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Research Article

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Hypoglycemic effect of ethanolic leaves extracts of *Anacardium occidentalis* and *Gomphrena globosa* plants on alloxan induced-diabetic rats

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ABSTRACT

This study was undertaken to determine the hypoglyceamic effects of ethanolic extract of G. globosa and A. occidentalis leaves in aLloxan induced diabetic wistar albino rats. The results of the phytochemical screening for both plants reveal the presence of alkaloid, flavonoid, tannins and saponins. The results of acute toxicity test show LD50 of 125mg/ml for A. occidentalis while no death recorded for all the concentration used for G. globosa. In one set of experiment graded doses of the leaves extract (200, 100, 50, and 25mg/kg b. w.) were separately administered to groups of fasted alloxan induced diabetic rat. The hypoglyceamic effect of the ethanolic leaves extracts of both plants were compared with that of diabenese (100mg/kg b.w) of fasted alloxan induced rats, following treatment relatively moderate to high doses of both plants (25, 50, 100, 200mg/kg b. w.) produced a dose-dependant significant reduction ($p<0.05$) in blood glucose levels of fasted alloxan induced diabetic rat after 1-3 days compared to control. In conclusion, the ethanolic extract of the leaves of Gomphrena globosa and Anacardium occidentalis possess hypoglyceamic activity in alloxan induced diabetic wistar albino rat. It may be safe to conclude that , the plants may be useful in the management of diabetes.

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Key words: Diabetic, ethanolic, extracts, hypoglycemic, *Gomphrena globosa*, *Anacardium occidentalis*, alloxan.

INTRODUCTION

Diabetes mellitus is a serious health challenge affecting many people worldwide. According to world health organization reports, the global prevalence of diabetes will increase from 2.8% in 2000 to 4.4% in 2030 [1]. Currently, there are over 150millions diabetics world-wide and this is likely to increase to 300 million or more by the year 2030 [2;3]

Diabetes mellitus is known to be characterized by deficiencies in insulin secretion and or impairment of insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism.[4;5] Carbohydrate metabolizing machinery defects and consistent effort of physiological system to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system persistent deterioration of endocrine control exacerbates the metabolic disturbance and primarily leads to hyperglyceamia [6;7, 8].

Currently, pharmacological strategies in the treatment of diabetes mellitus are aimed at management rather than prevention and cure. Evidences, provided by traditional medicine have indicated that natural products may be better treatment to ailments like diabetes than currently used conventional drugs [9] Moreover, polyherbal therapies i.e., the combination of various types of agents from different plant sources, can be used to enhance efficacy given their synergistic potential, agonistic or antagonistic, pharmacological effects and minimum side effects [6,10]. Its in the light of this, with the hindsight of indigenous knowledge, that the

present: work on the hypoglyceamic activities of *Gomphrena globosa* and *Anacardium occidentalis* leaves was undertaken.

Gomphrena globosa L. (Amaranthaceae) is originally an annual plant, native to Panama and Guatamala in central America. It has being widely introduced to area outside its range including Africa, west Indies and Brazil. The phytochemical investigation shows that it contains saponins, tannins flavonoids and alkaloids [11] *Gomphrena globosa* is used in the treatment of high blood pressure, renal failure, bronchial asthma, acute and chronic bronchitis and whooping cough and diabetes mellitus [11].

Anacardium occidentalis belongs to the family of Anacardiaceae. The leaves are spirally arranged, leathery textured, 2 to 15cm broad with a smooth margin, there are usually 3 to 14 leaves in each terminal stem, leaves become fully mature 20-25 days after emerging. It has a significant concentration of potassium and manganese [12] Phytochemically it contains coumarins, quininies, anthocyanidins, triterpenes, steroid and Tannins. Its used profusely for treatment of many ailments in Fork medicine. The aim of this study is to evaluate the hypoglyceamic effects of ethanolic extract of *Gomphrena globosa* and *Anacardium occidentalis* on Alloxan induced diabetic rat.

