams of powder dried were macerated in 2L of distilled water for 24 hours at room temperature. After filtration the filtrate was dried at 50°C and the resulting powder (8.1g) was dissolved in Tween 80 at 5% (w/v).

Chemical analysis of the aqueous extract

Presence of different chemical families of compounds was tested in the crud aqueous extract (CAE) according to methods described in Harbone[11] and Bruneton [3]. Analysis of total sugar was carried out according to Dubois[12]. Phenolic compounds was analysed as described in Martin and Larry[13]. Proteins were estimated according to Kjeldahl method of[14]. The amount of mineral elements (Na, K, Ca, Ba and Li) was analyzed through flame spectrophotometer.

Animals

Male Wistar rats weighing 250-275 g were used. Animal's breeding was done at the animal house of the faculty of Sciences, University of Mascara, (Algeria), The animals were fed with standard laboratory diet and water was provided *ad libitum*. Prior to the experiment, animals were subjected to fast for 18 hours with free access to water.

Toxicity evaluation

Acute toxicity of CAE was tested in male Wistar rats. Different mono doses of the drug (0.3, 0.6, 1, 3, and 4g/kg) were administrated orally to different groups of rats (5rats/group). Control groups received Tween 80 at 5 %.

Induction of diabetes

Diabetes was induced in fasted rats injecting 50 mg/kg streptozotocin (STZ) in the tail vein. STZ was dissolved in 0.1 M citrate buffer (pH 4.5). Fasted blood glucose level was assessed 14 days after STZ injection as well as glucosuria to confirm the diabetic state. Only rats with a fasting blood glucose level at least 2.0 g/L and positive urine glucose were used in the experiments[15].

Collection of blood and determination of serum glucose level

Blood samples were drawn by retro-orbital puncture. Serum was separated by blood centrifugation at 3,000g for 15min and the glucose level was measured by the glucose-oxidase method[16].

Antidiabetic effect evaluation

Animals were divided into six groups of five rats:

- Normal control rats received Tween 80 at 5%,
- Normal treated rats by 300mg/kg of CAE,
- Normal treated rats by 0.1mg/kg of glimepiride
- Diabetic control rats received Tween 80 at 5%,
- Diabetic treated rats by 300mg/kg CAE,
- Diabetic treated rats by 0.1 mg/kg of glimepiride.

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 (3g/kg). Serum glucose levels were measured at 0, 60 and 120 min after glucose loading[17].

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

Chemical analysis of the aqueous extract

Chemical tests for the presence of different classes of compounds in aqueous extract confirm the presence of tannins, saponins, flavonoids, cardiac glycosides, coumarines, alkaloids, mucilage and amino acids.

Quantitative analysis of the aqueous extract is resumed in table 1. It demonstrates important contents of total sugar and ash.

Toxicity evaluation

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

Antidiabetic effect

Effect of the aqueous extract on glycaemia

In the light of the results shown in table 2, we observe the effect of aqueous extract on glycaemia in normal and diabetic rats. Treatment has on diabetic treated rats an important blood-glucose lowering effect starting from the first week. Decrease level is of 52% during the second week and 55% at the end of the experiment. This variation is significant on the 21st and the 28th day. In normal treated rats, variation of glycaemia during last week is significant ($p < 0.05$). Results of glimepiride show a smaller and no significant decrease.

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 week. On diabetic rats, treatment with aqueous extract significantly restored loss of body weight caused by streptozotocin.

Effect of aqueous extract on the OGTT

Oral glucose bolus of 3g/kg increases glucose level on diabetic and normal controlled rats. During the second hour, this state is corrected on normal controlled rats. However, on normal treated rats glycaemia rate increases a little and is rapidly restored.

DISCUSSION

Crude aqueous extract is rich with tannins, saponins, flavonoids, cardiac glycosides, coumarines, alkaloids, mucilage and amino acids. All these compounds can be responsible of antidiabetic effects. *Trigonella foenum-graecum* is a hypoglycemic plant, its active composites are trigonellin (Alkaloid)[5,6], mucilage[18] and 4-hydroxyisoleucine (amino acid) [19]. In addition, momordicosides (saponins) isolated from hydroetanolic extract *Momordica charantia* have a significant antidiabetic effect[20].

Crude aqueous extract contains some mineral elements like K, Ca which are involved in insulin secretion [21, 22].

The treatment of diabetes involves exercise, diet and current therapeutic agents including sulfonylureas and related compounds, biguanides, thiazolidenediones and α-glucosidase inhibitors[23]. 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[23, 24]. 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[25].

