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POTENTIAL ANTIDIABETIC ACTIVITY OF THE FRUIT EXTRACT OF *HARUNGANA MADAGASCARIENSIS* LAM. EX POIRET

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ABSTRACT

The aim of this study was to determine the hypoglycemic effect of the ethanol extract of *Harungana madagascariensis* fruit on alloxan induced diabetic rats. Following preliminary phytochemical screening of the ethanol fruit extract, an acute toxicity evaluation of the plant extract was carried out. Diabetes was induced by intraperitoneal administration of 160mg/kg of alloxan monohydrate and animals with blood glucose level greater than 200mg/dl were used for the antidiabetic activity evaluation. These were divided into six groups of five rats each. Group A animals were administered deionized water, groups B, C, and D were treated with 250, 500 and 1000mg/kg of the extract, respectively, while group E were treated with glibenclamide and group F were left untreated. All drugs were administered orally via an orogastric tube for three weeks. Blood glucose level was determined on days 0, 1, 3, 7, 14 and 21 using glucose test strip method. Phytochemical screening showed the presence of alkaloids, anthraquinone, tannins, saponins, flavonoid and terpenoids. The extract at 250, 500 and 1000mg/kg induced a statistically significant ($P<0.05$) reduction in blood glucose level on day 14 when compared to the diabetic untreated group. This study suggests that the fruit extract of *Harungana madagascariensis* possess a significant anti-diabetic activity.

KEYWORDS: *Harungana madagascariensis,* Antidiabetic, Ethanol, Fruit, Alloxan.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease which affects about 10% of the world's population. It is considered a "modern day epidemic" and as a global public health issue.[1]

Currently, there are over 240 million diabetics worldwide and about 40% of them develop severe diabetic nephropathy.[2] There is a significant increase in the number of patients suffering from this disease due to lifestyle changes such as less physical activity.[3] Despite the research that has been done, diabetic kidney epidemic is increasing rapidly requiring painful dialysis or kidney transplantation.[4] Management of diabetes without any side effect is still a challenge in the medical community. The use of drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects.[5] This situation has led to an increase in exploration of complementary and alternative medicines from natural resources having potent antidiabetic as well as nephroprotective activity with fewer side effects.

In recent years, interest in the treatment of diabetes using herbal drugs which are generally non-toxic has been renewed. World Health Organization has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs. Plants derivatives which have hypoglycemic properties had been used in folk medicine and traditional healing systems around the world from very ancients times.[6] Traditional medicine continues to provide health coverage for over 80% of the world population especially in the developing world.[7] Ethanol fruit extract of *Harungana madagascriensis* was reported by[8] to possess anti-anaemic activity. Hence, this study aimed to investigate the potential antidiabetic activity of the fruit of *Harungana madagascariensis* on albino rats.

MATERIALS AND METHODS

Plant Materials

The fruit of *Harungana madagascariensis* was collected from Omuoko forest, in Rivers state and it was authenticated by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, with herbarium number UPH/P/080; UPH/V/1,219.

Preparation of extract

The fruits of *Harungana madagascariens*is were freshly collected, cleaned, shade dried for two weeks and pulverized using a mechanical grinder. 1.2kg powder of *Harungana madagascariensis* was subjected to solvent extraction for seventy-two hours with absolute ethanol. The extract was concentrated using rotary evaporator and carefully evaporated to dryness over a water bath at a temperature of 45°C. The yield obtained was stored in a refrigerator and the percentage yield determined.

Animals used

Fifty eight healthy albino rats of average weight (150g) were used throughout the experiments. The animals were procured from the animal house of the Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State. All the animals were fed with rodent feeds and water ad-libitum under hygienic condition. Ethical approval was obtained from the research ethics committee of the University of Port Harcourt.

Chemicals and drugs used

Alloxan monohydrate (Manufactured by Aldrich Chemistry, a division of Sigma Aldrich Company (U.S.A)

Absolute Ethanol (Analytical grade) JHD.

Glibenclamide (Manufactured by Sanofi Aventis).

Phytochemical screening

Preliminary screening of crude extract of *Harungana madagascariensis* was carried out to determine the presence of its constituents using the method as described by.[9]

Acute toxicological evaluation

The acute toxicity study of the plant extract was done according to[10], using 18 healthy albino rats. In the first phase, three groups of three rats each were treated with the extract at doses of 10, 100 and 1000mg/kg body weight orally. They were observed for 24 hours for signs of toxicity and death.

