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Full Length Research Paper

Hypoglycaemic effect of aqueous stem extract of *Anisopus mannii* in normal rats

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The hypoglycaemic effect and acute oral toxicity of graded doses of aqueous extract of *Anisopus mannii* was investigated in rats. The revised limit dose test of Up and Down procedure was used to determine the acute oral toxicity of the plant. The result revealed that the Median lethal dose of the plant is greater than 3000 mg/kg body weight. The treatment of the rats with varying doses of the plant extract for 28 days in this studies resulted in increases in body weight although not statistically significant (P > 0.05). The repeated administration of graded doses of the extract also produces significant (P < 0.05) decreases in the blood sugar levels of the treatment groups compared to their respective day zero values thus justifying its folkloric use as a potential hypoglycaemic agent.

Key words: *Anisopus mannii*, hypoglycemic effect, rats.

INTRODUCTION

Dependence on herbs as medicine in the treatment of disease is common among a large proportion of the rural populace because of its availability and affordability (Sani et al., 2009). Due to the increasing awareness of the importance of traditional medicine in human and animal health care, researches into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile. This is not only to supplement modern drugs with effective local herbs for economics reasons but also to fill the gap created by recurring shortage of conventional drugs (Sofowora, 1982). WHO (1993) support the use of effective and safe remedies and accept traditional medicine as a valuable and readily available resources for primary health care. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Hyperglycaemia is a condition characterized by a high amount of serum glucose causing damage to small vessels, hence increase the risk of late stage diabetes complications such as neuropathy, heart attack, hypertension, stroke (Henning and Jan, 2004), Atherosclerosis and microangiopathic vascular diseases (Murray et al., 1998). In the pursuit of the global health challenge for further evaluation of the folkloric methods of managing disease, several medicinal plants are being screened for their potential hypoglycaemic efficacies. One of such plant is *Anisopus mannii* of the family Asclepiadaceae).

The plant is a glabrous twining shrub, strong climber with greenish flower in globose, lateral umbelliform cymes and horizontally opposite follicle 6 - 8 inch long and about half inch thick, tapering to a slightly hooked point at the apex (Hutchinson and Dalziel, 1963). It is known as 'Sakayau' or 'Kashe zaki' (meaning sweet killer) among the Hausas of the northern Nigeria, where a cold decoction of the stem is traditionally used as hypoglycaemic agent (Sani et al., 2009) . Despite the widespread use of this plant, there is little information in scientific literature providing information on its hypoglycaemic as well as its toxicological effect. Recent studies show that aqueous stem extract of *A. mannii* contains pharmacological active compounds (like saponins, flavonoids, alkaloids, glycosides, terpenes and steroids) and possess anti bacterial activity (Sani et al., 2009).

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This present study investigates the hypoglycaemic effect of *A. mannii* in normal male rats.

MATERIALS AND METHODS

Collection, identification and processing of plant material

Stems of *A. mannii* were collected from Kuwayange village in Damboa Local Government area of Borno state, Nigeria, in January, 2008. The plant was identified and authenticated by Prof. S.S. Sanusi of Botany Department, University of Maiduguri, Borno state, Nigeria where a voucher specimen was deposited for reference with specimen no. DSN 01. The stems were sorted, cleaned and air dried at room temperature for 2 weeks and then crushed to powder using pestle and mortar. The powdered samples were then collected and stored in a clean polythene bag until required for extraction.

Preparation of aqueous extract

About 300 g of the powdered sample was mixed with 1.5 L of distilled water, in a conical flask. The mixture was homogenously mixed, allowed to stay overnight at room temperature and then filtered using Whatman filter paper (No 1). The filtrate was then evaporated to dryness using oven preset at 50°C and was later stored at 4°C. The yield of the extract was 24.7% w/w.

