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HYPOGLYCAEMIC ACTIVITY OF EXTRACTS OF THE AERIAL PARTS OF *SATUREJA PUNCTATA* BENTH. BRIQ. IN STREPTOZOTOCIN-INDUCED DIABETIC MICE

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ABSTRACT: The ethnopharmacological use of herbal remedies for the treatment of diabetes mellitus is an area of study ripe with potential as a starting point in the development of alternative, inexpensive therapies for treating the disease. In the traditional health care system of Ethiopia, the aerial parts of *Satureja punctata* (Benth.) Briq. (Lamiaceae) are used for the treatment of diabetes mellitus and various other ailments. This study reports the antidiabetic activity of the total hydroalcoholic and aqueous extracts as well as the various solvent fractions of *S. punctata* in streptozotocin-induced diabetic mice. The results which were compared with glibenclamide, a standard hypoglycaemic agent, and control groups revealed that the total extracts as well as the most polar solvent fractions possess significant antidiabetic effect while those of the the nonpolar fractions are devoid of any activity. At a concentration of 300 mg/kg the total hydroalcoholic extract reduced blood glucose level from 441.3 to 339.0 mg/dl while the aqueous extract lowered the level from 458.3 to 351.7 mg/dl on the 3rd hr after drug administration. Similarly, both the methanol and aqueous fractions showed activity with the former displaying a much better action. Thus, the methanol fraction showed a maximum reduction (38.1%) of blood glucose level while a reduction of 19.4% was observed for the aqueous fraction during the 3rd hr following the application. Moreover, the activity of the methanol fraction was proved to be in a dose-dependent manner. From the present study, it can be concluded that the aerial parts of *S. punctata* have genuine antidiabetic activity, and their use in traditional medicine to control diabetis mellitus may be justified.

Key words/phrases: Antidiabetic activity, Glibenclamide, Hydroalcoholic extract, Lamiaceae, *Satureja punctata* solvent fractions.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycaemia. The metabolic disturbance involves disturbance in the metabolism of fats, proteins and carbohydrates, reflecting a state of insulin deprivation. This occurs due to deficient insulin secretion or due to factors opposing the tissue effects of insulin or both.

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Although, to date, metformin is the only ethical drug approved for the treatment of type 2 diabetes mellitus, which is derived from the medicinal plant *Galega officinalis* (Eshrat, 2002), researches conducted over the last several decades have shown that plant and plant-based therapies have high potential to treat and control diabetes and its complications (Ivorra *et al.,* 1989; Grover *et al.,* 2001). The merit of using effective crude traditional herbal therapies to treat diabetic patients is a well-appreciated objective especially in countries like Ethiopia where accessibility to allopathic therapy is hindered due to low income. However, if plants are to be used in their original traditional recipes, their consistency and efficacy have to be ensured through properly conducted scientific investigations.

There are over 1,200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabetic activity (Matthew *et al.,* 2006). Among those plants traditionally used for the treatment of diabetes in Ethiopia, *Satureja punctata* (Benth.) Briq. (Lamiaceae) is one (Getahun Abate, 1989).

S. punctata is a sub-shrub or small shrub which grows up to 10-100 cm high, often with long unbranched branches (Ryding, 2006). It is known to occur only in Ethiopia and other parts of Africa growing on stony slopes mainly on limestone. Locally it is known as *"Lomishet"* (Amharic) and the decoction of the dried aerial parts of the plant is used for the treatment of various diseases, including diabetes (Getahun Abate, 1989).

Literature review indicates that *S. punctata* contains essential oil, the major constituents being geranial and neral (Chagonda and Chalchat, 2005; Yinebeb Tariku *et al*., 2010). One of these studies further revealed that the oil possess antileishmanial activity (Yinebeb Tariku *et al*., 2010). However, there has been no scientific report concerning the hypoglycaemic activity of this plant. Hence, it was deemed prudent to scientifically investigate the antidiabetic activity of this medicinal plant.