In the second phase, three groups of three rats each were treated with the extract at doses of 1600, 2900 and 5000mg/kg body weight orally. They were observed for 24 hours for signs of toxicity and death.

Induction of diabetes mellitus

Diabetes was induced by intraperitoneal administration of alloxan monohydrate at a dose of 160mg/kg body weight dissolved in normal saline. After seventy two hours, the rats with fasting blood-glucose levels more than 200mg/dl were considered diabetic and selected for the study.[11]

Evaluation of anti-diabetic activity

Seventy-two hours after alloxan administration, rats with glucose level greater than 200mg/dl were selected for the anti-diabetic study. The animals were grouped into six groups of five

rats each. Group A (non-diabetic) animals were administered with deionized water, groups B, C, and D were treated with 250, 500 and 1000mg/kg extract respectively while group E (diabetic rats) were treated with 10mg/kg glibenclamide and group F (diabetic untreated) were given 5ml/kg of water. All administration was done through the oral route daily for three consecutive weeks. Blood glucose level was determined on days 0, 1, 3, 7, 14 and 21 using glucose strip method. The change in body weight and fasting blood glucose levels of all the rats were recorded at regular intervals during experimental period. Fasting blood glucose level was determined on day 0, 1, 3, 7, 14, 21, every morning before food administration. Acute study of the blood glucose level of the rats was done at 0hr, 2hrs, 3hrs and 4hrs on day 1.[11]

Blood glucose samples of the rats were collected by tail tipping with the aid of glucose test strip using a glucometer (Active) in mg/dl.

Statistical analysis

Data obtained are expressed as Mean ± SEM (Standard error of mean). Statistical significance was determined by Two-way analysis of variance test (ANOVA). Student's t- test was used to determine the level of significance between treated and control groups. P-values <0.05 were considered to be statistically significant.

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, flavonoids, anthraquinone, tannins, saponin, reducing sugar and triterpenes in the extract as shown in the table below.

Table 1: Phytochemical screening of ethanol fruit extract of *H. Madagascariensis.*

CHEMICAL CONSTITUENTS	OBSERVATION
Alkaloids	+
Flavonoids	+
Anthraquinone (free anthraquinone)	+
Saponins	+
Tannins	+
Phlobatanins	-
Carbohydrate (Reducing sugar)	+
Terpenoids	+

+ Presence, - absence

Acute toxicity study

From the acute oral toxicity tests, the animals showed signs of itching on administration of the extract which phases out after some time and the LD_{50} of the fruit extract of *Harungana madagascariensis* was found to be greater than 5,000mg/kg.

Effect of ethanol extract of *Harungana madagascariensis* on body weight

There was an increase in the body weight of all the rats treated with 250, 500,1000mg/kg extract and glibenclamide. However, the increase in body weight was not statistically significant (P>0.05) when compared to the diabetic untreated group. There was a statistically significant (P<0.05) difference in weight gain in the control group when compared to the diabetic untreated group.

Table 2: Effect of ethanol fruit extract of *Harungana madagascariensis* on body weight of alloxan-induced diabetic rats (Mean± SEM).

TREATMENTS	DOSE mg/kg	DAY 0	DAY 21	INCREASE/DECREASE IN BODY WEIGHT
EXTRACT	250	120.1±5.8	132.8±8.7	12.7
	500	119.7±10.8	146.5±12	26.8
	1000	153.7±0.8	158.7±7.3	5.0
GLIBENCLAMIDE	10	113.5±11.6	128.3±8.8	14.6
DIABETIC UNTREATED	5ml	102.1±4.3	116.9±15.5	14.8
CONTROL	5ml	118.3±2.7	168.6±2.7*	50.3

*=P<0.05 compared to diabetic untreated showed a statistically significant difference. n = 5

Antidiabetic activity evaluation

Table 3 below showed that the 500mg/kg extract treated group had a decrease in blood glucose level on day 7, 14 and 21 which was persistent when compared to diabetic untreated group and glibenclamide treated group while 250mg/kg and 1000mg/kg extract treated groups exhibited a decrease in blood glucose level on day 3,7,14 and day 7, 14 respectively with an increase on day 21.

However, the result showed that the blood glucose levels of 250mg/kg, 500mg/kg and 1000mg/kg extract-treated groups gave a statistically significant difference at P<0.05 when compared to the diabetic untreated and glibenclamide treated groups. Therefore, the effect seems to be dose-dependent.

Table 4: Effects of ethanolic extract of fruit of *Harungana madagascariensis* on blood glucose levels of alloxan-induced diabetic rats. (Mean ± SEM).