***Gomphrena globona* plant**

Anacardium occidentalis

EXPERIMENTAL SECTION

Collection of samples

Fresh leaves of *Anacardium occidentalis* and *Gomphrena globosa* were collected from National Root Crop Research Institute, Umudike (NRCRI) and were identified at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike by Dr. Jimoh Mulikat A. a taxonomist

Preparation of Plant Extract

Fresh leaves of *Anacardium occidentalis* and *Gomphrena globosa* were collected and dried under normal room temperature and grounded into powder using grinder. The ethanol extract were prepared by adding 50g of the plants powder with 200ml ethanol for 24hrs at room temperature and filtered with whatman no 1 filter paper. The filtrate was evaporated to dryness in water bath at 50c. 5g of stock solution was prepared and kept in an air tight bottle in a refrigerator until used.

Animals

Twenty-four (24) mice with an average weight of 15g and wistar albino rats average weight of 188g of both sex were used for this study. The mice and the rats were obtained from National Root Crop Research Institute, Umudike and housed in animal house of college of Natural and Applied sciences, Michael Okpara University of Agric Umudike, with 12hrs light and dark cycle. The mice and rats were kept in the

experimental animal cages for two weeks for acclimatization before the experiment, with free access to standard pellet diet and water.

Acute Toxicity Test

Investigation on the acute toxicity study LD50 of the extract was determined using the method described by Lorke D [13] 24 experimental mice were used and distributed into 6 groups with 4 animal in each group, doubling dilution of 1000mg/ml, 500mg/ml, 125mg/ml 62.5mg/ml and 31.25mg/ml of the extract were administered intrapertionally to the animals. The mice were observed for adverse reactions like abnormal behaviors and general body conditions, and mortality. The LD50 was calculated using the method described by Lorke [13]

Alloxan-Induced Hyperglycemia

The alloxan solution was prepared by dissolving 1g of alloxan powder in a normal saline which consist of 0.9Nacl and 100ml of water. Diabetes was induced by intraperitonial route administration of 1% alloxan solution to the wistar albino rats. 72hrs after alloxan injection, fasting blood glucose was assessed to confirm the diabetic state in the first five groups while group 6 was given water, used as negative control group.

Determination of Blood Glucose Level

Blood samples from rat was collected from the tip of the tail and dropped on a glucometer to determine the blood glucose level after an interval of 12hrs.

PHYTOCHEMICAL SCREENING

Alkaloid Determination

The alkaloid precipitation gravimetric method as described by [14] was used. 5g of the processed sample was dispersed in 100mls of 10% acid in ethanol solution, the mixture was shaken and allowed to stand for 4hrs at room temperature and shaken every 30mins at the end of this period, the mixture was filtered through whatman No 42 grade of filter paper. The filtrate, i. e the extract was evaporated to one quarter of its original volume by boiling on a hot plate. The extract was treated with drop wise addition of conc. NH3 solution to precipitate the alkaloid. The dilution was done until NH3 was in excess, the alkaloid precipitate was removed by filtration using weighed whatman No42 filter paper, and then wash with 1% NH4OH solution, the precipitate in the filter paper was dried at 60c and weighed after cooling in desiccators. The alkaloid content was calculated as shown below:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Wt of sample}} \times \frac{100}{1}$$

Where W_1 = weight of empty filter paper
W_2 = weight of filter paper + alkaloid precipitate
Weight of sample analyzed.

