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Effect of *Strophanthus hispidus* Dc. (*Apocynaceae*) Roots Extract on Normal and Hyperglycemic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AOT and IMR managed the analyses of the study. Authors FEO and AO managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: To investigate the hypoglycemic activity of both ethanol extract *Strophanthus hispidus* root (EESHR) and chloroform extract *Strophanthus hispidus* root (CESHR) respectively.
Methods: Both ethanol extract *Strophanthus. hispidus* root and chloroform extract *Strophanthus. hispidus* root were evaluated respectively for hypoglycemic activity using Fasting blood glucose (FBG) method. Chloroform extract *Strophanthus hispidus* root was further fractionated using standard column chromatography techniques and the fractions were evaluated for hypoglycemic using standard oral glucose tolerance test method.
Results: In the experiments, the hypoglycemic activity of the chloroform extracts *Strophanthus hispidus* root was better than that of ethanol extract *Strophanthus hispidus* root at 500 mg/kg respectively for both extracts which were significant (p=0.05) and compared to gibenclamide.

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Conclusion: The results showed that chloroform extract *Strophanthus hispidus* root fraction (3-4) at 200 mg/kg possesses significant hypoglycemic activity better than that of the standard drug-glibenclamide. This supports its use as a potent anti-diabetic drug in alternative herbal medicine.

Keywords: Strophanthus hispidus; root extract; hypoglycemic; glibenclamide.

1. INTRODUCTION

The oral glucose tolerance tests (OGTT) indicates the body's ability to utilize a type of sugar named glucose that is the body's main source of energy. Oral glucose tolerance tests, a test of immerse value and sentiment, in favour of using fasting plasma glucose concentration alone as a practical attempt to simply the diagnosis of diabetes [1]. Hyperglycemia is an important factor in development and progression of the complication of diabetes mellitus [2].

The plant SPH, popularly known as brown *strophanthus,* and hairy *strophanthus* in West Africa, including Nigeria, is indigenous to Africa [3]. A deciduous shrub of 5 m tall and up to 100 cm wide, having its stem bark dark grey in colour, with few lenticels, has been reported to have diverse medicinal uses; for example, in the Savannah Zone of West Africa, the latex and seeds of *Strophanthus hispidus* are used as arrow poison, while decoctions of root, stem bark or leaf are used externally to treat skin diseases, leprosy, ulcers, malaria, dysentery, gonorrhea and diabetes [3]. The leaves have been reported to possess hypoglycemic properties [4]. Similarly, the anti-inflammatory properties of the aqueous of the root extract have been studied [5]. In Nigeria and Ghana, the root decoction is ingested to treat rheumatic diseases and diabetes, while in Togo; the root bark macerate is employed for treating oedema [3]. The trace elements and major elements present in the plant root have been reported by Akinlami et al. [6]. The seeds and the plant extract of *Strophanthus kombé*are beneficial as a cardiac drug or a diuretic [7]. These plants are mainly used in the treatment of congestive heart failure; however, their toxicity limits their extensive use. Ouabain, a more common name for G-strophanthin a poisonous glycoside obtained from the fruits of *Strophanthus gratus*, is used widely to block the sodium pump for in vitro studies. K-strophanthin, isolated from Strophanthus kombé, was also used for the treatment of heart diseases until its adverse side effects were observed. The cytotoxicity and structural characterization of various cardenolide glycosides have been extensively studied [7]. Cowan et al isolated lignans from the stem of Strophanthus gratus [8]. Three lignans; pinoresinol, 8-hydroxypinoresinol and olivil have been isolated from the stem [8]. In this study, the blood lowering effects of chloroform and ethanol crude extracts of *S. hispidus* respectively were conducted through oral glucose tolerance test in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh roots of *Strophanthus hispidus* (Plate.1) were purchased from the market, in Ondo southwest, Nigeria and identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria and specimen sample were kept in the hermarium.

2.2 Extraction

The identified plants materials were air dried for four weeks and pulverized into fine powder using clean and sterile Mortar and Blender. The powdered samples were sieved and quartered to obtain a representative of 1000g used for this study. Two hundred grams of the powered roots of *S. hispidus* were extracted with 1L of 96% ethanol or chloroform respectively at room temperature (25-30ºC) by maceration for 72h. After the extraction, the solvents were removed from the extracts by distillation and drying at 60ºC in a water bath, leaving the extracted portion as a residue. The crude Ethanol Extract *S. hispidus* Root (EESHR) and Chloroform Extract *S. hispidus* Root (CESHR) were stored and kept in air tight bottle in a refrigerator until used for bio-assay. The crude EESHR was dissolved in normal saline to obtain the required concentration of 500mg/kg while CESHR was first dissolved in Tween-80 and followed by normal saline to also obtain the needed concentration of 500mg/kg that were required for the bioassay.