Treatment Days	Blood Glucose Level Mg/Dl *Harungana Madagascariensis* Extracts			Glibenclamide	Diabetic Untreated	Control
	250mg/kg	500mg/kg	1000mg/kg	10mg/kg	5ml/kg	5ml/kg
DAY 0	438±42.2	499±97.8	358±31.2	375±36.3	428±86.2	101±7.4
DAY 1	519±41.3	531±69.7	448±49.8*	544±28.6	588±12.4	112±4.4
DAY 3	507±49.3	537±63.7	460±43.4*	583±17.0	553±25.0	122±3.1
DAY 7	429±30.7	483±88.3	345±24.7	423±11.6	438±88.0	95±3.5
DAY 14	243±46.4*	398±12.0*	282±32.2	369±15.2	327±38.1	100±3.1
DAY 21	352±68.2	351±21.1*	286±81.0	457±10.7	429±58.2	89±3.5

*=P<0.05 showed a statistically significant difference when compared with diabetic untreated and glibenclamide. n = 5.

DISCUSSION

Despite the presence of known antidiabetic drugs in the pharmaceutical market, herbal drugs are frequently considered to be less toxic, free from side effects and cheaper than synthetic ones. Alloxan causes enormous reduction in insulin release through the destruction of cells of the islets of langerhans, thereby inducing hyperglycemia.[12] It is also reported to induce diabetes by forming highly reactive superoxide radicals which destroy the insulin producing cells in the pancreas.[13]

Decoctions from different parts of *Harungana madagascariensis* are highly valued in the treatment of various human diseases including drug related renal disease in African traditional medicine as reported by.[14]

Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, reducing sugars, terpenoids and steroids. This can be related to a study which stated that the ethanol extract of *Mimosa pigra* possessed antidiabetic activity as a result of the presence of flavonoids and tannins in the extract.[8] The findings of this study is also similar to that of Mirsha which reported that plant constituents under the category of polysaccharides, peptides, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthones, flavonoids, lipids, phenolics, coumarins, iridiods, alkyl disulphides, inorganics ions and guanidines have antidiabetic activity.[15]

The acute toxicity of the ethanol extract of fruit of *Harungana madagascariensis* showed no adverse effect or mortality even at 5000mg/kg which showed that it is relatively safe.[10] This can be corroborated with the study of Saidu which showed that the oral LD_{50} of the ethanolic

extract of *Cassia arereh* was greater than 5000mg/kg.[16]

From the result obtained and analysis carried out (P<0.05), there was an observable change in body weight of all the extract treated groups and glibenclamide. Although the increase in weight was not statistically significant when compared to diabetic untreated. Only the control group showed a significant weight gain. This can be related to the study which reported that the increase in weight may be a reflection of efficient insulin action.[17]

During prolonged study, the blood glucose level of 250mg/kg extract treated group decreased on days 3, 7 and 14, that of 500mg/kg extract treated group decreased on days 7,14 and 21 while the blood glucose level of 1000mg/kg extract treated group decreased on days 7 and 14. The 250mg/kg extract treated group gave a significant decrease in blood glucose level on day 1 and 14, the 500mg/kg extract treated group gave a significant decrease in blood glucose level on day 1, 14 and 21 while 1000mg/kg extract treated group gave a significant decrease in blood glucose level on day 1, 3 and 21 when compared to diabetic untreated group. The extract treated groups at 250mg/kg showed a statistically significant difference (P<0.05) in blood glucose level reduction on day 14, the 500mg/kg exhibited a statistically significant decrease on days 14 and 21 while the 1000mg/kg had a statistically significant difference in blood glucose level on day 3 when compared to the positive control group.

A study done by Norberg showed that saponins demonstrated glucagon decreasing effect which may enhance glucose utilization and lower blood glucose level.[18] Also saponin stimulates insulin release from pancreas. It has been demonstrated that flavonoids may reduce hyperglycemia and exert protective effects against non-enzymatic glycation of proteins in animals.[19] Tannins have been reported to inhibit insulin degradation and improves glucose utilization in addition to their α-glucosidase inhibitory activity.[20] The phytochemical constituents of the ethanol fruit extract of *Harungana madagascariensis* with their different mechanism of action may have contributed to the antidiabetic activity of the plant.

CONCLUSION

It may be concluded that the fruit extract of *Harugana madagascariensis* possess antidiabetic activity which may be due to the tannin, flavonoid, triterpenes, anthraquinone, saponin and alkaloid present in the plant.

CONFLICT OF INTEREST

Authors have declared that no conflict of interests exist.

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