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ACUTE TOXICITY STUDIES AND HYPOGLYCEMIC ACTIVITY OF THE METHANOL EXTRACT OF THE LEAVES OF *HYPTIS* *SUAVEOLENS* POIT. (LAMIACEAE)

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ABSTRACT

Hyptis suaveolens Poit. (family Lamiaceae) commonly called curry leaf, is an erect, annual and aromatic herb that may grow up to 2 m high. A savanna plant that is found in abandoned farmlands. Traditionally, the plant is used in the treatment of diabetes mellitus, fever, eczema, flatulence, cancers and headache. Acute toxicity studies of the methanol extract showed an LD₅₀ of 2154.1 mg/Kg body weight in rats. The anti-diabetic study of the same extract in alloxan-induced diabetic rats, showed significant ($p < 0.05$) reduction in the blood glucose concentration. The result tends to suggest that the methanol extract of *H. suaveolens* leaves possess anti-diabetic activity in alloxan-induced diabetic rats and may justify the claimed by the traditional healers in Northern Nigeria.

Keywords: *Hyptis suaveolens*; LD₅₀; Hypoglycemia; Methanol extract

INTRODUCTION

Hyptis suaveolens Poit. is an erect, annual and aromatic plant that belong to the family Lamiaceae. It is commonly called curry leaf, and locally, in northern Nigeria as *Daddoyata-daji* in Hausa; *Efiri* in Yoruba and *Tanmotswangi-eba* in Nupe. It may be found in abandoned farmlands in West Africa especially in northern Nigeria (Abdullahi, *et al.*, 2003). It is also found in bushes abundant in open and waste land at both low and medium altitude. The leaves of the plant are opposite and ovate, about 4 – 9 cm long, with an obtuse apex, bilateral base and are dorsiventrally arched. It has alternate and stipulate veins. The flowers are axillary with long stalk, hairy calyx, and about 4 mm long. It is striate and erect. The corolla is blue, zygomorphic and bilabiate. The stamens are four, declinate and about 8 mm

long, the seeds are flat and mucilaginous (Pankaj, 2005). A decoction of the leaf is used by traditional healers in Northern Nigeria, especially around Niger, Nassarawa, and Kaduna States in the treatment of diabetes mellitus and fever associated with cold among others (Abdullahi, *et al.*, 2003). It is also used as an aromatic herb by traditional healers. This herb holds a reputed position among the traditional healers that are expert in the treatment of different types of cancers in India. Its different parts are used both internally and externally for dermatitis and eczema (Pankaj, 2005). The leaves of the plant have been shown to contain alkaloids, terpenes and volatile oils (Gills, 1992). This study aims at investigating the acute toxicity and the anti-diabetic activity of methanolic extract of *Hyptis suaveolens* leaves.

MATERIALS AND METHODS

Experimental animals

Wister strain albino rats of both sexes weighing 103 – 185g were obtained and kept in the animal house of the Department of Pharmacology and Clinical Pharmacy Ahmadu Bello University, Zaria. The animals were kept under well-ventilated conditions, 12 hours light/dark cycle, room temperature of $25 \pm 2^{\circ}\text{C}$ and fed on standard Feeds (Excel Feeds Plc) and had access to water *ad libitum*.

Collection and Preparation of Plant Material

The leaves of *H. suaveolens* were collected around Samaru Zaria, Nigeria in July 2005. It was authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where a sample (voucher specimen number 518) has been deposited. The leaves were air-dried and powdered with the aid of local mortar and pestle.

Extraction of Plant Material

150g of the powdered leaves of *H. suaveolens* was macerated in 750ml of methanol for 72 hours. The mixture was filtered and the filtrate was evaporated to dryness on a water bath, which gives a reddish-brown, semi-solid residue.

