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Full Length Research Paper

Hypoglycaemic activity of the ethanol extract of *Ageratum conyzoides* linn. shoots on alloxan-induced diabetic rats

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Owing to the increasing prevalence of diabetes in Nigeria, there is a continuous search for antidiabetic phytomedicines. The hypoglycaemic activity of the ethanol extract of shoots of *Ageratum conyzoides* Linn. was determined using alloxan-induced rats. Ethanol extract was prepared using a soxhlet extractor. The hypoglycaemic activity of ethanol extract of *A. conyzoides* was determined using alloxan-induced diabetic albino rats of either sex weighing 140 to 200 g. The rats were divided into 6 groups of 5 rats each. Group 1 was the negative control experiment (no alloxan, no treatment). Group 2 was the positive control, while Groups 3, 4 and 5 were injected with alloxan to induce diabetes and treated with different doses of the plant extracts namely; 100 mg/kg (Group 3), 200 mg/kg (Group 4), and 400 mg/kg (Group 5). Normoglycaemic rats in Group 6 were also administered with 400 mg/kg of the extract for a period of two weeks. The phytochemical screening of powdered shoots of *A. conyzoides* was carried out using standard procedures. The plant extract had hypoglycaemic effect on each group of diabetic rats as it lowered the fasting blood sugar (FBS) levels from 390.6 to 90.2 mg/dl (Group 3), 590.4 to 45.8 mg/dl (Group 4) and 466.2 to 42.4 mg/dl (Group 5). The plant extract had no observable adverse effect on normoglycaemic rats and it reduced the FBS level from 55.0 to 49.3 mg/dl within two weeks. The phytochemical screening of the shoot of *A. conyzoides* revealed the presence of alkaloids, cardenolides, tannins, saponins and flavonoids. Considering the hypoglycaemic activity of *A. conyzoides* on the diabetic albino rats, the plant could be a source of antidiabetic phytomedicine.

Key words: *Ageratum conyzoides,* hypoglycaemic activity, alloxan- diadetic rats, phytochemical screening.

INTRODUCTION

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused either by lack of insulin secretion or decreased sensitivity of the tissue to insulin, which is secreted by the pancreas (Guyton and John, 2000). The World Health Organization reported that diabetes mellitus (DM) is fast becoming pandemic (WHO, 1985). In 1980, the WHO expert committee on diabetes listed as one of its recommendations, further investigation into traditional methods of management and treatment of diabetes due to side effects of insulin and other synthetic hypoglycaemics (WHO, 1980).Scientific investigation has confirmed the efficacy of some medicinal plants in the treatment of diabetes. Such plants include: *Pterocarpus marsupium* and *Mormodica charanti*a (Ruchi, 2005), *Cyamposis tetragonoloba* (Bhandari and Sharma, 1999) and *Ocimum gratissimum* (Ibironke and Ekpo, 1999).

Ageratum conyzoides is widely utilized in traditional medicine by various cultures worldwide although application varies by the region.

In Nigeria, the leaf juice is used for dressing wounds, ulcers, skin infections and as remedy for inflammation and redness of the eye (Gill, 1992). This study investigated the ethanol extracts of *A. conyzoides* for hypoglycaemic activity on alloxan- induced diabetic rats and screened the plant material for the presence of

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Table 1. Basal blood sugar (BBS) and fasting blood sugar (FBS) (mg/dl) of control and diabetic rats.

Experimental animals (rats)	BBS (mg/dl)	FBS (mg/dl)
Group 1	41.0±0.49	-
Group 2	42.2±0.34	202.8±0.47
Group 3	42.4±0.45	390.6±0.33
Group 4	42.8±0.57	509.4±0.67
Group 5	45.6±0.11	66.2±0.47

Values represent mean ± SE (n = 3).

secondary metabolites.

MATERIALS AND METHODS

Plant material

The shoots of *A. conyzoides* were collected at the nursery of the Department of Botany and Microbiology, University of Ibadan, Nigeria. The identity of the plant was confirmed at the University of Ibadan Herbarium (UIH). The plant sample was air-dried, powdered and stored in a clean air-light glass container for further use.