Plate 1. Roots of *Strophanthus hispidus* DC. (*Apocynaceae*)

2.3 Phytochemical Screening of Crude Plant Extracts

Qualitative phytochemical screening of ethanol extract *S. hispidus* root (EESHR) and chloroform extract *S. hispidus* root (EESHR) respectively were carried-out by standard procedures [9,10,11] and [12].

2.4 Test Animals

A sum total of 50 male albino rats weighing about 120-220 gm. were procured from Animal house of Federal University of Technology, Akure, Nigeria. All the animals were kept in standard polypropylene cages and maintained at 27ºC ± 2ºC under 12 h. dark/light cycle. The animals were fed with standard rat feed and water was given ad libitum. Ethical clearance for handling the animals was strictly adhered to as described by Akhtar et al. [13].

2.5 Experimental Design

Initial screening of the ethanol extract *S. hispidus* root (EESHR) and chloroform extract *S. hispidus* root (CESHR) respectively was done to evaluate their hypoglycemic potential with dosage of 500 mg/kg for each of the extracts. These were administered orally by gavage in normal rats by conducting fasting blood glucose (FBG) study. Oral glucose tolerance test (OGTT) studies were done on separated components of chloroform extract *S. hispidus* root (CESHR), which was subjected to Column chromatography. The chloroform extract *S. hispidus* root (CESHR) was identified as the most effective hypoglycemic than ethanol extract *S. hispidus* root (EESHR) by initial screening.

2.6 Assessment of Hypoglycemic Activity in Normal Healthy Rats

The animals were fasted for 18 h with free access to water prior to the administration of the extracts. After fasting the blood glucose levels was measured using the glucose-oxidase principle and the fasting blood glucose greater than 50 mg/dL was included in the study for the normoglycemic group. The normoglycemic rats were randomly assigned into four groups (1-4) of five rats (n = 5) each as follows,

Group 1: Normal treated with 500 mg/kg of CESHR
Group 2: Normal treated with 500 mg/kg of EESHR
Group 3: Normal treated with 5 ml/kg of normal saline
Group 4: Normal treated with 5 mg/kg of gibenclamide

Fasting blood glucose (FBG) was taken initially and then blood samples were collected from tail vein at 2, 4, 6 and 8 h after administering the extracts.

2.7 Column Chromatography Separation of Chloroform Extract *S. hispidus* Root (CESHR)

Thin Layer Chromatography (TLC) was employed using silica gel 60 F_{254} precoated plates and solvent system: hexane: ethyl acetate (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8). TLC was carried out on the crude extract to estimate the number of components. One drop of glacial acetic acid was added to each solvent system to aid distinct separation of spots into their different components. After the

development, the chromatogram was then allowed to dry before it was transferred into the iodine tank. Initial spotting of the crude chloroform extract *S. hispidus* root obtained on TLC plates indicated more than one components, hence the need for proper separation with a column chromatograph. Column chromatograph was used to separate crude chloroform extract *S. hispidus* root. 3 cm x 100 cm column size and a silica gel of mesh size 60-200 were used. Silica gel (180 g) was made into slurry by adding the solid adsorbent to a quantity of packing solvent (hexane). The slurry was swirled until it was homogeneous and relatively free of entrapped air. Prepared slurry was slowly poured into the column with the tap opened such that it gently settled uniformly. The column was constantly tapped to get rid of any trapped air bubbles and also ensure a leveled layer of the adsorbent. 6 g of crude extract was pre-adsorbed on silica gel. Hexane was used to wash the packed column prior to analysis. This pre-adsorbed sample was allowed to dry before loading onto the column. The ratio of the silica gel to that of extract was 30:1. The loaded sample was then covered with pure white sand and a small piece of cotton wool. The top of the column was usually filled with enough solvent/mixture of solvents to avoid cracking. The elution started with 100% non-polar hexane after which the polarity of the solvent(s) was increased gradually with EtOAc until 100% in a gradient elution chromatographic technique. The solvent system used in eluting the column was as follows: hexane 100%, ethyl acetate/hexane 5%, 10%, 15%, 20%, 25%, 40%, 50%, ethyl acetate 100%. A total of 40 eluents of 50 ml each were collected. TLC of the eluents collected was also carried out using the same solvent system employed in the column chromatography. This was done to ascertain the number of components in it and also to pool together similar eluents which contain the same component based on their Rf values. The fractions obtained (50ml) were grouped and coded to afford four main fractions: CESHR (3-4), CESHR (5-9), CESHR (10-12), and CESHR (18-25).