Experimental animal

Thirty (30) adult male albino rats, 9 - 10 weeks old, weighing between 84 and 200 g were procured from the laboratory animal house of the Department of Veterinary Pathology of the University of Maiduguri, Borno, Nigeria. The rats were kept in metal cage, fed with commercial standard growers feed (Vital Feeds, Bukuru, Jos, Nigeria) and water *ad libitum* and maintained under standard laboratory condition prior to the procedure. The animals were handled in accordance with the international guiding principles for biochemical research involving animals (C.I.O.M.S 1985).

Acute oral toxicity study

The limit dose test, up and down procedure as revised by Dixon (1965, 1991) was used to evaluate the acute oral toxicity of aqueous extract of *A. mannii* stem in adult male rats. Five (5) adult male rats were randomly selected for the experiment. They were marked and housed individually in cages in the laboratory for 7 days to allow for acclimatization to the laboratory conditions.

The rats were fasted overnight but allowed free access to water prior to dosing on each occasion. A rat from group A was picked, weighed and dosed orally with a limit dose 3000 mg/kg body weight of the freshly prepared aqueous extract. Another animal from the same group was given the same dose of the extract until all the animals in the group were fed with the same dose of the extract. Each animal was observed each time for instant death and then watched for the successive 24 h for the short-term outcome and finally for the next 12 days for any delayed toxic effects.

Effect of aqueous stem extract of *Anisopus mannii* on blood glucose level of normal rats

Twenty five adult male rats weighing between 84 and 200 g was randomly assigned into five groups of five rats each. Groups B, C, D and E were orally administered graded doses, namely 100, 200, 300 and 400 mg/kg respectively of the aqueous extract of *A. mannii* for 28 days, while group A (control) received normal saline for the same period. A drop of blood samples from the tail vein of the rats was applied to the test spot of one touch glucose meter (Asatoor and Kings, 1954) for estimation of blood glucose levels on zero day (before extract administration) and weekly thereafter, prior to subsequent extract administration. The blood glucose levels of the rats before extract administration (at day zero) ranges between 70.6 and 78.6 mg/dl.

Statistical analysis

All data obtained during this study was analysed using Graph Pad Instat version 3.0 (2003) computer statistical software package and expressed as mean ± standard error of mean (S.E.M.). $P \leq 0.05$ will be considered significant.

RESULT

Acute oral toxicity studies

There were no deaths of rats dosed 3000 mg/kg body weight of the aqueous plant extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1) . The LD_{50} was calculated to be greater than 3000 mg/kg body weight /orally.

Effect of *Anisopus mannii* aqueous stem extract on bodyweight of rats

The treatment of the rats with varying doses of the plant extract for 28 days in this studies resulted in increases in body weight which is dose and time dependent although not statistically significant ($P > 0.05$), (Table 2).

Effect of *Anisopus mannii* on blood glucose level of normal male rats

The extract produces significant ($P < 0.05$) dose and time dependent decreases in the blood sugar levels of the treatment group compared to their respective day zero values (Table 3).

DISCUSSION

The observed hypoglycaemic effect of *A. mannii* in this study is an indication that the plant contains active constituents with potent hypoglycaemic property which could be acting via increased peripheral glucose utilization. Recent studies have revealed that the aqueous stem extract of *Anisopus mannii* contains various pharmacological active compounds like saponins, flavonoids, alkaloids, glycosides, terpenes and steroids (Sani et al., 2009). Literature has shown the biological

Table 1. Result of limit dose test of aqueous stem extract of *A. mannii* in rats.

Test sequence	Animal ID	Dose (mg/kg)	Short-term result (48 h)	Long-term result (12 days)
1	A	3000	Survival	Survival
2	B	3000	Survival	Survival
3	C	3000	Survival	Survival
4	D	3000	Survival	Survival
5	E	3000	Survival	Survival

ID identification number.

Table 2. Effect of intake of aqueous stem extract of *A. mannii* on bodyweight (g) of albino rats.