MATERIALS AND METHODS

Plant material

The aerial parts of *S. punctata* were collected in December 2007 from north eastern part of the Italian Embassy, in Addis Ababa, Ethiopia. The plant material was authenticated by Ato Melaku Wendafrash, the National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia, where voucher specimen (Collection Number WT001) was deposited.

Experimental animals

Swiss albino mice of either sex weighing 25-30 g were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa. Before and during the experiment, the mice were allowed free access to standard pellet diet and water. After randomization into various groups and before initiation of the experiment, the mice were acclimatized to the animal house conditions (Kumar *et al*., 2006) at the Department of Drug Research (DDR), EHNRI. Prior to each study, the animals were made to fast for 12-14 hrs but had free access to water (Ragavan and Krishnakumari, 2006). All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines and rules of EHNRI. All the animal experiments were conducted at DDR, EHNRI.

Extraction, fractionation and isolation

Methanol extract

The plant material (100 g) of *S. punctata* (shade dried and powdered aerial parts) were extracted with 80% methanol for 72 hrs by percolation. The miscela collected every 24 hrs was filtered (Whatman No. 3, Whatman Ltd. England), and concentrated under reduced pressure using Rota Vapor (Buchi Rota vapor R- 200) at 40^0C. The resulting semi-solid extract was placed on a water bath set at 40^0C till it dried. The dried extract (20.8 g) was then transferred into vials and kept in a refrigerator until further use.

Aqueous extract

Powdered plant material (200 g) of the study plant was boiled with 2000 ml of distilled water for 30 min. The decoction was then filtered twice with cotton gauze. Lypholizer (Heto Power Dry LL3000 Wag Tech) was used to dry the aqueous extract. The freeze-dried extract (15.2 g) was kept in a desicator so as to remove moisture and then stored in a refrigerator until further use.

Solvent fractionation

The powdered plant material (30 g) of the study plant was fractionated in a Soxhlet apparatus with different solvents of increasing order of polarity starting from petroleum ether and then chloroform, acetone and methanol. Soxhlet extraction in each solvent system was carried out for 48 hrs. After completion of soxhlet extraction, the marc was allowed to dry in an open air. This was transferred to a flask and boiled for 30 min to prepare the aqueous fraction. The different fractions were concentrated under reduced

pressure and dried in a water bath at a temperature not exceeding 40^0C. In the case of the aqueous fraction a lypholizer (Heto Power Dry LL3000 Wag Tech) was used for drying. The dried fractions were then transferred into vials and stored in a refrigerator for further use.

Induction of experimental diabetes

Swiss albino mice were fasted overnight (12-14 hrs) and their weight and fasting blood glucose levels were recorded. Mice were then made diabetic by a single intraperitoneal injection of 150 mg/kg body weight of streptozotocin (STZ). STZ was first weighed for each group of animal according to their average weight and then solublized in 0.11 M sodium citrate buffer solution (pH=4.5) just prior to injection (Williamson *et al.*, 1996). Food and water were presented to the animals 30 min after drug administration (De Carvalho *et al*., 2003). Seventy-two hrs after STZ injection, fasting blood glucose level of each animal was determined and animals with a fasting blood glucose level above 200 mg/dl (Kumar *et al*., 2006) were used for the study. Blood samples were taken from the tail of the mice and glycaemia was determined by the glucose-oxidase peroxidase method with reagent strips and their evaluation was made on the ACCU-CHEK Active glucometer (Roche Diagnostics, Basel, Switzerland). In this method, glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a coloured product. The intensity of the pink colour is proportional to the original glucose concentration.

Experimental design

In the experiment, STZ-induced diabetic mice (blood glucose level >200 mg/dl) were used. The mice were divided into 11 groups of 6 mice each. The first group served as diabetic control treated with equivalent volumes of vehicle alone orally (distilled water); the other 9 groups were given plant extracts/fractions orally in varying doses (100 - 300 mg/kg). The last group which served as a positive control received glibenclamide dissolved in distilled water at a dose of 40 µg/kg body weight. Animals were fasted for 12-14 hrs prior to extract/drug administration allowing them access only to water. Blood samples were collected from the tail tip of mice at 0.5, 1, 2, 3 and 4 hr after extract administration (Rao, 2006).