Tannin Determination

Tannin content of the sample was determined by Folin Denis colometric method [15]. 5g of the processed sample was mixed with distilled water in the ratio of 1:10 (w/v). The mixture was shaken for 30mins at room temperature and filtered to obtained the extract, a standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric flask to serve as standard and reagent blank respectively, 2ml of each of the sample extract was put in a labeled flask: the content of each flask was mixed with 35ml of distilled water and 1ml of Folin Denis reagent was added to each; this was followed by 2.5ml of saturated Na_2Co_3 solution. After which, each flask was diluted to 50ml mark with distilled water and incubated for 90mins at room temperature, their absorbance was measured at 760nm in a spectrophotometer with the reagent blank at zero. Tannin content was calculated as:

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{Au}{As} \times C \times \frac{Vt}{Va}$$

Where
W = Weight of sample
Au = Absorbance of standard tannin solution
C = Concentration of standard tannin solution
Vt = Total volume of extract
Va= Volume of extract analyzed.

Saponin Determination

This was done by the double solvent extraction gravimetric method as described by [14] 5g of the processed sample was mixed with 50mls of 20% aqueous ethanol solution and incubated for 12hrs at a temperature of 55c with constant agitation. The mixture was filtered through whatman No 42 grades filter paper. The residue was re-extracted with 50ml of ethanol solution for 30mins and the extracts weighted together.

The combine extract precipitate was washed with 5% NaCl solution and evaporated to dryness in a previously weighed evaporation dish. The saponin was then dried in the oven at 60c (to remove any residue solvent) cooled in a desiccators and re-weighed. The saponin content was calculated as:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

Where W =Weight of sample used
W_1 = Weight of empty evaporation dish
W_2 = Weight of dish + saponin extract.

Flavonoid Determination

Flavonoid was determined using the method described by Harbone [14] 5g of processed sample was boiled in 100mls of 2M HCL solution under reflux for 40mins, and allowed to cool and then filtered. The filtrate was treated with equal volume of ethylacetate and the moisture was transferred to a separate funnel. The flavonoid extract (contained in the ethylacetate portion) was received by filtration using weighed filter paper. The weight was obtained after drying in the oven and cool in a desiccators. The weight was expressed as a percentage of the weight analyzed; and was calculated as:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Where
W_1 = Weight of filter paper
W_2 = Weight of filter paper + flavonoid precipitate
W = Weight of sample analyzed.

RESULTS

Composition of the phytochemical constituent of leaves of *Anacardium occidentalis* and *Gomphrena globosa.*

The phytochemical screening revealed the presence of tannins, saponins, flavonoid and alkaloids. The percentage composition of the phytochemical constituent of *Anacardium occidentalis* and *Gomphrena globosa* are shown in table 1.

Quantitative Test **Table 1**

Sample	Saponin	Tannin	Flavonoid	Alkaloid
Anacardium occidentalis	0.47	0.66	1.01	0.55
Gomphrena globosa	0.29	0.32	0.67	0.46

Table 2

Qualitative Test

Sample	Saponin	Tannin	Flavonoid	Alkaloid
Anacardium occidentalis	+	+	+	+
Gomphrena globosa	+	+	+	+

Effect of ethanolic leaf extract of *Anacardium occidentalis* and *Gomphrena globosa* on alloxan induced diabetic mice