2.8 Oral Glucose Tolerance Test in Chloroform Extract *S. hispidus* Root (CESHR)

This test was performed according to [14]. Rats were divided into five groups (n = 6). They were fasted overnight and accessed to water only. Blood was taken from the lateral veins of the tail and the blood sugar levels were initially monitored with a glucometer (One touch Horizon). Group I served as control groups were treated with vehicle (0.5% Tween 80 solution), group II treated with 5mg/kg of gibenclamide as a standard drug. Chloroform Fractions CESHR (3-4) serves as group III, CESHR (5-9) served as group IV, CESHR (10-12) served as group V and CESHR (18-25) served as group VI each were treated with a dose of 200 mg/kg and After 30 min, the animals were treated with 5% (wt/v) glucose orally. Blood glucose levels were monitored from lateral tail veins at 30, 90 and 150 min intervals after post glucose challenge. Data were expressed as mean ± standard error of mean. Statistical comparison were performed by one-way ANOVA followed by Dunnett's multiple comparison test, and the values were considered statistically significant when p =.05.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Screening

Table 1 presents the result of Qualitative phytochemical screening of ethanol extract *S. hispidus* root (EESHR) and chloroform extract *S. hispidus* root (EESHR) respectively. Both extracts contained flavonoids, alkaloids, saponins. Cardiac glycosides, tannins and anthraquinones. The results are in agreement with similar research work carried out by Agbaje and Fageyinbo [15]. Phytochemicals compounds present as secondary metabolites play vital roles in the bioactivity exhibited by medicinal plants used ethno botanically [16]. Some plant compounds have pharmacology effects, which forms the basis of chemical synthesis of drugs used in modern medicine [16]. Flavonoids are polyphenols compounds that are structurally classified as flavonols, flavones, isoflavones, catechins, anthocyanidins and chalcones [17]. Numerous flavonoids have been identified; many of which occur in fruits, vegetables and beverages tea, coffee, beer, wine and fruit drinks [18]. Flavonoids (Fig. 1) have potential beneficial effects on human health. They have been reported to have anti-viral, anti-platelet, ant-inflammatory, anti-tumors, anti-diabetic and antioxidant activities [18,19].

3.2 Hypoglycemic Effect of Extracts on Normal Rats

Fig. 1 describes the result of hypoglycemic effect of chloroform extract of *S. hispidus* roots (CESHR) and ethanol extract of *S. hispidus* roots (EESHR) respectively, on fasting blood glucose

(FBG) of normal rats. Rats treated with 500 mg/kg CESHR showed a maximum fall of 39.2% in fasting blood glucose (FBG) after 8hrs of oral administration, while the fall of 26.62% was observed with 500 mg/kg of EESHR respectively. The hypoglycemic activity of the chloroform root extract was better than that of ethanol root extract. The result was in agreement with similar report by of Ojiako et al. [20].

3.3 Hypoglycemic Effect of Chloroform Extracts Fractions on OGTT of Normal Rats

Fig. 2 depicts the hypoglycemic effect of a single oral administration of 200 mg/kg each of different chloroform extract fractions of *S. hispidus* root on OGTT of normal rats. Experimental induction of hyperglycemia by intragastric ingestion of glucose resulted in a 1.5 to 2-fold increase in plasma glucose levels (comparing bar groups of 0 minute with the bar groups of 30 minute, (Fig. 2). The CESHR fraction (3-4) produced a maximum fall of 42.45% at two hours after glucose administration, whereas fall of 32.74%, 34.66% and 28.93% were observed with the CESHR fraction (5-9), CESHR fraction (10-12) and CESHR fraction (18-25) respectively. A standard antihyperglycemic drug, glibenclamide, when administered to glucose loaded rats, reduced blood glucose level by 49.00%. The results demonstrated that the CESHR fraction (3-4) possesses antihyperglycemic potential more than the others fractions. The CESHR fraction (3-4) was comparable to that of the standard drug, glibenclamide, in its effectiveness in lowering blood glucose. This suggests that the fraction contains antihyperglycemic constituents.