Dose	Days of treatment				
mg/kg	0	7	14	21	28
Control	124.6 ± 10.2	140.4 ± 6.3	142.6 ± 6.2	153.4 ± 8.8	162.0 ± 8.4
100	115.6 ± 13.3	131.0 ± 15.6	145.0 ± 16.7	156.4 ± 19.1	162.2 ± 19.7
200	113.8 ± 8.4	129.0 ± 12.1	144.8 ± 12.5	160.2 ± 14.6	168.6 ± 15.0
300	140.0 ± 5.1	152.8 ± 8.4	166.8 ± 8.7	176.4 ± 9.2	184.8 ± 8.5
400	115.2 ± 17.6	129.8 ± 16.3	139.0 ± 14.0	145.0 ± 11.5	149.6 ± 10

Mean ± SEM, n = 5.

Table 3. Effect of intake of aqueous stem extract of *Anisopus mannii* on blood glucose levels (mg/dl) of normal albino rats.

Dose (mg/kg)	Days of treatment				
	0	7	14	21	28
Control	70.6±2.7	71.0±4.7 (+0.57)	71.2±4.9 (+0.85)	71.2±4.6 (+0.85)	71.6±4.7 (+1.42)
100	78.6±1.5	76.4±4.5 (-2.80%)	58.4±6.6 (-25.70)	53.6±5.7[a] (-31.80)	63.6±6.0 (-19.08)
200	78.0±4.7	74.8±2.5 (-4.10)	59.6±3.3 (-23.59)	54.8±2.4[a] (-29.74)	67.0±3.5 (-14.10)
300	71.8±1.8	69.6±4.7 (-3.06)	54.8±5.4 (-23.68)	48.2±3.4[a] (-32.87)	60.4±3.3 (-15.88)
400	75.6±5.1	72.4±2.4 (-4.23)	56.8±4.6 (-24.87)	50.8±1.7[a] (-32.80)	53.4±3.4[a] (-29.37)

Mean ± SEM, n = 5.
a = significantly ($P < 0.05$) lower than day zero value.
Numbers in bracket indicates percentage increase (+) or decrease (-) in blood glucose levels when compared with day zero.

activities of alkaloids and flavonoids to include hypoglycaemia, hypolipidemia, hypoazotemia, hypotension among other biological effects (Oladele et al., 1995; Sudheesh et al., 2005; Sanni, 2007) . Alkaloids have been reported to produce hypoglycaemic effect in mice (Kubo et al., 2000). Saponins have also been reported to produce hypoglycaemic effect in mice (Li et al., 2002), while flavonoids was reported to produce hypoglycaemic effect in rats (Anila et al., 2002). The presence of these active biological principles in *Anisopus mannii* stem extract could be responsible for the oral hypoglycaemic effect obtained in this study. The oral administration of the aqueous stem extract of *A. mannii* at limit dose of 3000 mg/kg body weight did not produced any sign of acute toxicity or instant death in any of the five (5) rats tested. This suggests that the extract has low acute toxicity when administered orally. According to Bruce (1985, 1987), any substance with LD_{50} estimated to be greater than 2000 - 5000 mg/kg body weight given orally could be considered of low toxicity and being safe.

Similarly, the chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organization for Economic Co-operation and Development (OECD, Paris, France) (Walum, 1998) are as follow: very toxic, <5 mg/kg; toxic, >5 <50 mg/kg; harmful, >50 <500 mg/kg; and no label, >500 <2000 mg/kg. Therefore, the high LD_{50} (>3000 mg/kg body weight) of the aqueous extract obtained, is an indication that the extract could be considered relatively safe especially when administered orally where absorption may not be complete due to inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984).

The treatment of the rats with varying doses of the plant extract for 28 days in this studies resulted in increases in body weight which is dose and time dependent although not statistically significant ($P > 0.05$). The extract also produces significant ($P < 0.05$) dose and time dependent decreases in the blood sugar levels of the treatment group compared to their respective day zero values.

Conclusion

The extract possesses a potential hypoglycaemic property thus justifying its folkloric use as a hypoglycaemic agent.

Recommendation

Further research into the potential usefulness of the plant extract as anti diabetic agent is required using drug induced diabetic animal model in order to validate its use in Medicare and isolation of the active principle(s).

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