African Journal of Biotechnology Vol. 8 (20), pp. 5589-5594, 19 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Hypoglycemic and antihyperglycemic effects of *Anabasis articulata* (Forssk) Moq (Chenopodiaceae), an Algerian medicinal plant

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Accepted 2 May, 2008

Anabasis articulata **leaves decoction is widely used by Algerian traditional medicine practitioners as a remedy for the treatment of diabetes. The aqueous extract was found to be non-toxic at 1000 mg/kg, as no deaths or hazardous signs were recorded during treatment or the observation period (24 and 72 h) in either control or treated groups of mice. Experiments were performed in non-diabetic mice, and in hyperglycemic mice (glucose treated and alloxan treated mice) to confirm the antidiabetic potential of *A. articulata*. Our results showed that the orally administration at a dose of 400 mg/kg decreased the glycaemia by 29.89% after 6 h ($p < 0.05$), corresponding to the greatest decrease of blood glucose in normoglycaemic mice. This dose also lowered blood glucose concentrations in diabetic mice revealing antihyperglycemic effect of *A. articulata* leaves. The class of phytochemical responsible for antidiabetic effects in aqueous leaf extract was also investigated. Phytochemical screening showed that the aqueous extract contains alkaloids (1.25%) and saponin (1.30%). Our findings showed that saponin (5 mg/Kg) was the active fraction, since it restores the normal blood glucose levels after 21 days of treatment. The alkaloid fraction did not significantly reduce the blood glucose level. The present study confirms the antidiabetic proprieties of *A. articulata* leaves previously reported by Algerian healers.**

Key word: *Anabasis articulata*, antihyperglycemic; diabetic mice; antidiabetic effect; saponin, alkaloids.

INTRODUCTION

Diabetes is a serious complex chronic condition that is a major source of ill health worldwide. The number of people in the world with diabetes has increased dramatically in recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world (Carter, 2004). In some parts of the world, before the advent of insulin injections and other pharmaceutical preparations, healers relied heavily upon medicinal plants and herbs to treat diabetes. In this context, more than 1200 plants have been described to be experimentally or ethnopharmacologically used in the treatment of diabetes (Aida et al., 1990; Hur, 1999; Alarcon-Aguilara et al., 1998; Sepici et al., 2004; Kang et al., 2005). Currently, a few of these medicinal plants have received scientific or medical scrutiny, despite the fact that the World Health Organization (WHO, 2006) has encouraged and recommended that traditional treatment for diabetes warrant further evaluation.

Algeria has a rich heritage of medicinal plants of wide diversity which are prescribed by the traditional healers to treat several diseases including diabetes and cardiovascular diseases (Bellakhdar et al., 1991; Ziyyat et al., 1997; Eddouks et al., 2002). *Anabasis articulata* locally named as 'ajrem' is a wild plant widely used in Algerian

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traditional medicine to treat diabetes, fever, headache and skin diseases such as eczema (Hmamouchi, 1999; Hammiche et al., 2006). Its is taken orally after decoction in water as a single herb or with other medicinal plants. No scientific investigations concerning the pharmacological properties of *A. articulata* has been done.

The phytochemical constituents of *A. articulata* revealed the presence of saponin. Among them triterpenoid saponin glycosides have been isolated and identified (Sandberg and Shalaby, 1960; Sandberg and Michel, 1962; Segal et al., 1969). Literature data indicates that some saponin isolated from medicinal plants significantly reduces blood glucose levels (Gina et al., 1989; Cherian et al., 1992; Nakashima et al., 1993; Petit et al., 1995; Zarzuelo et al., 1996; Nojima et al., 1998; Abdel-Hassan et al., 2000; Abdel-Zaher et al., 2005). Several biological activities have been attributed to saponin such as their immuno-stimulant effect (Estrada et al., 2000), cytotoxicity (Zou et al., 2000; Heisler et al., 2005) and antitumoral properties (Zheng et al., 2006).

The present study was conducted to confirm the anti-diabetic effect of *A. articulata* leaves previously reported by Algerian healers.

MATERIALS AND METHODS

Plant material

The leaves of *A. articulata* are collected from local inhabitants having knowledge of the curative properties of this plant in September 2006. The plant materials were identified and authenticated with assistance of Prof. Hadjadj-Aouls, M.S (Botanic Department, Oran, Algeria). Voucher specimen of this plant was deposited in the Agricultural Institute (INA) in Algeries.

