International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

ANTIDIABETIC ACTIVITY OF THE AQUEOUS EXTRACT OF *ANCHUSA STRIGOSA* LAB IN STREPTOZOTOCIN DIABETIC RATS

Ahmed Muhammed[1*] and Nuray Arı[2]

[1]Department of Biology, Faculty of Education, Tikrit University, Tikrit, Iraq
[2]Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey

***Corresponding author e-mail:** akmali78@yahoo.com

ABSTRACT

Flowers of *Anchusa strigosa* Lab. (Boraginaceae) used traditionally in Iraq for curing many kinds of diseases including diabetes. The present study was carried out to evaluate the antidiabetic activity of aqueous extract of flowers of *A. strigosa* in streptozotocin (STZ)-diabetic rats. The aqueous extract of *A. strigosa* flowers was administered orally (250 mg/kg and 500 mg/kg) to diabetic rats for 30 days. *A. strigosa* caused a dose-dependent fall in blood glucose. Also there was an improvement in serum insulin levels. Cholesterol and triglyceride levels showed significant reduction in comparison with diabetic control group. The extract treatment also showed significant increase in hepatic glycogen levels. The results suggest that *A. strigosa* possesses antidiabetic effects in STZ-induced diabetic rats.

Keywords: *Anchusa strigosa*, antidiabetic activity, STZ-diabetic rats, insulin

INTRODUCTION

Diabetes mellitus (DM) can be defined as group of metabolic diseases that are characterized by hyperglycemia due to defects in insulin secretion, insulin action or both [1]. Two types of DM are recognized; Type1, that results in an absolute deficiency of insulin secretion and Type 2, that results in a combination of resistance to action of insulin and inadequate insulin secretion [1,2]. Treatment of DM conventional is based on insulin and oral hypoglycemic agents, both treatments failed to restore a normal pattern of glucose homeostasis, in addition to its restrictions because of side effects [3,4]. This leads to growing interest in looking for alternatives for these drugs, medicinal plants consider one of the main source for these alternatives.

The ancient Iraqi people knew and used over 250 medicinal remedies and understood how much they could be supported by sound diet and good hygiene, one of these important plants was *Anchusa strigosa* Lab. (Boraginaceae) which called Lisan-el-thor in Arabic, and its dry flowers which are used locally for curing many kinds of diseases [5]. Total lipids and pure proteins isolated from the water extracted dry flowers of *A. strigosa* have been showed antimicrobial activity [6,7].

Disi et al. [8] has reported that the aqueous root extract of *A. strigosa* was effective against gastric-ethanol-induced ulcer in laboratory animals. Later six new pyrrolizidine alkaloids and glycoside compounds have been isolated from *A. strigosa* roots, and its antifeedant activity was investigated [9]. The present study was undertaken to evaluate the antidiabetic activity of *A. strigosa* in STZ diabetic rats.

MATERIALS AND METHODS

Plant material: The flowers of *A. strigosa* were collected during May 2006 from Tikrit, Iraq. The plant were identified and authenticated by Dr. Khalil I. Al-Shemmary, Plant taxonomist, Biology Department, Faculty of Science, Tikrit University, Tikrit, Iraq. The voucher specimen (No.6381) was deposited at the herbarium of Faculty of Science, Tikrit University.

Preparation of plant extract: Aqueous extract of *A. strigosa* flowers was prepared using the method described by Abdel-Barry et al. [10]. Briefly, dried plant materials were grinded by electric grinder to a fine powder. 50g of the powder were suspended in 250ml of distilled water and then stirred magnetically for 24 hours at 50 °C. Subsequently, the suspension was filtered (Whatman No.1) and concentrated under reduced pressure at 40°C, to get dry residue 8.36g (16.7%).

Preliminary phytochemical screening: The preliminary phytochemical tests revealed the presence of alkaloids, lipids, glycosides, proteins and phenolic compounds in the flowers [11,12].

Experimental animals: Adult male Wistar albino rats (2-3 months old, weighing 200-250 g) were obtained from the animal house of College of Education, University of Mosul, Mosul, Iraq. Animals were acclimatized and maintained under standard laboratory conditions (12 h light and 12h dark cycle, 22±2 °C) access to food and water *ad libitum* for 7 days prior to the experiments. All animal experiments were conducted in accordance with the "principle of laboratory animal care" (NIH Publication No. 85-23 revised 1985).guidelines and procedures [13], and protocol approved by the Institutional Animal Ethics Committee, Tikrit University, Tikrit, Iraq.