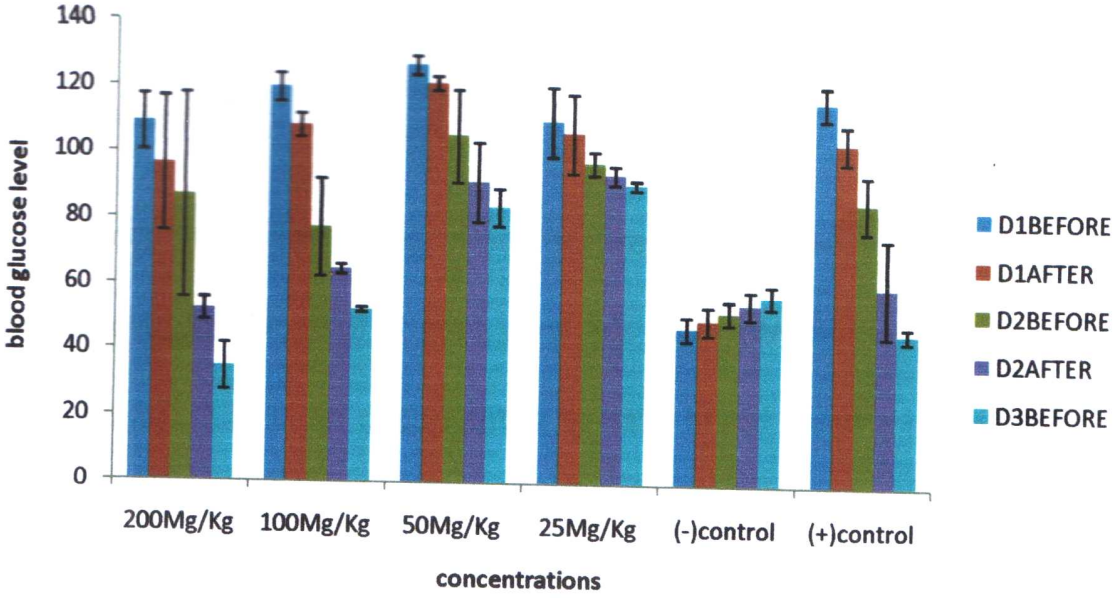
The result showed a dose dependent reduction in blood glucose level, Animals administered with extracts of concentration (200mg/ml) and (100mg/ml) showed a significant ($p<0.05$) reduction in blood glucose

level and there was no significant difference ($p<0.05$) in the activity of the extract when compared with the positive control results as shown in table 3.

Table 3 showed the blood glucose means and standard deviations at different concentrations of the extract.

***Anacardium occidentalis* Leaf Extract**

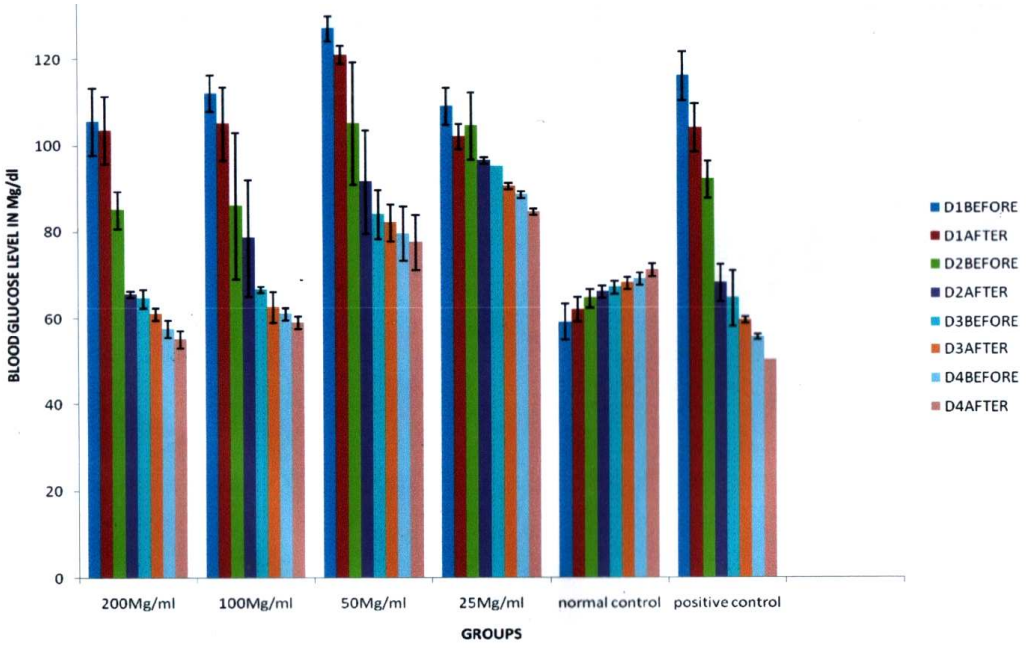
Concentration of extract in mg/ml Before	Day 1	Day2	Day3
200	109 ± 8.5	87 ± 31.1	35 ± 7.1
100	120 ± 4.2	77.5 ± 14.9	52.5 ± 1.0
50	127 ± 2.8	105 ± 14.1	84.0 ± 5.7
25	110.5 ± 10.6	97.5 ± 3.5	91 ± 1.4
Positive control	116.5 ± 4.9	86 ± 8.5	46.5 ± 2.1
Negative control			



