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Research Article

ANTIHYPERGLYCAEMIC EFFECT OF *VETIVERIA ZIZANIOIDES* (L.) NASH ROOT EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT
To evaluate the antihyperglycaemic activity of the ethanol extract of roots of *Vetiveria zizanioides* (L.) Nash, in alloxan induced diabetic rats. Alloxan monohydrate (150 mg/kg) was administered to Wister albino rats via the interperitoneal route. The diabetic rats were then placed into 6 groups, following stabilization of hyperglycaemia. The first group was untreated; second group received a reference standard, glibenclamide (10 mg/kg), and the next four groups received ethanol extract of *V. zizanioides* roots at the doses of 100, 250, 500 &750 mg/kg, respectively each day. Treatment was provided via the oral route for 28 days and fasting sugar level was monitored over this period. Acute toxicity (oral and interperitoneal) studies as well as phytochemical screenings of the extracts were carried out. It was observed that ethanol extract of *V. zizanioides* (100, 250, 500 & 750 mg/kg p.w.o) significantly reduced the blood-glucose level at the end of 28th days in alloxan induced diabetes rats. The antihyperglycaemic effects were compared with those of glibenclamide (10 mg/kg p.w.o), the standard reference hypoglycaemic agent. The present study indicates that ethanol extract of *V. zizanioides* root posses better antihyperglycaemic activity compared to other extracts of it in both normal and alloxan induced diabetic rats. The study provides a proof for the ethno medical claims and reported biological activities of *V. Zizanioides* in respect to its very good therapeutic and antidiabetic potential.
Keywords: Antihyperglycemic activity; *Vetiveria zizanioides*; alloxan; diabetes.

INTRODUCTION

Vetiveria zizanioides (L.) Nash (family: Poaceae) is commonly known as 'Khas-Khas' in Bangladesh and India. It is a perennial grass with thick fibrous adventitious roots[1]. This species is native of Indian subcontinent and has been introduced in many tropical countries. Roots are stimulant, tonic, cooling, stomachic, diuretic, antispasmodic and emmenagogue, and used in fevers, inflammations and irritation of the stomach. Essence of the root is used to check vomiting in cholera. Smoke of grass is inhaled to relieve headache. Apart from its use as an insect repellent and soil erosion management tool, vetiver grass has numerous traditional uses such as root paste for headaches and leaf paste for rheumatism and sprains. Commercial uses of vetiver grass mainly pertain to the extraction of vetiver oil through distillation of the roots. Vetiver oil has extensive applications in the soap and cosmetic industries, food flavouring and is also used as an anti-microbial and anti-fungal agent in the pharmaceutical industry[2]. This oil is principally used in high class perfumery where its persistent odour makes it of great value as a fixative in admixture with other perfumes. Vetiver grass is also cultivated for the production of a commercially important essential oil used in perfumery and aromatherapy[3-6]. Various tribal people in the subcontinent use different parts of the grass for many ailments such as mouth ulcer, fever, boil, epilepsy, burn, snakebite, scorpion sting, rheumatism, headache, etc[7]. Over 150 compounds have been isolated and characterized from vetiver oil so far. The complex odour of vetiver oil is dominated by a woody balsamic tonality of a very special kind. This tonality indicates the presence of some volatile compounds that have been reported to be mainly sesquiterpenes and their derivatives. Among these, the major active constituents identified are khusimol, vetivone, eudesmol, khusimone, zizaene, prezizaene, β-vetispirene, khusimol, vetiselinenol and α-vetivone. As, there is no report available on the literature survey regarding the antidiabetic activity, the present investigation was undertaken to determine the antihyperglycaemic effect of *V. zizanioides* root extract in alloxan induced diabetic rats to substantiate the folklore claim[8].

Diabetes mellitus is the most common metabolic disorder characterized by hyperglycemic, glucoseurea and negative nitrogen balance and it is mainly due to lack of insulin secretion in beta cells of pancreas and desensitization of insulin receptors for insulin. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people. It is expected to touch 300 million marks by 2025. In diabetes, hyperglycaemia generates reactive oxygen species (ROS), which in turn causes lipid peroxidation and membrane damage and these free radicals play an important role in the production of secondary complications in diabetes mellitus (kidney, eye, blood vessel, and nerve damage)[9]. Herbal drugs are gaining popularity in the treatment of diabetic mellitus. The major advantages of herbal medicine seem to be their efficacy, low incidence of side effects, low cost and easily available.

The preliminary phytochemical studies on ethanol extract of *V. zizanioides* showed the presence of glycosides, steroids, saponin, tannin, flavonoids and phenolic compounds.

EXPERIMENTAL

Plant materials

The roots of *V. zizanioides* were collected in the month of August, from the Simlipal Biosphere in the district of Mayurbhanj, North Odisha, India. The collected plant was authenticated by Botanical survey of India, Central National Herbarium, Botanical garden, Shibpur, Howrah, India, vide letter no CNH/1-1/231/2008/Tech. II/261.