Acute toxicity study: The acute oral toxicity study was carried out according to OECD guideline 423 [14]. Normal healthy male rats fasted for 12 h were randomly divided into test groups and control groups. *A. strigosa* (1.0, 2.0 and 4.0 g/kg body weight) was separately administered orally to the rats in each of the test groups. Each of the rats in the control groups was treated with vehicle alone. Then the rats in both the test and control groups were allowed access to food and water. The animals were closely observed for the first 12 h for any toxic symptoms and for 72 h for mortality rate.

Oral glucose tolerance test: Oral glucose tolerance test was performed in overnight fasted (16 h) normal rats in order to select the optimal dose of *A. strigosa* to be used in the study. Rats were divided into five groups (n = 6): Group I rats received vehicle alone; Group II rats received glibenclamide (0.6 mg/kg); Group III rats received *A. strigosa* (125 mg/kg); Group IV rats received *A. strigosa* (250 mg/kg); Group V rats received *A. strigosa* (500 mg/kg). The rats were orally gavaged with 2 g/kg body weight of glucose dissolved in water (40%, wt/vol) 30 min after to the oral administration of the vehicle, standard drug and extracts. Blood samples were collected from the tail vein at 0 (prior to glucose administration), 30, 60 and 120 min [15,16].

Induction of experimental diabetes: Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg body weight) in 0.1 M cold citrate buffer (pH. 4.5). Rats were supplied with 5% glucose solution for 24h after STZ injection in order to prevent hypoglycemia. After 7 days fasting blood glucose levels were determined, animals with blood glucose level greater than 250 mg/dl were considered diabetic and included in the experiments [17,18].

Experimental design: Rats were divided into five groups of six animals each. The extract was administered orally by gavage once daily for 30 days. Group I: normal control administered vehicle alone (carboxy methyl cellulose CMC 0.5%; 1ml/kg bw); Group II: diabetic control administered vehicle alone; Group III: diabetic rats administered glibenclamide (0.6 mg/kg); Group IV: diabetic rats administered *A. strigosa* aqueous extract (250 mg/kg); V: diabetic rats administered *A. strigosa* aqueous extract (500 mg/kg). Fasting blood glucose and body weight were estimated on days 0, 7, 14, 21 and 30 of extract administration. At the end of the experimental period, overnight fasted rats were anaesthetized and sacrificed. Blood samples were collected for insulin, cholesterol and triglycerides determination, also, liver was dissected out for glycogen determination.

Biochemical assays: Fasting blood glucose levels were determined at days 0, 7, 14, 21 and 30 using the glucometer (Accu-Chek Active, Roche, Germany) based on glucose dye oxido-reductase mediator reaction.. The level of serum insulin was estimated by using a radioimmunoassay kit (DiaSorin, Saluggia, Italy). Serum cholesterol and triglyceride levels were determined by the commercial kits (Bicon, Germany). Whereas the hepatic glycogen levels determined by the method of anthrone reagent [19].

Statistical analysis: The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test using computerized software SPSS (version 13.0). The values are given as mean ± S.E.M. for six rats in each group. *p*-Values <0.05 were defined as statistically significant.

RESULTS

Acute toxicity study: Acute toxicity study revealed the non-toxic nature of the aqueous extract of *A. strigosa.* No mortality was observed in the extract-

treated rats and observations showed the normal behavior of the treated rats. There was no lethality or any toxic reactions found with the selected dose until the end of the study.

Effect of A. strigosa on oral glucose tolerance test: The mean blood glucose levels of all groups did not show significant difference before glucose administration . After oral glucose administration, *A. strigosa* (250 and 500 mg/kg) and glibenclamide (0.6 mg/kg) significantly ($p<0.01$) inhibited the rise in blood glucose levels in glucose-loaded rats when compared with control group. At 100 mg/kg recorded reduction was not significant. The effects of the *A. strigosa* were dose dependent (Figure 1).

Effect of A. strigosa aqueous extract on blood glucose levels: Induction of diabetes in experimental rats produced significant elevation in fasting blood glucose (FBG) levels as compared to the normal control group. *A. strigosa* (250 mg/kg) produced significant decrease ($p<0.05$) in 7th day, hypoglycemic effect was also significant ($p<0.01$) in 14th, 21st and 30th days. Diabetic rats treated with 500 mg/kg of *A. strigosa* showed significant decrease ($p<0.01$) in 7th, 14th, 21st and 30th days. *A. strigosa* effect on FBG was in a dose-dependent manner. Glibenclamide, standard oral hypoglycemic drug, decreased significantly ($p<0.01$) FBG as compared to the diabetic control group (Table 1).

