Evaluation of the Anti-hyperglycemic Activity of Three Medicinal Plants Extracts in Agadir Region (South of Morocco)

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Abstract: Our study was carried out to evaluate the hypoglycaemic and anti-diabetic activity of organic extracts of three plants: \textit{Argania spinosa} (leaves), \textit{Ceratonia siliqua} (aerial part) and \textit{Pistacia atlantica} (leaves), traditionally used in herbal medicine in Agadir region. Oral administration of extracts was tested at 50, 150 and 250 mg/kg and the anti-diabetic effect was studied by inducing diabetes by pancreatectomy and by alloxan injected at a dose of 180 mg/kg. Ethanol extract of \textit{Argania spinosa} induced a significant transient hypoglycemia with a maximum reduction of 12\% for the concentration 250 mg/kg compared to the control. Analyses of the hypoglycemic activity show a very highly significant effect for extracts of \textit{Argania spinosa} and \textit{Ceratonia siliqua} at the two concentrations tested (250 and 150 mg/kg). On the other hand, the extract of \textit{Pistachia atlantica} did not show any remarkable results. In the same sense, the results of the anti-hyperglycemic activity showed a remarkable activity for the extract of \textit{Argania spinosa} and \textit{Ceratonia siliqua} and a slight activity for \textit{Pistachia atlantica}.

Key words: Biological activity, organic extract, bioactive substances, antihyperglycemic, hypoglycemic.

1. Introduction

Diabetes is one of the most serious diseases that affect public health. It is considered to be the fourth leading factor in mortality worldwide followed by cardiovascular diseases, cancers and chronic respiratory diseases. As the diabetes care is very expensive for the majority of patients in developing countries, particularly on the African continent, traditional medicine remains the most requested means to relieve or even cure this disease [1]. Scientists around the world have largely focused on finding new therapeutic molecules of natural origin. As the diabetes care is very expensive for the majority of patients in developing countries, particularly on the African continent, traditional medicine remains the most requested means to relieve or even cure this disease [1]. Scientists around the world have largely focused on finding new therapeutic molecules of natural origin. This trend is explained by the urgent need to test new treatments to overcome the side effects, sometimes serious, of synthetic drugs [2].

In Morocco, the study of medicinal plants has experienced a significant boom in recent years through ethnopharmacological surveys which have covered almost the whole country. The Moroccan cultural wealth, through its history and its abundant biodiversity linked to its geographical location and its Mediterranean climate, has allowed it to have a marked traditional knowledge in herbal medicine for chronic diseases, including diabetes [1]. The search for new candidate molecules from medicinal plants constitutes a substantial step in the development of new drugs.

Several works have been carried out on the biological activities of different extracts of leaves of these plants using almost the same protocol and the same experimental conditions although they are carried out in different regions in the world and in our country [3-9]. These studies have proven the hypoglycemic, antihyperglycemic and antioxidant
effect of these extracts. On the other hand, other authors have not found remarkable results on this subject [10, 11].

The aim of our study is to evaluate the hypoglycemic and antihyperglycemic activities of leaves extracts from three medicinal plants in Agadir region: *Argania spinosa*, *Ceratonia siliqua* and *Pistachia atlantica*.

2. Materials and Methods

2.1 Plant Material

The leaves of *Pistacia atlantica* used in our experiment were collected in April 2018 in Amskroud (north of Agadir) and those of *Argania spinosa* and *Ceratonia siliqua* in the Science Faculty’s Botanical Garden. Their authentication was made PrMsanda Fouad from Laboratory of Biotechnology and Valorization of Natural Resources (Faculty of Sciences, Ibn Zohr University, Agadir, Morocco).

2.2 Preparation of the Extract

After picking, the leaves were washed with tap water and dried in the oven at 40 °C for 24 h. Then, they were ground into a fine powder using an electric grinder and stored carefully in airtight plastic boxes until their use.

Five grams (5 g) of fine powder from the leaves of each plant were introduced into 100 mL of a water-ethanol mixture (20-80, V/V). The whole was stirred for 15 min by a magnetic stirrer and then it was put in the sonicator (ultrasonic bath) to accelerate the dissolution of the extract. This latter was then centrifuged at 3,500 rpm for 15 min. The supernatant obtained was collected and 100 mL water-ethanol mixture was added to the pellet, repeating the same steps to have a good extraction. The second supernatant was evaporated under reduced pressure using a rotary evaporator at 40 °C. The powder obtained was kept cool at 4 °C until use.

2.3 Animals

Female Wistar rats (200-260 g) housed in standard environmental conditions of temperature (22 °C), relative humidity (40-60%) and dark/light cycle (12/12) were used. They were fed with standard diet (cereals, fish meal, oilseed meal, minerals and vitamin supplements (Vit. A, D3 and E)) and water ad libitum. Pregnant, lactating, or unhealthy females were excluded from the study.