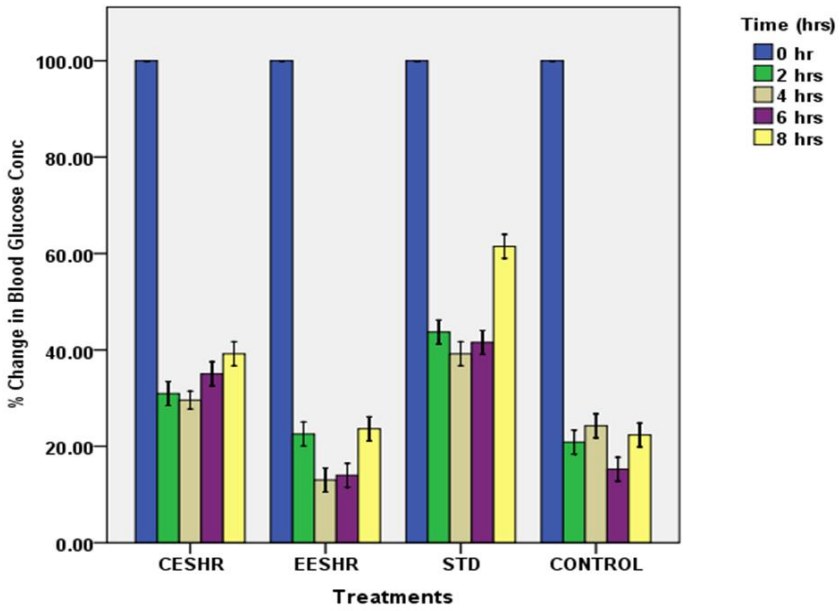

Fig. 1. Hypoglycemic effect of *S. hispidus* root extracts on BGL of normal rats

Each value shown in mean ±S.D. (n = 5). Control (distilled water); Chloroform extract S. hispidus roots (CESHR-500 mg/kg); Ethanol extract S. hispidus roots (EESHR-500mg/kg); STD (gibenclamide -5 mg/kg)

Table 1. Qualitative phytochemical screening of ethanol extract *S. hispidus* root (EESHR) and chloroform extract *S. hispidus* root (CESHR) respectively

General test	Specific test	EESHR	CESHR
Test for flavonoids	Ferric chloride test	+	+
Test for glycosides	Keller-kiliani test	+	+
Test for tannins	Ferric chloride test	+	+
Test for alkaloids	Dragendoffs test	+	+
Test for anthraquinones	Hydrogen chloride test	+	+
Test for saponins	Frothing test	+	+

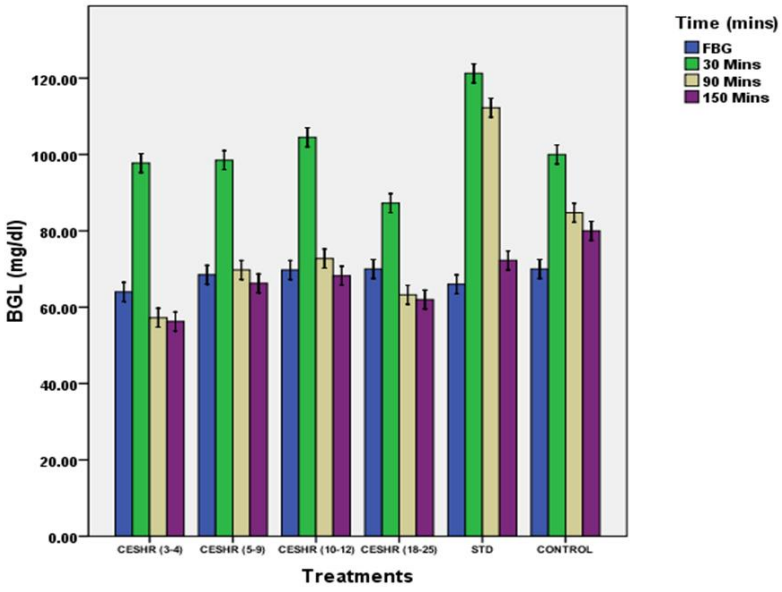

Fig. 2. Hypoglycemic effect of Chloroform extract fractions *S. hispidus* roots (CESHR) on BGL of normal rats during OGTT

Each value shown in mean ±S.D. (n = 6). Control (0.5% Tween 80 solution); Chloroform extract fractions S. hispidus roots (CESHR-200 mg/kg); STD (glibenclamide-5 mg/kg)

However, it can be suggested that the mode of action of action of fractions CESHR (3-4) is probably mediated by an enhanced secretion of insulin, like glibenclamide a biguanides. In the light of the above pharmacology results, further works is on-going in our laboratory to possible isolate and characterize the possible hypoglycemic compounds from the most active fraction using Infra-red spectroscopic, 1H, 13C, 2D-NMR spectroscopic and Electron Spray Mass spectroscopy. Finally, the present study has given idea of the hypoglycemic compounds present in the reported plant fraction.

4. CONCLUSION

The result showed that CESHR fraction (3-4) at 200 mg/kg possesses significant hypoglycemic activity better than that of the standard drug-glibenclamide. This supports its use as a potent anti-diabetic drug in alternative herbal medicine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors declare that this work was not against public interest. Animal experiments were conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub. No. 85-23, Revised).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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