Effect of A. strigosa aqueous extract on body weight: As shown in Table 2 there was a significant reduction in diabetic control group weight in comparison with normal control group. Oral administration of *A. strigosa* (250 and 500 mg/kg) produced significant increase ($p<0.01$) in body weight since 7th day (Table 2). Diabetic rats treated with glibenclamide also exert significant increase ($p<0.01$) in body weight.

Effect of A. strigosa aqueous extract on lipid profile, serum insulin and hepatic glycogen levels: STZ-induced diabetic rats showed a significant increase ($p<0.01$) in lipid profile, while serum insulin and hepatic glycogen levels showed a significant decrease ($p<0.01$) in comparison with normal control animals. The aqueous extract of *A. strigosa* (500 mg/kg) lowered cholesterol level significantly ($p<0.01$), while the dose 250 mg/kg did not show significant changes in cholesterol levels (Table 3). Both doses (250 and 500 mg/kg) of *A. strigosa* produced significant reduction ($p<0.01$) in triglyceride levels. Serum insulin and hepatic glycogen levels showed significant increase ($p<0.05$) and ($p<0.01$) respectively in diabetic animals treated with plant extract (Table 3).

DISCUSSION

The present study designed to evaluate the antidiabetic effect of the aqueous extract of *A. strigosa* in STZ-diabetic rats. STZ is toxic glucose analogue that preferentially accumulate in pancreatic β-cells via the GLUT2 glucose transporters. The alkylating activity of STZ methylnitrosourea moiety results in the fragmentation of the DNA of pancreatic cells. In the attempt to repair DNA, poly (ADP-ribose) polymerase is overstimulated. As a result there will be depletion in cellular NAD^+, and subsequently ATP stores which leads to β-cells necrosis. Also STZ ability to liberate NO and ROS play a role in its diabetogenic activity [20]. Administration of *A. strigosa* to diabetic rats showed a significant decrease in the fasting blood glucose and an increase in the serum insulin levels. The possible mechanism by which the extract shows its antihyperglycemic activity is by increasing either the pancreatic secretion of insulin from existing β-cells or by its release from the bound form. The glucose lowering activity was compared with glibenclamide, a standard oral hypoglycemic agent. Glibenclamide has been used as conventional drug for type 2 diabetes and show its activity through stimulation of insulin secretion from β-cells [21]. Induction of diabetes with STZ is associated with characteristic decrease in body weight, which is due to the excessive breakdown of fats and proteins, even though the food consumption is more in diabetic animals than normal [22,23,24]. Oral administration of *A. strigosa* improved the body weight in diabetic rats, may be due to improvement in insulin secretion and proper glycemic control. The marked increase in serum cholesterol and triglycerides of untreated diabetic rats is in agreement with the findings of Daisy et al. [25] increased cholesterol levels can be due to cholesterol acyl transferase, this enzyme will be activated in the absence of insulin [26]. Under normal conditions, the enzyme lipoprotein lipase hydrolysis triglycerides. In the present study, administration of *A. strigosa* significantly improved these abnormalities may be by increasing insulin levels in STZ-diabetic rats. Lowering of these parameters is may be useful for preventing of atherosclerosis [27]. The hepatic glycogen content significantly decreased in diabetic rats [28], probably due to lack of insulin in the diabetic condition which results in the inactivation of glycogen synthase system. Also in experimental diabetes state there will be no inhibition to glycogenolysis that controlled by

insulin therefore hepatic glycogen content will be decreased [29].

The significant increase in the glycogen levels of the diabetic animals treated with plant extract may be because of the reactivation of glycogen synthase system as a result of increased insulin secretion.

CONCLUSION

The results of study suggest that oral administration of *A. strigosa* aqueous extract has antihyperglycemic and insulin secretory effects in STZ-diabetic rats. However, more chemical and pharmacological studies are required to find out the active constituent(s) responsible for these effects.

