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Antihyperglycemic Effect of Zygophyllum Geslini Aqueous Extract in Streptozotocin-Induced Diabetic Wistar Rats

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Abstrtact: Diabetes mellitus is a major public health concern. Finding a cure for the disease without its side-effects is the objective of modern medicine. The plant is a raw material for these studies. *Zygophyllum geslini* is a species widely used in Algeria to treat this disease. Our aim is to investigate the antidiabetic activity of aqueous extract and its fractions in induced diabetic Wistar rats by streptozotocin. The three drugs caused a decrease in blood sugar for 14 days. Butanolic fractions (BF) fraction gives significant results on blood glucose after seven days and significant regulating oral glucose tolerance. This preliminary study shows that *Z. geslini* is endowed with a remarkable antidiabetic activity and that further studies are needed.

Key words: *Zygophyllum geslini*, streptozotocin, induced-diabetic rats, antihyperglycemic.

1. Introduction

Diabetes mellitus is a metabolic disease caused by a disorder in insulin secretion or action with chronic hyperglycaemia [1]. This disease affects several millions of population all the world.

Management of diabetes mellitus by existing therapeutic agents without any side effects is still a challenge for the modern medical system. This leads to search for new and efficient antidiabetic drugs without side effects.

Since ancient times, the plant is an important source of medication. In Algerian traditional medical practice many, plants are used to treat diabetes mellitus in south Algeria and most of these medicinal plants are not scientifically evaluated for their therapeutic efficiency. Scientific studies on these plants are recommended by the World Health Organisation [2].

Zygophyllum geslini is a plant used as a remedy for diabetes mellitus in Algerian traditional medicine. Further, there is no scientific study on the medicinal property of this species [3].

The present work was undertaken to study the glucose-lowering effects of the aqueous extract of this plant and its fractions.

2. Materials and Methods

2.1 Chemicals

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

2.2 Plant Material

Fresh aerial parts of *Z. geslini* were collected in August from Adrar (South of Algeria). Authentication of the plant was carried out by Dr. Mahboubi and Belaskri, Department of Biology, Faculty of Sciences, University of Tlemcen, Algeria. A sample of this plant is available in the department of Biology.

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The plant material was dried in the laboratory at room temperature and mixed in a grinder.

2.3 Preparation of Aqueous Extract and Fractionation

Twenty grams of this obtained powdered dried were macerated in 100 mL H_2O during 24 hours at room temperature. After filtration the filtrate (5.26 g) was extracted by liquid-liquid partition with n-butanol. The resulting fractions, aqueous (3.24 g) and organic (1.66 g) were evaporated and tested for their antidiabetic effects.

2.4 Phytochemical Study

The aqueous extract (AE) and the two aqueous and butanolic fractions (AF, BF) were tested for the presence of different families of compounds according to the method described in Harbone [4]. The dosage of phenolic compounds as carried out as described by Martin and Larry [5] and the total sugars is carried out according to the method of Dubois [6].

2.5 Animals

Male Wistar rats weighing 215-275 g from the animal house of the faculty of Science, University of Mascara, were used. The animals were fed with standard laboratory diet and given water *ad libitum*. Prior to the experiment, the animals were subjected to fast for 16 hours with free access to water.

2.6 Streptozotocin-Induced Diabetic Rats

Diabetes was induced in fasted rats by tail vein injection of streptozotocin (STZ: 50 mg/kg, iv) dissolved in 0.1M citrate buffer (pH 4.5). Fasted blood glucose level were analyzed 14 days after STZ injection as well as glucosuria to confirm the diabetic state. Only rats with a fasting blood glucose level at least 200 mg/dL and positive urine glucose were used in the experiment [7].

2.7 Determination of the Efficacy of the Plant Drugs in Diabetic Rats

After induce of diabetes, animals were divided into eight groups with five animals in each group as follows: Group I: normal control rats received Tween 80 at 5%; Group II: diabetic control rats received Tween 80 at 5%; Group III: normal rats treated with AE (500 mg/kg b.w/d); Group IV: diabetic rats treated with AE (500 mg/kg b.w/d); Group V: normal rats treated with AF (300 mg/kg b.w/d); Group VI: diabetic rats treated with AF (300 mg/kg b.w/d); Group VII: normal rats treated with BF (200 mg/kg b.w/d); Group VIII: diabetic rats treated with BF (200 mg/kg b.w/d).