Ethanol extract

The powdered sample was extracted in absolute ethanol using a soxhlet extractor. The extract was concentrated to dryness in a rotary vacuum evaporator at 50°C and then air-dried to constant weight. The extract was refrigerated at 4°C prior to use.

Pharmacological screening

Experimental animals

Albino rats of either sex, weighing between 140 and 200 g were divided into six groups of five animals each. They were fed with commercially prepared feed made by Ladokun Feeds Limited, Ibadan. Good drinking water was also provided freely. The animals were given a two-week period of acclimatization to laboratory conditions before the commencement of the experiment.

Alloxan

Alloxan has been shown to destroy the beta cells of the pancreas. The uric acid derivative initiates free radical damage to DNA in the beta cells of the pancreas, causing the cells to malfunction and die (Snell, 2000). Experimental animals were made diabetic by a single intraperitoneal injection of 150 mg/kg of alloxan monohydrate (Dhanabal et al., 2004) dissolved in normal saline (0.9%). The volume of alloxan administered was calculated thus:

$$\frac{\text{Weight of animal (g)} \times \text{dose of drug (150 mg/kg)}}{1000 \times \text{concentration of drug (100 mg/ml)}}$$

Experimental groupings

The animals were grouped into 6, with each group having 5 animals, and treated as follows:

Group 1: Normal (negative control).
Group 2: Diabetic but not treated (positive control).
Group 3: Diabetic; treated with 100 mg/kg of extract.
Group 4: Diabetic; treated with 200 mg/kg of extract.
Group 5: Diabetic; treated with 400 mg/kg of extract.
Group 6: Given 400 mg/kg of extract alone.

The basal blood sugar levels of all the animals were checked using a glucometer, after fasting them for 18 h. The procedure involved cutting a bit of their tails for a drop of blood and dropping this blood on a test strip attached to the glucometer. The blood sugar level was given in mg/dl.

Animals in Groups 2, 3, 4 and 5 were injected with alloxan and they became diabetic after 48 h. This was confirmed by checking their glucose level using a glucometer and animals with glucose levels greater than 100 mg/dl were selected. They were treated with the appropriate dose of crude extract, administered twice daily using an oral canula, for two weeks. A concentration of 100 mg/dl of the crude extract was used and the volume administered was calculated as that of alloxan. The fasting blood sugar levels of all the animals were first checked after 24 h of treatment, and then subsequently, every 38 h and readings were taken.

Phytochemical screening

Plant sample was screened for the presence of alkaloids, cardenolides, anthraquinones, saponins, tannins and flavonoids (Evans, 1997; Odebiyi and Sofowora, 1978).

Statistical analysis

Analysis of variance and comparison of means were carried out on all data using statistical analysis system (SAS).

RESULTS

The pharmacological screening of animals injected with alloxan became diabetic as their fasting blood sugar (FBS) level increased from 42.2 to 202.8 mg/dl (Group 2), 42.4 to 390.6 mg/dl (Group 3), 42.8 to 509.4 mg/dl (Group 4) and 45.6 to 466.2 mg/dl (Group 5). The diabetic state of the animals was further confirmed when the FBS level of Group 1 animals (negative control: no alloxan) still remained at basal (41.0 mg/dl) as the FBS levels of alloxanised animals increased (Table 1). The hypoglycaemic effects of different doses of ethanol extract of *A. conyzoides* on fasting blood sugar levels of diabetics rats is as shown in Table 2. Table 3 shows the hypoglycaemic effect of the ethanol extract at 400 mg/kg on normolglycaemic rats in Group 6. The powdered shoots of *A. conyzoides* contained alkanoids, cardenolides, saponins, tannins, flavonoids but anthraquinone was absent (Table 4).

DISCUSSION

The ethanolic extract of *A. conyzoides* lowered FBS

Table 2. Fasting blood sugar (mg/dl) of diabetic rats on administration of different doses of *A. conyzoides* extract.