Statistical analysis

Blood glucose levels in groups were expressed as mean ± standard error of mean (S.E.M). The data were statistically analyzed by repeated measures

ANOVA followed by Dunnett`s test. P-values less than 0.05 were considered significant (Ozbek *et al.,* 2004; Kumar *et al.,* 2008).

RESULTS AND DISCUSSION

In Ethiopia, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. To date, however, only a few of such medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken (WHO, 1980). *S. punctata* is one of such medicinal plants commonly used in Ethiopian traditional medicine. In addition to the treatment of diabetes, preparations made from the aerial parts of several of these species are used in Ethiopian traditional medicine to treat headache, stop menstruation, relieve stomach pains, and improve the quality of milk (Getahun Abate, 1989; Ryding, 2006). Since streptozotocin is known to destroy insulin-producing pancreatic β-cells, the STZ-treated mice model appears to represent a good laboratory non-Insulin-dependent diabetes mellitus (NIDDM) experimental diabetic state, with residual or remnant insulin production by the pancreatic β-cells. The diabetic state of STZ-treated diabetic mice is, therefore, not the same as that obtained by total pancreatectomy, as daily administration of insulin is not required for survival in STZ-treated diabetic animals (Adeyemi and Agbaje, 2008). In this experiment administration of the STZ (150 mg/kg mg/kg: i.p.) led to significantly elevated levels of serum glucose.

Activities of crude extracts

The fasting mean blood glucose level values before and after treatment for four hrs in diabetic control and diabetic mice treated with aqueous and 80% methanol extracts of *S. punctata* are presented in Table 1. Comparisons were made at 0 time vs 0.5, 1, 2, 3, and 4 hr.

The blood glucose in diabetic mice was reduced sharply after oral administration of aqueous and 80% MeOH extracts of the aerial parts of *S. punctata* and glibenclamide. When comparisons were made at 0 time (initial blood glucose level) vs different time intervals, blood glucose levels declined sharply from 441.3 - 339.0 mg/dl on the 3rd hr after oral feeding of the 80% methanol extract (300 mg/kg). Similarly, on the 3rd hr after oral administration of 300 mg/kg of the aqueous extract the level was reduced from 458.3 - 351.7 mg/dl. Glibenclamide (40 µg/kg) also showed maximum effect on the 3rd hr reducing blood glucose level from 383.5 - 162.5 mg/dl.

Table 1. Effect of oral administration of crude extracts 300 mg/kg of *Satureja punctata* on fasting blood glucose levels (mg/dl) in STZ-induced diabetic mice.

Group	Blood glucose level in mg/dl					
	0 h	0.5 h	1 h	2 h	3 h	4 h
Diabetic control	455.0±7.7	463.7±8.0	469.3±8.5	476.0±8.1	458.0±6.3	446.7±6.6
80% MeOH extract	441.3±10.6	409.7±12.6^{NS}	393.2±15.1^{NS}	368.0±19.0**	339.0±28.0** (23.1)	341.7±28.8**
Aqueous extract	458.3±11.8	428.0±12.1^{NS}	393.5±21.1*	381.2±26.5**	351.7±22.9** (23.3)	352.7±23.3**
Glibenclamide (40 µg/kg)	383.5±23.0	292.8±20.0**	276.3±10.0**	227.0±19.0**	162.5±18.0** (57.8)	163.2±19.0**

Values are given as mean ± S.E.M for groups of six animals. Values are statistically significant at *p< 0.05 and **p<0.01. Comparison is made at 0 time (initial) Vs 0.5, 1, 2, 3, and 4 hr. Figures in parenthesis indicate maximum percent reduction in blood glucose levels. NS - Non significant.