Chronically hyperglycaemic rats were obtained by intraperitoneal injection of 180 mg/kg body of alloxan (99%) dissolved in distilled water. Diabetes was detected after 72 h. Rats with a blood glucose level greater than or equal to 2 g/L are considered diabetic and are therefore included in our tests.

The diabetic controls in our study were obtained by partial pancreatectomy performed on rats. This is carried out in three stages: splenic, pyloric and duodenal according to the technique described by Foglia in 1944. Diabetes does not appear until one to two months after the procedure.

Untreated controls, diabetic rats received a daily dose of the organic leaf extract of *Argania spinosa*, *Pistacia atlantica* and *Ceratonia siliqua* for a period of 15 days.

2.4 Experimental Protocol

The rats were fasted for 14 h and then blood samples are taken at time T-90 min before the oral administration of glucose (4 g/L). At time T-30 min, they were force-fed with a small syringe with an esophageal probe with saline solution (10 mL/kg) or with one of the extracts at different concentrations: 50, 150 and 250 mg/kg. At time T-0 min, the rats were force-fed with a glucose solution at a dose of 4 g/kg to induce a state of temporary hyperglycemia. Tail blood was taken every 30 min for 180 min. Blood samples (0.2 mL) were immediately taken into Eppendorf tube containing 6% EDTA and centrifuged at 4 °C at 3,000 rpm for 5 min. The plasma is collected and stored at 0 °C until determination of blood sugar.

2.5 Plasma Glucose Analysis

The plasma glucose measurement is carried out
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colorimetrically using spectrophotometer (spectrocolorimeter (Spectronic Genesys 2) and glucose oxidase-peroxidase reactive strips (Biotest Medical Corporation, Taiwan).

The principle of the reaction is based on an enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts by the catalytic action of a peroxidase, with a phenol and 4-aminophenazone to form a red violet compound of quinoneimine which serves as a colored indicator.

One hundred (100) µL of plasma from each sample is added to 1 mL of reagent (phosphate buffer pH 7.0 (100 mM), glucose oxidase (1,800 IU), peroxidase (1,000 IU), 4-aminoantipyrine (0.4 mM) and phenol (0.1 mM)). For the blank, 100 µL of distilled water is used. The plasma-reagent assembly is then homogenized, then the optical density (DO) of the tests and standard glucose is read against the blank at the wavelength of 505 nm. The following formula allows determining the blood sugar in g/L: \[ [C] = \frac{\text{DO Test}}{\text{DO Standard}} \text{ g/L.} \]

2.6 Statistical Analysis

Results are reported as mean ± S.E.M. The result of anti-diabetic effects of the test samples was compared to control and standard groups. Statistical analysis was carried out using analysis of variance ANOVA. Differences were considered significant at the 5% probability level \( p < 0.05 \).

3. Results and Discussion

3.1 Hypoglycemic Activity

The use of medicinal plants is today a form of medicine that is increasingly used throughout the world. The use of herbal treatment and the search for new substances with biological activity is one of the greatest scientific concerns.

Ethanol extracts from the leaves of *Argania spinosa*, *Pistacia atlantica* and *Ceratonia siliqua* have been tested for hypoglycemic activity.

The search for the hypoglycemic activity of the organic extract of the leaves of *Argania spinosa* showed a highly significant hypoglycemic effect with two concentrations tested: 250 and 150 mg/kg body weight \( p = 0.004 < 0.01 \), while oral administration of the 50 mg/kg concentration showed no significant difference \( p > 0.05 \) (Fig. 1).

These values appear approximate to those observed in a comparative study conducted in Tunisia by (El Adib et al. [12]. Indeed, the study demonstrated the

![Fig. 1 Evolution of glycemia in hyperglycemic rats treated with ethanolic extract of *Argania spinosa* at 50, 150 and 250 mg/kg. The results are presented as averages ± S.E.M. * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \) (n = 8).](image-url)
effectiveness of the oil, coming from the leaves of *Argania spinosa* on the reduction of the blood sugar level by reaching 15.34% reduction after 3 h and a half, while the oil did not exceed 2% reduction after 2 h. In another sense, the results reported in the study of Cherki [13] showed a high activity of this oil considering it as a hypoglycemic agent.

The effect of ethanolic extract from the leaves of *Pistacia atlantica* at various oral doses (50, 100 and 250 mg/kg) on fasting blood glucose levels in normal rats is shown in Fig. 2.

Blood glucose levels did not vary significantly over the duration of this study ($p > 0.05$) for the 150 and 50 mg/kg concentrations. The 250 mg/kg concentration, on the other hand, induces a slight decrease in blood glucose levels in treated rats of 8.39% at 90 min.