Chemical reagents

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka (Buchs, Switzerland) and Merck (Germany).

Preparation of aqueous extracts

Plant material was prepared according to the traditional method used in Algeria (decoction): 100 g of powdered aerial parts mixed with 2 L distilled water were boiled for 10 min and then cooled for 15 min and filtered. The filtrate was then freeze-dried to give 17.50 g of a brown extract powder and the desired dose (mg of lyophilized aqueous extract per kg body weight) was then prepared and reconstituted in 10 ml of distilled water.

The active aqueous fraction was subjected to chemical analysis to determine the class of compounds present in it (Trease and Evans, 1983). This fraction was tested for the presence of alkaloids (Dragendorff reagent and Mayer's reagent), steroids (Liebermann–Buchard test), and saponin.

Preparation of the crude alkaloid fraction

The alkaloid extract was obtained by an acid/basic modified extraction as described by Ott-Longoni et al. (1980), with minor modifications (Hughes et al., 2005). *A. articulata* leaves were dried and the air-dried plant material (500 g) was extracted three times with hexane (2 L/kg) for 48 h at room temperature with occasional shaking to eliminate apolar constituents. The extract was then filtered and the residue was flooded with methanol (1.5 L/kg) using the above process. The methanol extract was then concentrated under reduced pressure and acidified with 0.5 M H_2SO_4. The acidic extract was washed with chloroform to remove neutral components. The aqueous acidic fraction was then made basic with ammonia (pH 10) and extracted again with chloroform until the aqueous layer was free of alkaloids. The combined chloroform extracts were evaporated *in vacuo* to yield the crude alkaloid fraction as a brown residue (1.25% w/w of the dry starting material). This extract was developed by chromatography in a thin layer silica gel chromatography using chloroform/methanol (8:2) as a solvent system. Later, plates were air-dried, observed under UV light, sprayed with Dragendorf's reagents and heated at 100°C for 5 min (Wagner and Bladt, 1996).

Extraction of saponin

Saponins, contained in the leaves of *A. articulata* (100 g) were extracted with methanol in Soxhlet apparatus (Estrada et al., 2000). The extract was concentrated, freeze-dried and re-extracted with water and butanol saturated with water. The dried crystalline butanolic extract (1.3 g) was obtained. Thin layer chromatographic analysis was performed with the method of Wagner and Bladt (1996). The developing solvent was n-butanol : acetic acid : water (40:10:50). The butanolic crystalline extract was dissolved in the developing solvent and applied to aluminum-backed plates coated with silica gel 60 F_{254} (layer thickness 0.2 mm, 20x20 cm; E. Merck, Darmstadt, Germany). After development, plates were air-dried, observed under UV light, sprayed with methanol : acetic acid : sulphuric acid : anisaldehyde (85:10:5:0.1) and heated at 100°C for 5 min. On heating, saponin bands turned red and were readily visualized.

Animals

9 weeks old Swiss albino mice (10) ranging in weight from 40 – 45 g were used. The animals were housed with a 12 h light : 12 h dark schedule with free access to water and food (SDS Dietex-France) and maintained at a constant temperature (21 ± 2°C) and a relative humidity of 55 ± 10%. All animal procedures were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

Toxicity evaluation in mice

To study sub-acute (short term) toxicity, four groups of mice were used and were orally fed with the aqueous extract of *A. articulata* in increasing dose levels of 100, 500, 1000 mg/kg body weight. The animals were observed after 30 min and 2 h. The following profiles were observed (Turner, 1965). Behavioural profile: alertness, restlessness, irritability, and fearfulness. Neurological profile: spontaneous activities, reactivity, touch response, pain response and gait. Autonomic profile: defecation and urination.

Normal mice

Ability of the extract to lower blood glucose, in the normal or the hyperglycemic animals, reflects its hypoglycaemic effect. The animals were fasted for 6 h prior to the collection of blood for glucose estimation, though water was allowed *ad libitum* during this period. Ten minutes after collection of the fasting blood sample, the

aqueous extract containing, saponin and alkaloids were administered orally in the different doses (100 - 500 mg/Kg). The percentage change in the plant induced glycemia was calculated as a time function by applying the formula below:

$[(G_i - G_0)/G_0] \times 100$ [Dinesh, 2001]

G_0 is the initial glycemia and Gi is glycemia at the 'i' h (I = 1, 2 or 6 h). This parameter was used as an index of the hypoglycemic activity.