As shown in Table 1, this decline in blood glucose levels of drug treated groups when compared with 0 time was found to be statistically significant ($p < 0.01$). The maximum percent reductions in blood glucose levels for the aqueous extract, methanol extract and glibenclamide were found to be 23.3%, 23.1% and 57.8%, respectively, at the 3rd hr. When compared with the antidiabetic effect of the crude aqueous extract of *Catharanthus roseus* (20.2%) (Singh et al., 2001), both extracts showed better activity. The effect of both extracts is also similar to the one reported by Bahatia *et al*. (2011) where the maximum percentage reduction of about 23% for the methanol and ethanol extracts of the aerial part of *Boerhaavia diffusa* was reported. However, the extracts were less potent than the leaf extracts of *Smallanthus sanchifolius* (Genta *et al*., 2010) and root extracts of *Ibervillea sonorae* (Alarcon-Aguilar *et al*., 2005).

Activities of solvent fractions

As shown in Table 2, oral administration of (300 mg/kg body weight) of the methanol and aqueous fractions of *S. punctata* resulted in hypoglycaemic activity on STZ-induced diabetic mice. On the other hand, the petroleum ether, chloroform and acetone fractions did not show any hypoglycaemic activity.

Literature review reveals that percent decline in blood glucose level is used for comparison of the hypoglycaemic activity of different extracts, fractions, and isolated compounds (Prasad *et al.,* 2009). Hence if decline in blood

glucose levels is to be the only index, then treatment with methanol fraction of *S. punctata* and glibenclamide has proved highly effective in causing significant antihyperglycaemic response in STZ-induced diabetic mice.

Table 2. Percent blood glucose reduction of the various solvent fractions of *Satureja punctata* at a dose of 300 mg/kg and glibenclamide (40 µg/kg).

	Percent blood glucose reduction				
Group	0.5 h	1 h	2 h	3 h	4 h
Diabetic control	-1.9±0.0	-3.1±0.2	-4.6±0.0	-0.7±0.3	1.8±0.0
Pet. ether fraction	-6.4±0.2	-4.2±0.2	-2.6±0.3	2.7±0.1	1.9±0.0
$CHCl_3$ fraction	-20.1±0.9	-13.3±8.1	-9.8±5.1	-2.6±2.5	0.9±1.1
Acetone fraction	-6.4±0.4	7.7±4.1	2.8±4.0	3.1±2.3	0.1±0.0
MeOH fraction	14.6±0.0	23.2±1.3	33.2±4.3	38.1±3.1	37.9±3.0
Aqueous fraction	12.8±5.2	13.4±3.5	19.3±5.0	19.1±4.4	19.4±4.1
Glibenclamide	23.7±0.7	27.9±1.6	40.9±1.3	57.8±2.3	57.6±2.4

Values are given as mean ± S.E.M for groups of six animals.

Table 2 shows that the hypoglycaemic activity of the aqueous fraction of *S. punctata* (19.4% maximum reduction) is less than that of the aqueous extract (23.3% maximum reduction) at the 3rd hr. This can be due to the presence of additional compounds in the aqueous extract, which was directly obtained from the plant unlike the aqueous fraction which was obtained after fractionating the plant material with other polar and nonpolar solvents. On the other hand, the methanol fraction (38.1% maximum reduction) was more active than that of the 80% MeOH extract (23.1% maximum reduction) at the 3rd hr after oral administration.

As shown in Table 3, the methanol fraction of *S. punctata* showed significant hypoglycaemic activity ($p<0.01$) at the 3rd hr after oral administration of 100, 200 and 300 mg/kg body weight on STZ-induced diabetic mice. This hypoglycaemic activity corresponds to 14.5%, 24% and 38.1% maximum reduction in blood glucose levels, respectively, indicating the improvement in blood glucose homeostasis was in a dose-dependent manner. The results of this finding are slightly lower than the hypoglycemic effect reported for the aqueous and butanol fractions of the stem bark of *Phyllanthus sellowianus* (Hnatyszyn *et al*., 2002).

Table 3. Hypoglycaemic activity of the methanol fraction of *Satureja punctata* after oral administration of different doses on STZ- induced diabetic mice.