Preparation of extracts

The roots were dried under shade and powdered to coarse particles. Two kg powdered plant materials were defatted with petroleum ether (60-80 ºC) in a Soxhlet extraction apparatus and further the same plant materials was extracted

successively with ethanol. The yield of the ethanol (EVZ) extract was found to be 1.47% w/w and 1.63% w/w. EVZ on pharmacological screening gave the best results and hence was selected for the present study.

Drugs and chemicals

Alloxan and glibenclamide (Merck and S.N. Chemicals, respectively) were used for these investigations. All other chemicals, solvents and drugs used were of analytical grade.

Phytochemical screening of EVZ

Small amount of dried extract (EVZ) was appropriately treated to prepare sample solution and then subjected to the phytochemical tests. The phytochemical screening of EVZ was performed using the following reagents and chemicals: alkaloids with Dragendorff's reagent; flavonoids with the use of Mg and HCl; tannins and phenolic compounds with ferric chloride and potassium dichromate solutions; steroids with Libermann Burchard reagent; terpenoids with tin and thionyl chloride; amino acids with ninhydrin solution and saponins with ability to produce suds; glycosides with chloroform and concentrated sulphuric acid. These were identified by the characteristic colour changes as per standard procedures.

Experimental animals

Male Wistar rats weighing 250-350 g of both sexes were selected for the present investigation. They were housed in clean polypropylene cages and were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to normal laboratory condition for one week before proceeding of the experiment. The experiment was performed under the guidance of the Institutional Ethical Committee approval no 12/09/IAFC/SOAU.

Preparation of drug solution

EVZ was dissolved in normal saline and administered at the doses of 100, 250, 500 and 750 mg/kg body weight of rats.

Acute toxicity studies

Acute toxicity study was performed for the extract according to the acute toxic classic method of Litchfield and Wilcoxon (1949)[10]. The animals were divided into six groups containing 10 animals each. The EVZ suspension was administrated orally in increasing dose up to 1500 mg/kg, b.w. These animals were observed for mortality and toxicity for 72 h.

Induction of experimental diabetes

Diabetes was induced in wistar rats of either sex by a single intraperitoneal injection of aqueous alloxan monohydrate (150 mg/kg, i.p.)[11]. After 48 h of injection, animals with the serum glucose level above 200 mg/dl (diabetic) were selected for the study.

Collection of blood and determination of serum glucose

Blood samples were collected by cutting the tail vein of rats and blood glucose levels are checked by Glucometer (Dr. Morepen, 9F, 31 codes).

Experimental protocol

The method described by Dunn et al. (1943) was adopted to determine the serum glucose in alloxan-induced diabetic rats. The diabetic rats were fasted overnight and divide into six groups of six rats each[12].

Group-I - Vehicle (normal saline, 10 ml/kg p.o.)
Group-II - Glibenclamide (10 mg/kg p.o.)
Group-III - EVZ (100 mg/kg p.o.)
Group-IV - EVZ (250 mg/kg p.o.)
Group-V - EVZ (500 mg/kg p.o.)
Group-VI - EVZ (750 mg/kg p.o.)

For acute antihyperglycaemic study, blood samples were collected at 0, 2, 4, 6, 8 and 24 h, after administration of vehicle, glibenclamide and EVZ. Sub acute study involved administration of vehicle, glibenclamide and EVZ. At different concentrations, the blood-glucose levels were estimated on the day 0, 2, 14, 16, 18 and 24th day. Mean changes in blood-glucose levels were calculated.

Statistical analysis

Results are expressed as mean ± SD. Comparison between the groups with control was made by one way analysis of variance (ANOVA), followed by Dunnett's test. The values of $P<0.05$ were considered as statistically significant.

Table 1: Effect of multiple dose of ethanol extract of *V. zizanioides* roots (EVZ) and standard hypoglycaemic drug on blood glucose levels in normal fasted rats (acute study)

Drug	0 hr (mg/dl)	2 hr (mg/dl)	4 hr (mg/dl)	6 hr (mg/dl)	8 hr (mg/dl)	24 hr (mg/dl)
Control	104.2±2.79	83.67±3.98	95.83±2.65	91.83±3.55	97.33±2.16	100.8±3.76
Glibenclamide	100.1±2.61*	65.50±3.08*	84.67±2.81*	86.83±2.82*	93.17±2.32*	104.5±1.87*
EVZ (100 mg/kg)	101.8±4.36*	65.67±5.89*	75±2.61*	82.33±3.33*	86.50±3.08*	94.67±2.58*
EVZ (250 mg/kg)	93.67±4.41*	62±3.03*	81.17±3.06*	91.67±3.98*	91±3.03*	85.33±2.58*
EVZ (500 mg/kg)	103.5±3.83*	59.50±3.39*	70.50±2.43*	80.83±3.06*	82.67±3.39*	91.83±3.55*
EVZ (750 mg/kg)	86.17±2.40*	63.50±2.43*	65.67±2.94*	73±2.61*	82.67±3.01*	91.67±3.59*

Values are mean ± SD. (n=6), *P<0.05 considered significant in comparison to control group.