Dose-response was studied with reference to hypoglycemia by single oral administration of different doses (100 - 500 mg/Kg) of the aqueous extracts, saponin (5 mg/Kg) and alkaloids (5 mg/Kg). Blood samples for glucose estimation were collected after 1, 2 and 6 h intervals. The above doses were used for screening the hypoglycaemic action of the leaf extract in the normal (normoglycemic) mice. The control mice (negative control) were orally administered with vehicle (distilled water). All blood samples were collected from the caudal incision.

Glucose induced antihyperglycemic mice

Mice fasted for 6 h were given glucose (10% solution) at a dose of 3 g/kg. The extracts (aqueous extract: 400 mg/Kg, saponin: 5 mg/Kg and alkaloids: 5 mg/Kg) were given to the fasted animals, and 30 min after treatment the above glucose load was given. Blood samples were obtained for the glucose estimation at the same time intervals 1, 2 and 6 h after the glucose loading. The mean glucose tolerance test (GTT), so obtained after the treatment, was compared with the pre-treatment. The activity was assessed quantitatively by the improvement in the GTT response. The percentage change in the plant induced glycemia was calculated by the preceding formula (above).

Induction of alloxan diabetes

The mice were kept in a fasting state for at least 6 h and then rendered diabetic by injecting alloxan intraperitoneally (200 mg/kg). Seven days after the administration, the animals with fasting plasma glucose of 200 mg/dl, or more, were classified as diabetic and were included in the study.

Animals were divided into seven groups of six mice each. The extract was administered for 21 days. Group I: normal control mice administered water daily; Group II: diabetic control mice no treated; Group III: diabetic mice administered aqueous extract (500 mg/kg); Group IV: diabetic mice administered aqueous extract (400 mg/kg); Group V: diabetic mice administered saponin extract (5 mg/kg); VI: diabetic mice administered alkaloids extract (5 mg/kg); Group VII: diabetic mice administered reference drug glibenclamide (10 mg/kg). The effects of administration of leaves aqueous extract to normal and diabetic mice were determined by measuring fasting plasma glucose levels (Nicholas, 1956) and initial and final changes in body weight. Day 7 of induction was designated as day 1 for extract administration in diabetic mice. Fasting plasma glucose was estimated on days 7, 14, and 21 of extract administration and the percent glycemic changes were calculated.

Data analysis

All the data reported were expressed as mean ± S.E.M. Statistical analyses were performed using Wilcoxon test. The values were considered to be significantly different when the p value was less than 0.05 compared to the respective control. Statistical analyses were carried out using the Statview® 4.5 statistical package (Abacus Concepts, Inc). (Borenstein et al., 1997).

RESULTS

Preliminary chemical screening

The qualitative chemical analysis of the aqueous extract of *A. articulata* showed that preliminary alkaloid tests were positive for both tertiary and quaternary alkaloids according to Mayer's and Dragendorff's reagents. Moreover, the screening for saponin component showed positive results with $FeCl_2$ and $HgCl_2$.

The quantitative chemical analysis exhibited the presence of alkaloids and saponin with percentage 1.25 and 1.30%, respectively. After development of butanol extract in plates of silica gel and observed under UV light, four saponin glycosides were detected; saponin bands turned red and were readily visualized.

Toxicity evaluation in mice

No deaths or hazardous signs were recorded during treatment or the observation period (24 and 72 h) in either control or treated groups of mice with aqueous extract (100, 500 and 1000 mg/kg).

Normal mice

As shown in Table 1 a dose-response study was carried out. Indeed, different doses of the leaf extract were orally administered and falls in plasma glucose levels were observed. Administration of the extract at a dose of 100 mg/kg brought about fall in fasting plasma glucose from 95.6 ± 4.03 to 83 ± 4.71. (12% fall) at 6 h. A dose of 200 mg/kg improved the response by producing a more significant fall of 15.8% at 6 h. Thus, of the four doses tried, a dose of 400 mg/kg caused the greatest decrease in the blood glucose (29.87% fall at 6 h). However, further increase in the dose (500 mg/kg) did not improve the hypoglycemic response (17.66% fall at 6 h).