Bar charts showing effect of different concentrations of extract at different times

***Gomphrena globosa* Leaf Extract**

Concentration of extract in mg/ml	Before	Day1	Day2	Day3	Day4
200	105.5 ± 7.7	85.0 ± 4.2	65.5 ± 0.7	61.0 ± 1.4	56.0 ± 2.1
100	112.0 ± 4.2	105.0 ± 8.4	78.5 ± 13.4	62.5 ± 3.5	61.0 ± 1.4
50	127.0 ± 2.8	121.5 ± 2.1	91.5 ± 12.0	82.0 ± 4.2	79.5 ± 6.3
25	109.0 ± 4.2	104.5 ± 7.7	96.5 ± 0.7	95.0 ± 0.0	90.5 ± 0.7
Positive control	116.0 ± 5.6	104.0 ± 5.6	92.0 ± 4.2	67.0 ± 2.8	54.0 ± 1.4
Negative control	59.0 ± 4.2	62.0 ± 2.8	64.5 ± 2.1	68.0 ± 1.4	69.0 ± 1.4



Bar charts showing effect of different concentrations of extract at different times

DISCUSSION

The hypoglyceamic effect of *Gomphrena globosa* on an alloxan induced diabetic rat in this work showed that repeated administration of the extract lowered the blood glucose level. Intraperitoneal administration of the extract at a dose of 200mg/kg b. w and 100mg/kg b. w showed a significant reduction in the blood glucose level, compared with both positive and negative control, while animals administered with the concentration of 50mg and 25mg/kg b. w. showed less significant reduction in the blood glucose level . Administration of ethanolic extract of *Anacardium occidentalis* effectively lowered the blood glucose level in alloxan induced diabetic rats compared to their negative control.

The plants extract may act by direct stimulation of insulin secretion in the B-cells, in other hand; the action of the extract may also involve insulin like extrapancreatic mechanism such as stimulation of glucose utilization and the reduction of hepatic gluconeogenesis [16]

The phytochemical analysis of both plants showed the presence of flavonoid saponins, tannins alkaloid. A number of investigations have shown that flavonoid and other secondary metabolites of plants including arginine and glutamic acid possess hypoglyceamic effect in various experimental animal model [17 ,18,19].

Hypoglyceamic activities of the leaves of *Gomphrena globosa* and *Anacardium occidentalis* may probably be due to flavonoid content of the plants, which appear to be involved in the stimulation of the B-cells and the subsequent secretion of preformed insulin. The result of acute toxicity test (LD 50) of *Gomphrena globosa* extract on Swiss mice indicated that the plant was not toxic since no death of the mice was recorded and this supported by the study on the toxicity and antioxidant effect of the plant [11] The LD 50 result of 125mg/kg b. w for *Anacardium occidentalis* leaves extract showed that the plant is toxic at high concentration. Its therefore mean that its not safe at concentration over 125mg/kg b. w.

CONCLUSION

The present study showed that ethanolic leaves extract of *Gomphrena globosa* and *Anacardium occidentalis* possessed hypoglycemic properties in alloxan induced diabetic rat, which suggest the presence of biologically active components which may be worth further investigation and elucidation. The hypoglycemic activities were found to be dose-dependent. Further studies is been recommend to isolate and characterized the active component of the crude extract of these plants.

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