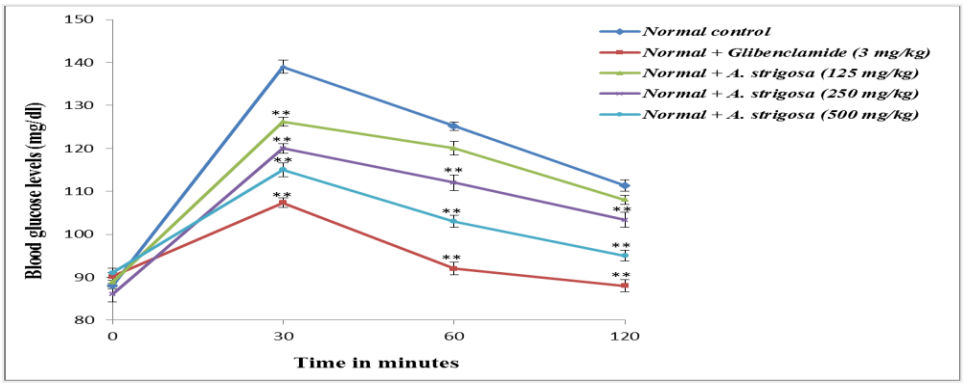

Figure 1: Effect of *A. strigosa* aqueous extract on oral glucose tolerance test
Readings are values ± S.E.M. for six rats in each group. * *p<0.05,* ** *p<0.01 vs. normal control.*

Table 1: Effect of *A. strigosa* aqueous extract on fasting blood glucose level in STZ-diabetic rats

Groups	Fasting blood glucose concentration (mg/dl)				
	0th day	7th day	14th day	21st day	30th day
Normal control	77.00 ± 1.15**	77.35 ± 2.86**	72.68 ± 1.59**	76.28 ± 1.37**	74.65 ± 1.41**
Diabetic control	319.31 ± 4.34	324.50 ± 17.95	318.41 ± 14.33	315.50 ± 7.02	312.41 ± 12.00
Diabetic + Glibenclamide (0.6 mg/kg)	322.48 ± 16.07	236.40 ± 2.38**	192.61 ± 3.71**	151.63 ± 3.53**	125.35 ± 2.31**
Diabetic + *A. strigosa* (250 mg/kg)	320.50 ± 20.73	298.61 ± 5.00*	237.25 ± 6.26**	190.28 ± 6.93**	166.40 ± 8.98**
Diabetic + *A. strigosa* (500 mg/kg)	314.68 ± 6.76	226.75 ± 3.14**	188.33 ± 3.00**	146.18 ± 3.65**	118.28 ± 3.06**

Readings are values ± S.E.M. for six rats in each group
** p<0.05 vs. diabetic control.*
*** p<0.01 vs. diabetic control.*

Table 2: Effect of *A. strigosa* aqueous extract on body weight in STZ-diabetic rats

Groups	Body weight (g)				
	0th day	7th day	14th day	21st day	30th day
Normal control	284.58 ± 4.62**	301.45 ± 4.93**	312.12 ± 8.01**	321.50 ± 5.77**	325.51 ± 6.99**
Diabetic control	185.53 ± 4.04	195.42 ± 2.37	197.50 ± 2.26	207.13 ± 2.70	206.28 ± 4.18
Diabetic + Glibenclamide (0.6 mg/kg)	196.30 ± 5.13	229.40 ± 5.18**	245.60 ± 4.38**	254.50 ± 8.36**	252.55 ± 6.12**
Diabetic + *A. strigosa* (250 mg/kg)	200.38 ± 4.01	235.53 ± 4.15**	248.55 ± 4.71**	252.46 ± 3.32**	253.46 ± 3.39**
Diabetic + *A. strigosa* (500 mg/kg)	191.61 ± 3.41	232.46 ± 3.71**	238.36 ± 4.46**	240.51 ± 3.80**	246.46 ± 5.20**

Readings are values ± S.E.M. for six rats in each group
** p<0.05 vs. diabetic control.*
*** p<0.01 vs. diabetic control.*

Table 3: Effect of *A. strigosa* aqueous extract on lipid profile, serum insulin and hepatic glycogen levels in STZ-diabetic rats

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Insulin (µU/ml)	Hepatic glycogen (g/100g)
Normal control	86.29 ± 1.67**	130.35 ± 3.83**	131.91 ± 2.18**	7.28 ± 8.98 **
Diabetic control	119.66 ± 2.69	223.63 ± 5.26	58.35 ± 1.80	1.31 ± 8.01
Diabetic + Glibenclamide (0.6 mg/kg)	66.38 ± 2.81**	148.50 ± 2.41**	121.62 ± 2.07**	6.05 ± 5.45**
Diabetic + *A. strigosa* (250 mg/kg)	114.49 ± 3.66	158.38 ± 2.53**	93.42 ± 2.19*	5.65 ± 0.17**
Diabetic + *A. strigosa* (500 mg/kg)	87.40 ± 3.76**	142.36 ± 2.45**	108.12 ± 1.12*	7.68 ± 0.15**