Glucose induced hyperglycemic mice

The mean blood glucose levels of fasted hyperglycaemic animals at 2, 4 and 6 h after oral administration of aqueous extract, alkaloids and saponin fraction are shown in Table 2.

In the glucose-induced diabetic mice, treatment with a dose of the 400 mg/kg of the extract to animals caused a fall of fasting plasma glucose (118.25 ± 0.85 mg/dl): the post-treatment values at 2, 4 and 6 h were 139.25 ± 6.11, 105.75 ± 5.64, and 105.00 ± 5.4 mg/dl, respectively. The percent of glycemic falls relative to the fasting plasma glucose were 10.55 and 11.19% at 4 and 6 h, respectively, which are significant ($p < 0.05$) and indicate a noteworthy hypoglycemic response at 6 h (Table 2). No such antihyperglycemic effect was observed in crude

Table 1. Dose-response effect of the leaves of *A. articulate* on plasma glucose values in the normoglycemic mice at 6 h.

Treatment	Pre-treated	Post-treated	Percentage
Control	76.8 ± 1.15	76.4 ± 3.58	-5.24
100 mg/Kg	95.60 ± 4.03	83.0 ± 4.71	-12
200 mg/Kg	85.0 ± 0.44	71.4 ± 3.5 *	-15.96
400 mg/Kg	91.20 ± 0.58	64.00 ± 3.43 **	-29.87
500 mg/Kg	83.4 ± 1.5	68.4 ± 4.61 *	-17.66

Plasma glucose values are given as means ± SE in mg/dl. The negative value (–) indicates a decrease in glycemia.
*P < 0.05; **P < 0.01.

Table 2. Mean plasma glucose values before and after admistration of the *A. articulata* leaves, saponin and alkaloids in the oral glucose tolerance in mice.

Treatment	Fasting	2 h	Change (%)	4 h	Change (%)	6 h	Change (%)
400 mg/kg	118.25 ± 0.85	139.25 ± 6.11	+17.72	105.75 ± 5.64 *	-10.55	105.00 ± 5.4 *	-11.19
Saponin (5 mg/kg)	110.8 ± 1.02	167.4 ± 7.34	+51.19	93.6 ± 1.93 *	-15.53	90.4 ± 3.14 * *	-18.44
Alkaloids (5 mg/kg)	100.2 ± 1.85	127.6 ± 8.91	+27.44	107.8 ± 4.39 *	+7.65	101.0 ± 5.27 *	+0.61
Control	85.4 ± 2.76	161 ± 23.6	+89.98	114.2 ± 3.8	+34.69	104.8 ± 1.96	+23.04

Values are means ± SE; N = 7. *P < 0.05; **P < 0.01. The negative value (–) indicates a decrease in glycemia.

alkaloids and the control group of mice after 4 h. The alkaloid extract did not significantly lower the fasting of glucose levels from 100.2 mg/dl at 0 h to 101 mg/ dl ml after 6 h.

The effects were more pronounced with saponin extract, which significantly ($P < 0.05$) lowered the fasting glucose levels after 4h, highly significant ($P < 0.01$) after 6 h. The percent of glycemic falls relative to the fasting plasma glucose were 15.53 and 18.44%, respectively at 4 and 6 h.

Induction of alloxan diabetes

As shown in Table 3, in the alloxan-induced diabetic mice, glibenglamide at 10 mg/kg i.p decreased the levels of hyperglycemia in 44.06 and 71.99%, after 15 and 21 days, respectively, of diabetes treatment. The aqueous extract at the dose of 400 mg/kg was found to reduce the increase of blood glucose levels in 45.23 and 74.48 % at 15 and 21 days, respectively. The saponin fraction at a dose of 5 mg/kg showed a maximum decrease (76.00%) in the blood glucose level in the diabetic mice after D+ 21 treatments. However, the treatment with the crude extract of alkaloids at same concentration of saponin (5 mg/kg) did not show any decrease in glycemia on the blood glucose level. After the treatment, the percentage change was increased in D+21 (4.95%). This variation indicates the absence of significant antihyperglycemic response in the alloxan diabetic mice.