Group	Dose (mg/kg)	Blood glucose level in mg/dl					
		0 h	0.5 h	1 h	2 h	3 h	4 h
Diabetic. control		353.3±7.5	366.7±8.3	370.0±8.3	376.0±8.4	358.3±6.4	345.5±6.7
MeOH fraction	100	349.0±9.5	331.3±9.0^{NS}	315.2±12.0*	307.3±8.9*	298.5±4.2*(14.5)	303.2±4.2*
MeOH fraction	200	375.5±13.9	340.3±7.8*	326.0±6.5*	316.7±6.4*	285.2±5.0*(24.0)	294.7±6.6*
MeOH fraction	300	426.5±23.5	264.2±20.0*	325.5±23.8*	285.0±32.2*	265.0±28.1*(38.1)	265.3±27.0*
Glibenclamide	40	383.5±23.0	292.8±20.0*	276.3±10.0*	227.0±19.0*	162.5±18.0*(57.8)	163.2±19.0*

Values are given as mean ± S.E.M for groups of six animals. Values are statistically significant at *p<0.01. Comparison is made at 0 time (initial) Vs 0.5, 1, 2, 3, and 4 hr. Figures in parenthesis indicate maximum percent reduction in blood glucose levels. NS - Non significant

CONCLUSION

In Ethiopia the aerial parts of *S. punctata* are traditionally used as a therapeutic agent for diabetes mellitus and other diseases. In the present study, it was observed that the total extracts as well as the polar solvent fractions of *S. Punctata* possess significant blood sugar lowering effect in streptozocin-induced diabetic mice. In addition, the methanol fraction of *S. punctata* was shown to display its antihyperglycaemic activity in a dose dependant manner. This study has revealed that *S. punctata* extracts are less potent than glibenclamide even at the time interval during which the extracts showed maximum blood glucose reduction, i.e. during the 3rd hr following administration of the test samples. Neverthless, the study indicated that the extract of the plant, particularly that of the methanol fraction require further investigation so as to isolate and characterize the component(s) responsible for activity. The preliminary results reported here further illustrate that correlations exist between the popular traditional use and genuine antidiabetic activities.

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REFERENCES

Adeneye, A.A and Agbaje E.O. (2008). Pharmacological evaluation of oral hypoglycaemic and antidiabetic effects of fresh leaves ethanol extract of *Morinda lucida* Benth. in normal and alloxan-induced diabetic rats. *Afr. J. Biomed. Res.* **11**: 65-71.

Alarcon-Aguilar, J.F., Jimenez-Estrada, M., Reyes-Chilpa, R. and Roman-Ramos, R.

(2000). Hypoglycaemic effect of extracts and fractions from *Psacalium decompositum* in healthy and alloxan-diabetic mice. *J. Ethnopharmacol*. **72**:21–27.

Bhatia, V., Kinja, K., Bishnoi, H., Savita, S. and Gnaneshwari, D. (2011). Antidiabetic activity of the alcoholic extract of the arial part of *Boerhaavia diffusa* in rats. *Recent Res. Sci. Technol.* **3:** 04-07.

Chagonda, L.S. and Chalchat, J. (2005). The composition of the leaf essential oil of *Satureja punctata* Benth. Briq. from Zimbabwe. *Flavour Fragr. J*. **20**: 316–317.

De Carvalho, E.N., Carvalho, N.A.S. and Ferreira, L.M. (2003). Experimental model of induction of diabetes mellitus in rats. *Acta Cir. Bras.* **18**: 60-64.

Eshrat, M. (2002). Hypoglycaemic, hypolipidemic and antioxidant properties of combination of curcumin from *Curcuma longa* Linn, and partly purified product from *Abroma augusta* Linn. in streptozotocin induced diabetes. *Indian J. Clin. Biochem.* **17**: 33-43.

Gentaa, B.S., Cabreraa, W.M., Mercadob, I.M., Grauc, A, Catalánb, C.A. and Sáncheza, S.S. (2010). Hypoglycaemic activity of leaf organic extracts from *Smallanthus sonchifolius*: Constituents of the most active fractions. *Chem-Biol. Interact.* **185:**143–152.