Number of days	Group 1	Group 2	Group 3	Group 4	Group 5
1	46.8±0.11	186.2±0.21	376.0±0.11	368.8±0.39	280.4±0.39
3	47.4±0.28	146.8±0.38	241.8±0.48	211.2±0.21	190.2±0.23
5	37.6±0.23	171.6±0.89	170.6±0.23	134.6±0.36	135.4±0.28
7	39.8±0.11	124.0±0.34	140.4±0.11	63.0±0.43	124.4±0.54
9	37.8±0.32	147.4±0.43	118.0±0.23	48.6±0.11	57.4±0.87
11	41.0±0.47	144.6±0.46	129.8±0.46	47.7±0.28	38.2±0.11
13	39.6±0.31	120.2±0.21	90.2±0.82	45.8±0.32	42.4±0.46

Values represent mean ± SE (n = 3).

Table 3. Fasting blood sugar (mg/dl) of normal rats administered with 400 mg/kg of *A. conyzoides* extract.

Number of days	Group 6
1	55.0±0.10
3	53.4±0.11
5	55.6±0.11
7	44.4±0.38
9	54.0±0.73
11	50.0±0.57
13	49.3±0.57

Values represent mean ± SE (n = 3).

Table 4. Phytochemical screening of shoots of *A. conyzoides.*

Phytochemical constituent	*A. conyzoides* shoots
Alkaloids	+
Cardenolides	+
Anthraquinones	-
Saponins	+
Tannins	+
Flavonoids	+

levels of the diabetic animals significantly. The FBS level of Group 3 (treated with 100 mg/kg) dropped from 390.6 to 90.2 mg/dl, Group 4 (treated with 200 mg/kg) dropped from 509.4 to 45.8 mg/dl while Group 5 (treated with 400 mg/kg) dropped from 466.2 to 42.4 mg/dl by the end of the study period. The extract lowered the FBS level of the animals to below 100 mg/dl as compared with the animals in Group 2 (alloxanised but not treated), which still remained above 100 mg/dl by the end of the study. Apart from remaining hyperglycaemic throughout the period of the experiment, Group 2 animals also showed diabetic symptoms such as retinopathy, sores, weight loss and excessive urination with ants clustering around their urine. Some of these alloxanized and untreated animals did not survive the two-week period of experiment probably due to the damage done by alloxan and intensified by lack of treatment, as these signs were not manifested in treated animals. Thus, it can be concluded that *A. conyzoides* exhibited hypoglycaemic activity.

The animals were considered treated when their FBS levels returned to basal. FBS levels of animals in Groups 4 and 5 had returned to basal by the ninth day of the experiment, whereas, the FBS levels of animals in Group 3 treated with 100 mg/kg had still not returned to basal by the end of the two weeks study period. The shortest time of treatment was therefore observed in Groups 4 and 5 that is, those animals treated with 200 and 400 mg/kg of the extract respectively. Thus, it can be concluded that dosages best suited for the treatment of hyperglycaemia

on experimental animals using ethanolic extract of *A. conyzoides* were 200 and 400 mg/kg. The administration of plant extract on normoglycaemic animals (Group 6) was aimed at observing the hypoglycaemic effect on normal animals. By the end of the two weeks study, the FBS level of the normal animals administered with 400 mg/kg of the extract dropped from 55.0 to 49.3 mg/dl. Based on the results obtained in this study, it can be deduced that the extract had hypoglycaemic effect on normal animals.

The presence of secondary metabolites in plants is an indication of medicinally active constituents (Bonner, 1991). Phytochemical screening of *A.conyzoides* revealed the presence of alkaloid, cardenolide, saponin, tannin and flavonoid. Results from previous phytochemical screening of *A. conyzoides* confirm the presence of these secondary metabolites (Ming, 1999). Studies of some hypoglycaemic plants such as *Blighia sapida, Phyllanthus niruri, Bridelia ferruginea* and *Corchorus olitorus* show that they contain alkaloids, flavonoids, tannins, and cardiac glycosides as part of their active constituents (Iwu, 1983). *Momordica charantia,* which is a potent hypoglycaemic plant also contains flavonoids (Ruchi, 2005). Thus, it is possible to infer that the presence of these secondary metabolites places *A. conyzoides* in the category of hypoglycaemic agents.

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