DISCUSSION

A. articulata extract was tested at several doses in normoglycemic mice, and its dose-response showed that the maximum hypoglycaemic effect was found with the dose of 400 mg/kg (Table 1). Besides the hypoglycaemic effect, our results indicate that aqueous extract of *A. articulata* reduces the hyperglycemia level and improves glucose tolerance in both glucose diabetic mice and alloxan diabetic mice (severe diabetic mice) revealing the antihyperglycemic effect of this medicinal plant. Indeed, 400 mg/kg of extract resulted to the reduction of hyperglycemia levels in glucose induced hyperglycemic mice after 4 h of treatment (Table 2). The daily treatment with this dose for 21 days brought also fasting plasma glucose to near normal range in severe diabetic animals (Table 3). Moreover, the saponin fraction (5 mg/kg) displayed antihyperglycemic effect in oral glucose tolerance test, and exhibited the almost same effect as the well-known antidiabetic glibenclamide (10 mg/kg) in alloxan diabetic mice. However, the alkaloid extract did not significantly lower the blood glucose levels compared with control after treatment in diabetic mice.

The results obtained clearly show that the aqueous extract of *A. articulata* leaves possess an antidiabetic effect. Phytochemical analysis showed that the major chemical constituents of the extract were alkaloids and saponin. In the light of our findings, the antidiabetic activity of *A. articulata* could be attributed to saponin components since alkaloids did not present any effect on hyperglycemia levels, whereas aqueous extract and saponin had the nearly similar effects in diabetic mice.

The antihyperglycemic activity of *A. articulata* is through the release of insulin from the pancreas that is, it exerts a direct insulinotropic effect, or it could also be due to the insulin like effect of the active principle (saponin)

Table 3. The effect of 3-week treatment with various doses of aqueous extract of *A. articulata,* saponin and *Alkaloids extract* on glucose levels in alloxan diabetic mice.

Treatment	D-7	D_0	D+7	Percent change	D+14	Percent change	D+21	Percent change
Control	82.5 ± 4.97	82.66 ± 4.57	81.83 ± 5.02	1.16 ± 0.98	82.16 ± 4.95	0.63 ± 0.57	82.00 ± 4.97	0.86 ± 1.64
Diabetic N.T.	91.00 ± 6.36	281.66 ± 4.51	309.88 ± 4.66	+10.09 ± 2.02	383.00 ± 5.36	+36.09 ± 2.28	389.8 ± 5.43	+38.55 ± 2.73
500 mg/Kg	85.14 ± 3.48	245.85 ± 6.81	286.85 ± 5.87*	+17.24 ± 4.30	258.14 ± 12.89*	+5.07 ± 4.49	199.57 ± 2.59*	-18.40 ± 2.74
400 mg/Kg	94.50 ± 3.09	367.16 ± 4.04	338.50 ± 3.70*	-7.63 ± 2.23	200.83 ± 1.60*	-45.23 ± 0.91	93.50 ± 6.60*	-74.48 ± 1.88
Saponin	86.83 ± 4.86	365.66 ± 2.47	335.16 ± 2.04*	-8.31 ± 0.99	195.33 ± 2.52*	-46.58 ± 0.56	87.66 ± 4.44*	-76.00 ± 1.28
Alkaloids	85.0 ± 3.88	254.5 ± 4.21	306.28 ± 2.81*	+20.52 ± 2.34	295.42 ± 13.45*	+16.03 ± 4.79	266.57 ±6.22	+4.95 ± 3.32
Glibenclamide	101 ± 3.88	353.16 ± 8.95	331.16 ± 8.32*	-6.17 ± 1.48	196.83 ± 1.90*	-44.06 ± 1.66	98.16 ± *4.52**	-71.99 ± 1.81

The values are expressed as means ± S.E.M, n = 6 mice per group.
Percentage change indicates the percentage lowering of plasma sugar in comparison to the reading at J0.
The negative value (–) indicates a decrease in glycemia of control diabetic animal treated with water.
*P < 0.05, when compared to the baseline values.
N.T. = Diabetic not treated.

present in the extract. Our results confirm the antidiabetic proprieties of *A. articulata* leaves previously reported by Algerian healers. However, chemical and pharmacological investigations are necessary to isolate and elucidate the structure of the saponin and to confirm its mechanism of action and antidiabetic potential.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry for the higher education and scientific research (MESRS) and National Health Research Agency, Oran, Algeria (ANDRS) for their financial support.

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