CAE is endowed with a remarkable antihyperglycemic activity. On normal rats, it improves oral glucose tolerance. In addition, it presents no risk of acute toxicity. These findings express positive effects, essentially on fasting hyperglycemia, what could be explained by mechanisms on different levels. CAE improves fasting hyperglycemia resulting from the toxic effects of STZ. This could be the result increasing insulin or insulin-mimetic effects. Pharmacology indexes antidiabetic drugs acting as insulin effects potentiators. It is known mainly as biguanids class and is represented by metformin[26]. Metformin allows to normalize excessive glucose in presence of insulin. It inhibits gluconeogenesis[24] and glycogenolysis[23]. To support this hypothesis, we can add the example of thiazolidinediones, a new class of antidiabetic agents. These active ingredients are strong insulin potentiators. Binding PPAR (Peroxisome proliferator-activated receptors), 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[27], 3) reducing production of factors inducing insulin resistance such as TNF (Tumor necrosis factor alpha) and resistin[25].

Another mechanism is the antagonism hyperglycemic. of glucagon to its receptor leading to inhibit biological effects of this hormone hyperglycemic.

CONCLUSION

We can conclude that aqueous extract of *Anacyclus valentinus* L.is endowed with a remarkable antidiabetic activity especially on fasting hyperglycemia. Studied extract isn't toxic and is rich in substances potentially causing the effects found. It would therefore be interesting to investigate about the molecule responsible of the active antidiabetic effect and to understand its mechanism.

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Table 1. Quantitative analysis of the aqueous extract

	mg/100mg of extract
Total sugar	46.40±0.04
Phenolic compounds	2.08±0.03
Proteins	11.37±1.03
Total ash	24.66±0.01
Na	0.633±0.016
K	4.930±0.045
Ca	1.884±0.037
Ba	5.136±0.067
Li	0.024±0.004

Extract is rich in ash especially K, Ca and Ba.

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

	0 Day	7 Day	14 Day	21 Day	28 Day
Normal control	1.19±0.05	1.2±0.04	1.13±0.08	0.97±0.02	1.17±0.11
Normal treated by 300mg/kg CAE	1±0.20	1.23±0.20	1.10±0.15	0.96±0.04	0.79±0.03
Normal treated by glimepiride 0.1mg/kg	0.93±0.07	1.10±0.02	0.82±0.06	0.74±0.08	1.14±0.05
Diabetic control	2.29±0.36	2.83±0.365	2.61±0.37	3.60±0.41	2.90±0.41
Diabetic treated 300mg/kg CAE	3.14±0.98	2.38±0.68	1.64±0.34	1.52±0.51	1.41±0.39*
Diabetic treated by glimepiride 0.1mg/kg	2.08±0.31	2.93±0.30	1.55±0.52	3.12±0.39	2.42±0.47

Each value is the mean ± standard deviation (N=5), * is the significantly different by the t-test ($p<0,05$) between diabetic treated 300mg/kg CAE and diabetic control

Table 3. Effect of Effect of the aqueous extract on glycaemia (g/L) during 28 days

	0 Day	7 Day	14 Day	21 Day	28 Day
Normal control	260±20	285±14	294±16	312±18	306±21
Normal treated by 300mg/kg CAE	280±8	296±11	311±9	315±11	292±14
Normal treated by glimepiride 0.1mg/kg	287±9	299±9	302±17	300±23	297±28
Diabetic control	270±28	255±27	277±28	273±36	230±72
Diabetic treated 300mg/kg CAE	267±23	274±33	279±33	278±7*	245±43
Diabetic treated by glimepiride 0.1mg/kg	218±7	244±7	240±7	217±10	199±26

Each value is the mean ± standard deviation (N=5), * is the significantly different by the t-test ($p<0,05$) between diabetic treated 300mg/kg CAE and diabetic treated by glimepiride 0,1mg/kg

Table 4. Effect of Effect of the aqueous extract on glycaemia (g/L) during 28 days

	0 min	60 min	120 min
Normal control	0.97±0.01	1.67±0.19	1±0.041
Normal treated by 300mg/kg CAE	0.96±0.04	1.28±0.07	1.02±0.09
Normal treated by glimepiride 0.1mg/kg	0.75±0.08	1.35±0.05	0.86±0.05
Diabetic control	3.60±0.41	4.26±0.23	3.97±0.51
Diabetic treated 300mg/kg CAE	1.52±0.52	3.45±1.26	3.30±1.18
Diabetic treated by glimepiride 0.1mg/kg	3.12±0.39	4.64±0.31	3.81±0.25

Each value is the mean ± standard deviation (N=5)

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Figure.1. *Anacyclus valentinus* L. Photo taken from the area of bayadh (Algeria) in March 2012.