All groups were treated orally during 14 days. The drugs were dissolved in Tween 80 at 5%.

2.8 Estimation of Blood Glucose

Plasma glucose level was estimated by GOD-POD method of Trinder [8] before the treatment, after 7 and 14 days.

2.9 Oral Glucose Tolerance Test (OGTT)

At the end of the experiment, an oral glucose tolerance test was practised. Animals were loaded with glucose (3 g/kg). Blood glucose level was determined at 0, 60 and 120 min after glucose loading.

2.10 Statistical Analysis

Results were expressed as mean ± S.E.M. Differences between groups were considered to be significant at $P < 0.05$ using unpaired Student's *t*-test.

3. Results and Discussion

3.1 Phytochemical Study

In light of the phytochemical study (Table 1), the two fractions of aqueous extract are rich with metabolites that can exhibit the antidiabetic effects such as saponins, alkaloids [9], amino acids [10].

The butanol is frequently used to extract saponins from the crude extracts. Saponins are glycosides compounds [4, 11]. This can explain the high sugar level in FB.

3.2 Effect of the Drugs on Streptozotocin-Induced Diabetic Rats

Table 2 shows the evolution of blood glucose in normal and diabetic rats treated with the aqueous extract and its fractions. The results show a significant effect with the groups treated by AE and its BF fraction. The butanolic fraction gave the best effect in comparison with the aqueous fraction.

Table 3 shows the variation of body weight in rats. The decrease of body weight in diabetic rats was restored in diabetic treated by AF and BF. All normal rats have a standard variation of body weight.

Oral glucose tolerance test in diabetic and normal rats showed an increase in blood glucose level 1 hour after glucose administration. A significant reduction(restoration) was marked in diabetic treated ($P < 0.05$) by the butanolic fraction (Table 4) after treatment and suppressed the rise in blood glucose compared with the control diabetic group. The aqueous fraction is also efficient on the oral glucose tolerance.

Table 1 Phytochemical compounds in the aqueous extract and its fractions.

	Aqueous extract (AE)	Aqueous fraction (AF)	Butanolic fraction (BF)
Flavonoids	+	-	-
Tannins	+	+	+
Saponins	+	+	+
Mucilage	+	+	+
Amino acids	+	+	+
Cardiotonic glycosides	+	+	+
Alkaloids	+	+	+
Phenolic compounds		20 mg/g of AF	19.3 mg/g of BF
Total sugars		121 mg/g of AF	237 mg/g of BF

Table 2 Effect of *Zygophyllum geslini* extracts on blood glucose (mg/dL).

Groups	Glyceamia (mg/dL)		
	Before treatment	7 days	14 days
Normal control	118.6 ± 10.3	88.4 ± 4.3 $_{NS}$	64.75 ± 8.6 $_{NS}$
Diabetic control	365.5 ± 9.4	335.5 ± 10.6 $_{NS}$	227.25 ± 68.1 $_{NS}$
Normal+AE	147.8 ± 10.6	113.4 ± 15.7 $_{NS}$	103.8 ± 4.8 *
Diabetic+AE	365.6 ± 50.2	308.6 ± 84.3 $_{NS}$	162.4 ± 55.6 *
Normal+AF	130.9 ± 49.5	85.6 ± 5.3 $_{NS}$	60.8 ± 10.4 $_{NS}$
Diabetic+AF	354.2 ± 102.9	299.2 ± 117.2 $_{NS}$	155.7 ± 52.5 $_{NS}$
Normal+BF	118.6 ± 10.3	86.1 ± 2.5*	66.2 ± 8.2*
Diabetic+BF	398.6 ± 81.73	93.4 ± 5.1*	175.2 ± 48.8*

Values are given as mean ± S.D. for five rats per group. Values are statistically significant at * $P < 0.05$; NS, non significant. Statistical significance was compared in each group by following the evolution in time.