Readings are values ± S.E.M. for six rats in each group
* *p<0.05 vs. diabetic control.*
** *p<0.01 vs. diabetic control.*

REFERENCES

1. American Diabetes Association. Diabetes Care, 2008; 31: S12-S54.
2. Hansen M. Pathophysiology: Foundation of Disease and Clinical Intervention. 1st ed., Philadelphia; W.B.Saunders Company: 1998, pp. 851-52.
3. Melinda A. Non insulin dependent diabetes mellitus treatment with sulphonylureas. In: Des natures M. and Hale P. Clinical Endocrinology and Metabolism, London; Balliere-Tindall: 1988, pp. 443-53.
4. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, 1997; 20(7): 1183-97.
5. Chakravarty HL. Plant Wealth of Iraq. Vol. I, Baghdad; Ministry of Agriculture and Agrarian reform: 1976, pp. 29.
6. Al-Hassan IA, Al-Salihi FG, Al-Salihi NJ. Ibn Al-Haitham J Pure Appl Sci, 1999; 10(1): 62-70.
7. Al-Salihi FG. Tikrit J Pure Sci, 2000; 6(1): 105-17.
8. Disi AM, Tamimi SO, Abuereish GM. J Ethnopharmacol, 1998; 60(3): 189-98.
9. Braca A, Bader A, Sicilian T, Morelli I. Planta Med, 2003; 69(9): 833-41.
10. Abdel-Barry JA, Abdel-Hassan IA, Jawad AM, Al-Hakiem MH. East Mediterr Health J, 2000; 6(1): 83-8.
11. Sakar MK, Tanker M. Fitokimyasal Analizler. Ankara; Ankara Üniversitesi Eczacılık Fakültesi Yayınları No. 67: 1991.
12. Harborne JB. Phytochemical Methods. 3rd ed., London; Chapman & Hall: 1998, pp. 60.
13. NIH. Guide for the use of laboratory animals. DHHS, PHS, NIH Publication, 1985, No. 85-23.
14. OECD/OCDE Guideline for the testing of chemicals. Revised Draft Guideline 423: Acute Oral Toxicity, October 2000.
15. Chika A, Bello SO. J Ethnopharmacol, 2010; 129(1): 34-7.
16. Husain GM, Singh PN, Singh RK, Kumar V. Phytother Res, 2011; 25(12): 1806-12.
17. Ravi K, Ramachandran B, Subramanian S. Biol Pharm Bull, 2004; 27: 1212-17.
18. Kaleem M, Asif M, Ahmed QU, Bano B. Singapore Med J, 2006; 47(8): 670-5.
19. Plumer PT. An Introduction to Practical Biochemistry. 2nd ed., UK; Mcgraw-Hill Book Company: 1978, pp.345-6.
20. Lenzen S. Diabetologia, 2008; 51: 216-26.
21. Bolkent Ş, Yanardağ R, Tabakoğlu-Oğuz A, Özsoy-Saçan Ö. J Ethnopharmacol, 2000; 73: 251-9.
22. Kelly D. Diabetes Rev, 1995; 3: 366-77.
23. Murray R, Granner D, Mayes P, Rodwell V. Harper's Biochemistry. 25th ed., Connecticut, Stamford; Appleton & Lange: 2000, pp. 611-7.
24. Subash Babu P, Prabuseenivasan S, Ignacimuthu S. Phytomedicine, 2007; 14: 15-22.
25. Daisy P, Eliza J, Mohamed Farook KA. J Ethnopharmacol, 2009; 126(2): 339-44.
26. Maechler P, Wollheim CB, Bentzen CL, Niesors E. Ann Nutr Metab, 1993; 37(4): 199-209.
27. Luc G, Fruchart JC. Am J Clin Nutr, 1991; 53: 206S-9S.
28. Grover JK, Vats V, Rathi SS. J Ethnopharmacol, 2000; 73(3): 461-70.
29. Vats V, Yadav SP, Grover JK. J Ethnopharmacol, 2004; 90(1): 155-60.