Getahun Abate (1989). Etse Debdabe (Ethiopian Traditional Medicine), pp.95, 114 (Sebsebe Demissew, ed.). Artistic Printing Press, Addis Ababa, Ethiopia.

Grover, J.K., Vats, V., Rathi, S.S. and Dawar, R. (2001). Traditional Indian antidiabetic plants attenuate renal hypertrophy, urine volume and albuminuria in streptozotocin-induced diabetic mice. *J. Ethnopharmacol*. **76**: 233-238.

Hnatyszyn, O., Mino, J., Ferraro, G., and Acevedo, C. (2002). The hypoglycemic effect of *Phyllanthus sellowianus* fractions in streptozotocin-induced diabetic mice. *Phytomedicine* **9**: 556–559.

Ivorra, M.D., Paya, M. and Villar, A. (1989). A review of natural products and plants as potential antidiabetic drugs. *J. Ethnopharmacol.* **27**: 243-275.

Kumar, A., Ilavarasan, R., Jayachandran, T., Deecaraman, M., Aravindan, P., Padmanabhan, N. and Krishan, M. (2008). Antidiabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. *J. Med. Plant Res*. **2**: 246-249.

Kumar, G.P.S., Arulselvan, P., Kumar, D.S. and Subramanian, S.P. (2006). Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin-induced diabetic rats. *J. Health. Sci*. **52**: 283-291.

Matthew, K., Olugbenga, O., Olajide, O. and Doyin, A. (2006). The effect of *Bridelia ferruginea* and *Senna alata* on plasma glucose concentration in normoglycaemic and glucose-induced hyperglycaemic rats. *Ethnobot. Leaflets* **10**: 209-218.

Ozbek, H., Ceylan, E., Kara, M., Ozgokce, F. and Koyuncu, M. (2004). Hypoglycaemic effect of *Rheum ribes* roots in alloxan induced diabetic and normal mice. *Scand. J. Lab. Anim. Sci*. **31**: 113-115.

Prasad, S., Kulshreshtha, A. and Qureshi, T. (2009). Antidiabetic activity of some herbal plants in streptozotocin-induced diabetic albino rats. *Pakistan J. Nutr*. **8**: 551-557.

Ragavan, B. and Krishnakumari, S. (2006). Antidiabetic effect of *Termanalia arjuna* bark extract in alloxan-induced diabetic rats. *Ind. J. Clin. Biochem.* **21**: 123-128.

Rao, N. (2006). Anti-hyperglycaemic and renal protective activities of *Andrographis paniculata* roots chloroform extract. *J. Pharmacol. Ther.* **5**: 47-50.

Ryding, O. (2006). Gentianaceae to Cyclochilaceae. In: **Flora of Ethiopia and Eritrea,** Vol. 4, pp. 302-366 (Hedberg., I., Ensermu Kelbesa, Edwards, S., Sebsebe

Demissew and Persson, E., eds.). The National Herbarium, Addis Ababa University, Addis Ababa.

Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumria, M.M.L., Ranganathan, S. and Sridharan, K. (2001). Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* **76**:269–277.

Swanston-Flatt, S.K., Day, C., Flatt, P.R., Gould, B.J. and Bailey, C.J. (1989). Glycemic effects of traditional European plant treatments for diabetes: Studies in normal and streptozotocin diabetic mice. *Diabetes Res.* **10**: 69–73.

Williamson, E., Okpako, D. and Evans, F. (1996). **Selection, preparation and pharmacological evaluation of plant material**. pp. 155-168. John Wiley and Sons Ltd., Chichester.

World Health Organization (WHO) (1980). Expert committee on *Diabetes mellitus*: 2nd Report. Technical Report Series Number 646, pp 61, World Health Organization, Geneva.

Yenebeb Tariku, Ariaya Hymete, Asrat Hailu and Rohloff, J. (2010). Essential-oil composition, antileishmanial, and toxicity study of *Artemisia abyssinica* and *Satureja punctata* ssp. punctata from Ethiopia. *Chem. Biodiv.* **7**: 1009-1018.