Table 3 Effect of *Zygophyllum geslini* extracts on body weight (g).

Groups	Body weight (g)		
	Before treatment	7 days	14 days
Normal control	232 ± 10 $_{NS}$	220 ± 10 $_{NS}$	234 ± 11 $_{NS}$
Diabetic control	214 ± 14 $_{NS}$	184 ± 15 $_{NS}$	178 ± 15 $_{NS}$
Normal+AE	249 ± 9 $_{NS}$	227 ± 9 $_{NS}$	243 ± 9 $_{NS}$
Diabetic+AE	218 ± 16 $_{NS}$	199 ± 14 $_{NS}$	188 ± 17 $_{NS}$
Normal+AF	248 ± 14 $_{NS}$	241 ± 10 $_{NS}$	255 ± 11 $_{NS}$
Diabetic+AF	237 ± 17 $_{NS}$	216 ± 17 $_{NS}$	229 ± 24 $_{NS}$
Normal+BF	229 ± 10 $_{NS}$	230 ± 9 $_{NS}$	233 ± 9 $_{NS}$
Diabetic+BF	226 ± 14 $_{NS}$	217 ± 20 $_{NS}$	220 ± 19 $_{NS}$

Values are given as mean ± S.D for five rats per group. Values are statistically significant at * $P < 0.05$; NS, non significant. Statistical significance was compared in each group by following the evolution in time.

Table 4 Oral glucose tolerance test (OGTT) at the end of experiment (after 14 days).

Groups	Glyceamia (mg/dL)		
	T0	60 min	120 min
Normal control	64.7 ± 8.6	95.2 ± 15.4 $_{NS}$	137.2 ± 57.1 $_{NS}$
Diabetic control	227.2 ± 68.0	314.2 ± 40.5 $_{NS}$	327.7 ± 20.9 $_{NS}$
Normal + AF	60.8 ± 10.3	80.8 ± 7.5 $_{NS}$	76.0 ± 4.4 $_{NS}$
Diabetic + AF	155.7 ± 61.1	234.0 ± 76.6 $_{NS}$	215.5 ± 68.2 $_{NS}$
Normal + BF	66.0 ± 8.2	130.0 ± 7.8 *$_{b}$	95.0 ± 11.6 $_{NS}$
Diabetic + BF	175.2 ± 48.8	410.6 ± 38.4*$_{b}$	228.0 ± 59.2 $_{NS}$

Values are given as mean ± S.D for five rats per group. Values are statistically significant at $P < 0.05$; NS, non significant. Statistical significance was compared in each group by following the evolution in time. (*) the difference between T0 and 60 min or T0 and 120 min, (b) the difference between 60 min and 120 min.

Z. geslini is a plant widely used in Algerian folk medicine for the treatment of diabetes mellitus. The antidiabetic effect of the aqueous extract and its fractions has been investigated using STZ experimental model diabetes mellitus. The first objective is to search for the fraction that gives the best effect.

STZ hyperglycaemia-induced has been described as a useful experimental model to study the activity of antidiabetic agents [12-14].

The study reports, mainly, the antihyperglycaemic effect of the aqueous extract. We have marked the good effects of the butanolic fraction. The works of Jaouhari et al. [15, 16] show that the aqueous extract of *Zygophyllum gaetulum* has an antidiabetic activity in alloxan-induced diabetic rats and in patients with diabetes mellitus.

Our work is preliminary and partial. It provides a basis for further research on this plant. Few parameters are used because our objective is based on the screening of fractions of molecules with good antidiabetic activity.

4. Conclusion

To conclude, *Z. geslini* has an interesting antihyperglycemic effect and which must be studied. This effect can be attributed to the chemical compounds, the saponins, amino acids or the phenolic compounds. The present work remains a contribution and requires further research with *in vitro* and *in vivo* test to isolate the active ingredient and for a better understanding of the action. We can also conclude that the butanolic fraction of the aqueous extract deserves to be studied

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