In the same context, several authors [10, 11] did not find surprising results and concluded that the organic extract of *Pistacia atlantica*, at the concentration 200 mg/kg does not have hypoglycemic activity in either normoglycemic or hyperglycemic rats.

According to the results reported in Fig. 3, it appears that the ethanolic extract of the leaves of *Ceratonia siliqua* has a very highly significant hypoglycemic effect as early as 30 min after gavage ($p < 0.001$) and up to 90 min for the 250 mg/kg concentration, which leads to a reduction in blood glucose levels of 11%.

In addition, this botanical extract shows a similar highly significant effect ($p < 0.01$) for the two other concentrations tested (150 and 50 mg/kg), i.e. 10% and 1% reduction in blood glucose levels respectively. These results are consistent with those reported by Al-seeni [14] and Baragob [15] who demonstrated the hypoglycemic effect of an aqueous extract of carob in rats at different concentrations. These results also support the use of this plant in traditional medicine as a hypoglycemic agent, as reported in the literature [16].

### 3.2 Anti-hyperglycemic Activity

Results on *in vitro* and *in vivo* animal models have shown that plant extracts (aqueous, polyphenolic,
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Fig. 3 Evolution of glycemia in hyperglycemic rats treated with ethanolic extract of Ceratonia siliqua at different concentrations.

The results are presented as averages ± S.E.M. * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \) (\( n = 8 \)).

Fig. 4 Evolution of glycemia in untreated diabetic control rats throughout the treatment period. The ethanolic extract of Argania spinosa represents a highly significant anti-hyperglycemic activity with a percentage reduction of 17.02% after 5 days of treatment; 26.11% on day 10 and 32.10% on day 15. In the same sense, Ceratonia siliqua showed an interesting anti-hyperglycemic effect, decreasing from 2.20 ± 0.28 g/L on the 5th day to 1.69 ± 0.23 g/L on the 15th day with a very highly significant reduction: 16.15%, 24.75% and 33.17% respectively. On the other hand, Pistacia atlantica showed only a slight reduction during the three selected periods with 12.38%, 15.79% and 20.93% respectively.

In our experiment, the first remarkable result is the importance of alloxane in the induction of diabetes rats used. Thus, this substance produces a selective cytotoxicity in the \( \beta \) cells of the pancreas by the generation of free radicals leading to a reduced synthesis and release of insulin after 72 h of injection and with blood glucose levels greater than 1.26 g/L on an empty stomach [19]. In addition, the drug used as a positive control (glibenclamide) was found to be moderately effective against hyperglycemia compared to the extracts tested.

These results are in agreement with the study conducted by Refs. [12, 20] when evaluating the antidiabetic activity of leaf extracts of Ceratonia siliqua, Gnetum africanum and Gnetum buchlozzianum. These results confirm the antihyperglycemic effect of these extracts.

According to Bnouham et al. [21], oral administration of argan oil at a dose of 2.5 mL/kg showed in diabetic rats a significant reduction in plasma glucose levels of...
55% compared to untreated diabetic rats. However, this effect remains lower than that induced by glibenclamide at 2 mg/kg. Therefore, the consumption of argan oil as a traditional food provides some reduction in the pathogenesis induced by hyperglycemia. This also explains the basis of its traditional use by the rural community of western southern Morocco [21].

Furthermore, the results of *Pistacia atlantica* are consistent with the work of Hamdan and Afifi [10] who did not approve its hypoglycemic activity. Contrary to these results, Hashemnia et al. [7] showed that daily oral administration of 200 mg/kg of *Pistacia atlantica* extract for 15 days decreased blood glucose concentrations in the normal range in streptozotocin induced diabetic rats [7].

### 4. Conclusions

Phytotherapy can be an alternative medicine or at least as a complement to traditional pharmacy. The need to find new curative molecules remains a public health priority.

This study aims to evaluate the hypoglycemic, anti-hyperglycemic and antioxidant activity of the organic extract of the leaves of *Argania spinosa*, *Pistacia atlantica* and *Ceratonia siliqua* harvested in the Agadir region. The choice of these plants is based on the frequency of their use by the local population and on their economic value in the region exploited.

Our preliminary results are interesting and suggest that the extracts studied represent a promising source of biologically active molecules with several pharmaceutical and biological uses. Analyses of the hypoglycemic activity show a very highly significant effect for extracts of *Argania spinosa* and *Ceratonia siliqua* and a slight activity for *Pistacia atlantica*. In the same sense, the results of the anti-hyperglycemic activity showed a remarkable activity for the extract of *Argania spinosa* and *Ceratonia siliqua* and a slight activity for *Pistacia atlantica*.
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