Preliminary Phytochemical Screening

The powdered root of *H. suaveolens* was subjected to preliminary phytochemical analysis to test for the presence or absence of phytochemical constituents using the following methods. Carbohydrates [(500 mg plant material boiled in 30 ml distilled water, filtered); 1 ml filtrate + 1ml of Molisch's reagent +1ml conc. H_2SO_4 . A reddish ring indicates the presence of carbohydrate; 1 ml filtrate + 2 ml of Fehling's solution + boiled for 5 min. A

brick red precipitate indicates the presence of reducing sugars; 1 ml filtrate + 1ml Barfoed's reagent + heat. A red precipitate indicates the presence of monosaccharide (Evans, 1996)]. Tannins [2 ml filtrate + 1 ml FeCl_3 , blue-black or greenish-black precipitate indicates tannins; 1 ml filtrate, + 3 drops of lead sub acetate, a colored precipitate indicates the presence of tannins (Evans, 1996)]. Saponnins [frothing test: 0.5 ml filtrate + 5 ml distilled water, shaken for 30 sec, persistence frothing indicates saponnins (Evans, 1996)]. Flavonoids [Shinoda's Test: (200 mg plant material in 5 ml ethanol, filtered) 1 ml filtrate, + magnesium ribbon + conc. HCl a pink or red color indicates the presence of flavonoids. Terpenes/steroids [Liebermann – Burchard's Test: (200 mg plant material in 10 ml chloroform, filtered; 2 ml filtrate + 2 ml acetic anhydride + 1 ml of conc. H_2SO_4 . A blue – green ring indicates the presence of terpenes/steroids (Parekh and Chanda, 2007)]. Alkaloids [200 mg plant material boiled in 20 ml of 1% H_2SO_4 in 50% ethanol, filtered; filtrate + 5 drops conc. NH_4OH + 20 ml chloroform and the two layers separated. Chloroform layer was extracted with 20 ml dilute H_2SO_4 . Extract + 5 drops of Mayer's/Wagner's/Drgendorff's reagents, a creamy/brownish-red/orange-red precipitate indicates the presence of alkaloids (Evans, 1996). Anthraquinones [Borntrager's test: 100 mg of powdered plant in 5 ml of chloroform, filtered. 2 ml filtrate + 2 ml 10% NH_4OH . A bright pink colour indicates the presence of anthraquinones; Modified Borntrager's test: 200 mg plant material boiled in 5ml 10% HCl, filtered. Filtrate extracted with 5ml benzene, and benzene layer shaken with 5 ml 10% NH_4OH . A rose pink or cherry red colour indicates the presence of anthraquinone derivatives (Evans, 1996).

Acute Toxicity Studies

LD₅₀ determination was conducted using the Lorke method. Nine mice were divided into 3 groups of 3 mice each. The first group received the extract (*i.p.*) at a dose of 1000 mg/Kg; group 2 received the extract at a dose of 100 mg/Kg (*i.p.*), while the last group received the extract at the dose of 10 mg/Kg body weight. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours. In the second phase 4 mice were divided into 4 groups of one mouse each. The extract was administered at the dose of 600, 1000, 1600, and 2900 mg/Kg (*i.p.*) to group 1, 2, 3 and 4 respectively, based on the result of the first phase, and the final LD₅₀ was calculated as the square root of the geometrical mean of highest non lethal dose and the lowest lethal dose (Lorke, 1983).

Induction of Hyperglycaemia

Hyperglycaemia was induced to the animal by an intraperitoneal injection of 100 mg/kg of alloxan monohydrate reconstituted in distilled water after an overnight fast. 3 days later, blood samples were obtained by the tail snip method and the sugar level of each animal was determined by the dextrostrix test strips, all rats with blood glucose concentration of greater than 100 mg/dL were considered hyperglycaemic (Ojewole, 2003).

Evaluation of the Hypoglycaemic Activity of the Extract

The methanol extract of the leaves of *H. suaveolens* reconstituted in normal saline was administered to alloxan-induced diabetic rats (group I) at a dose of 750 mg/Kg body weight after estimating their initial fasting blood glucose concentration. The blood glucose concentration was then assayed at time interval of 1, 2, 4, 6 and 24 hours. Chlorpropamide 250 mg/Kg and normal saline were administered to group II

(positive control) and III (negative control) respectively. Blood glucose concentrations were also evaluated at the various time intervals (1, 2, 4, 6 and 24 hours) by the tail snip method using dextrostrix test strips (Akah and Okafor, 1992).