Table 2: Effect of multiple dose of ethanol extract of *V. zizanioides* roots (EVZ) and standard hypoglycaemic drug on blood glucose levels in alloxan induced diabetic rats (acute study)

Drug	0hr (mg/dl)	2hr (mg/dl)	4hr (mg/dl)	6hr (mg/dl)	8hr (mg/dl)	24hr (mg/dl)
Control	241.0±2.61	159.0±4.43	175.5±1.83	184.0±3.23	218.2±3.91	227.7±5.54
Glibenclamide	261.3±2.81*	168.3±2.16*	181.5±1.87*	199.5±1.86*	223.3±3.78*	254.7±2.34*
EVZ (100 mg/kg)	255.5±1.87*	172.3±2.58*	166.5±2.88*	199.5±1.84*	219.0±7.51*	245.0±4.47*
EVZ (250 mg/kg)	263.5±3.73*	165.8±2.14*	172.5±1.82*	212.3±3.01*	233.3±3.78*	261.2±7.57*
EVZ (500 mg/kg)	272.2±4.79*	186.3±2.81*	190.8±5.12*	218.0±2.37*	244.5±3.27*	265.5±2.17*
EVZ (750 mg/kg)	276.5±3.08*	149.2±4.36*	167.2±2.32*	197.5±1.87*	241.3±6.62*	277.5±5.08*

Values are mean ± SD. (n=6), *P<0.05 considered significant in comparison to control group.

Table 3: Effect of multiple dose of ethanol extract of *V. zizanioides* roots (EVZ) and standard hypoglycaemic drug on blood glucose level in alloxan induced diabetic rats (sub acute study)

Drug	Day0 (mg/dl)	Day1 (mg/dl)	Day7 (mg/dl)	Day14 (mg/dl)	Day21 (mg/dl)	Day28 (mg/dl)
Control	207.5±1.82	233.0±3.74	280.5±1.87	285.5±1.86	294.5±1.87	298.3±2.16
Glibenclamide	205.2±2.48*	211.0±3.74*	261.5±1.87*	141.0±3.74*	124.5±1.84*	119.2±2.32*
EVZ (100 mg/kg)	221.5±3.27*	222.2±2.48*	208.0±2.61*	241.0±3.74*	198.5±1.87*	193.0±3.74*
EVZ (250 mg/kg)	240.5±1.88*	184.5±1.86*	176.0±2.61*	180.5±1.87*	158.5±1.80*	141.7±4.63*
EVZ (500 mg/kg)	208.8±3.76*	213.0±3.74*	188.5±1.81*	205.5±1.86*	171.5±1.89*	155.0±3.58*
EVZ (750 mg/kg)	255.4±3.08*	236.2±1.47	228.5±1.87*	231.5±1.84*	187.5±1.85*	176.3±3.14*

Values are mean ± SD. (n=6), *P<0.01 considered significant in comparison to control group.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of EVZ showed the presence of flavonoids, sterols, saponins and polyphenolic compounds.

The effects of root extract of *V. zizanioides* (L.) in normal fasted rats after multiple doses were presented in Table-1. It was observed that EVZ showed significant antidiabetic activity at 2^{th} and 4^{th} hour after administration of drug compared to diabetic control. The results were comparable with reference standard glibenclamide. The results related to blood-glucose level in alloxan induced diabetic rats after the multiple dose in acute and sub acute studies are presented in Table 2 and Table 3, respectively.

It was observed that only EVZ showed more significant antidiabetic activity than other extracts of *V. zizanioides*. EVZ showed more significant antidiabetic activity at 2^{th} and 4^{th} h compared to diabetic control.

On prolonged treatment, EVZ showed more significant antidiabetic activity at 7^{th}, 21^{th} and 28^{th} day compared to control (Table-3). The results of present experiments revealed that EVZ has significant antihyperglycaemic activity. Effects of oral administration of EVZ in euglycaemic rats compared with glibenclamide are presented in Table 1. Glibenclamide reduced blood sugar levels significantly and showed hypoglycaemic effects considerably.

Preliminary phytochemical analysis of ethanol extracts revealed the presence of flavonoids, sterols, saponins and polyphenolic compounds. Flavonoids and saponins containing plants were known to exhibit antidiabetic activity[13]. Hence, the presence of flavonoids and sterol in the ethanolic extract of *V. zizanioides*, may be responsible for such activities. Isolation and characterization of some new active constituents, which may give new lead molecules for the treatment of diabetes in a more acceptable manner is the scheme for further investigation.

CONCLUSION

Based on the aforementioned results, we concluded that EVZ has hypoglycaemic and antihyperglycaemic activity as it lower blood-glucose level in diabetic rats. However, the molecule(s) responsible for such an effect requires further investigation.

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