Statistical Analysis

Results were expressed as Mean \pm Standard Error of Mean. Statistical analysis was carried out using student's t-test and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The results of the acute toxicity of the methanolic extract of the plant showed an LD₅₀ of 2154.1 mg/Kg body weight in rats, the extract can therefore, be considered to be relatively safe (Lorke, 1983). Alloxan has been known to produce hyperglycaemia in laboratory animals. The reduction of the glycaemia produced by the methanol extract of the plant and chlorpropamide (positive control) in alloxan-induced diabetic rats is shown in table 1. The methanol extract at 750 mg/kg has significantly ($p < 0.05$), reduced the blood glucose level at 4th hour post treatment from 178.33 ± 9.13 at 0 hour to 120.00 ± 4.47 and significant hypoglycaemia was maintained for another 2 hours. Similarly, the standard drug has reduced the level of glycaemia from 165.00 ± 20.74 at 0 hour to 110.00 ± 2.58 at the 4th hour and maintained a hypoglycaemic level for another 2 hours. However, the level of blood sugar at 24hrs, was noticed to have gone up which may suggest that continuous dosing of the extract may be required. The preliminary phytochemical screening tests for the methanol extract of *H. suaveolens* leaves (table 2) revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, steroids and/or terpenes. Any of these secondary metabolites, singly or in combination with others could be

responsible for the anti-diabetic activity of the plant. As has been reported by Evans, that the polysaccharides glycans of some plants species (e.g. *Aconitum carmichaelii* root; *Ephedera distachya*, and *Gymnema sylvestra* leaves) to have oral hypoglycaemic activity. Mucilage from some members of Malvaceae family also has shown anti-diabetic property. Although, only a small

number of alkaloids have shown anti-diabetic activity, it is possible they may contribute to the overall activity of the plant. Flavonoids and related compounds, steroids from the bark of some ficus species, diterpenes from *Chuytia richadiana* have also been shown to have hypoglycaemic activity.

Table 1: Effect of methanol extract of *Hyptis suaveolens* leaves on blood glucose level of alloxan-induced diabetic rats

Group (n = 6)	Mean blood glucose level mg/dL ± SEM					
	0 Hour	1 Hour	2 Hour	4 Hour	6 Hour	24 Hour
I (750 mg/Kg extract)	178.33 ± 9.13	176.67 ± 8.16	141.67 ± 13.29	120.00 ± 4.48*	138.33 ± 7.49*	171.67 ± 7.53
II (Chlorpropamide, 250 mg/Kg)	165.00 ± 20.74	148.33 ± 21.37	136.67 ± 16.33	110.00 ± 2.58*	93.33 ± 3.33*	131.67 ± 14.72
III (Normal saline)	148.33 ± 29.27	143.33 ± 28.05	146.67 ± 26.58	155.00 ± 8.85*	161.67 ± 7.92	166.67 ± 15.06

*p<0.05 = significant; ± SEM: Standard Error of Mean; n = 6 (number of animals per group).

Table 2: Result of the preliminary phytochemical screening of powdered leaves of *H. suaveolens*

Constituent tested	Result
Carbohydrates:	
Molisch's test	Present
Barfoed's test	Present
Fehling's test	Present
Selivanoff's test	Absent
Cyanogenetic glycosides:	
Guignard's test	Absent
Anthraquinones:	
Borntrager's test	Absent
Modified Borntrager's test	Absent
Saponins:	
Frothing test	Absent
Cardiac glycosides:	
Keller-Keliani test	Absent
Kedde's test	Absent
Legal test	Absent
Terpenes and/or steroids:	
Liebermann-Burchard test	Present
Salkowski's test	Present
Flavonoids:	
Shinoda's test	Present
Sodium hydroxide test	Present
Tannins:	
Ferric chloride test	Present
Lead sub-acetate test	Present
Alkaloids:	
Mayer's test	Present
Wagner's test	Present
Dragendorff's test	Present

CONCLUSION

The presence of alkaloids, carbohydrates, flavonoids, tannins, steroids and/or terpenes in the leaves of *H. suaveolens* either as single constituents or in combination may be responsible for the observed anti-diabetic activity. The methanolic extract of the leaves of this plant shows some promising anti-diabetic activity looking at the level of blood glucose reduction tendency of the extract. This finding may justify the use of

the plant in the traditional treatment of diabetes mellitus.

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