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Research Article

The Effects of The Leaf Extracts of Vernonia Amygdalina, Ocimum Gratissimum and Phyllanthus Amarus on Blood Glucose Level of Alloxan-Induced Diabetic Guinea Pigs

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Abstract

This research work was meant to explore the hypoglycemic potencies of different tropical herbal aqueous leaf extract of Vernonia amygdalina (Bitter leaf), Phyllanthus amarus (Stone breaker) and Ocimum gratissimum (Sent leaf) on blood sugar level of alloxan induced diabetic guinea pigs. Thirty-five guinea pigs of both male and female were randomly selected and grouped accordingly using Glibenclamide solution (a known oral hypoglycemic drug) as a positive control and physiological solution (0.9% Normal Saline) as a negative control. The average weight of the guinea pigs was 250g. Each guinea pig was made diabetic by induction with a single dose of 5% alloxan monohydrate dissolved in 0.9% normal saline at 200mg/kg body weight intraperitoneally. This concentration of alloxan used for diabetic induction in these guinea pigs was determined during the preliminary test. Three different groups had oral administration of the aqueous leaf extract via Canula at 300mg/kg body weight per day for two weeks after alloxan induction. The positive and negative control groups had oral administration of glibenclamide solution (a known oral hypoglycemic agent) at 0.25mg/day and 0.9% NS (a physiological solution) at 15ml/day via canula for two weeks respectively. On the average, the results of this study revealed appreciable percentage reduction of fasting blood sugar level of the diabetic guinea pigs that had oral administration of aqueous leaf extracts of Phyllanthus amarus, Vernonia amygdalina and Ocimum gratissimum which was comparable to the fasting blood sugar level of positive control group (Glibernclamide solution), thereby showing an appreciable hypoglycemic effect. In conclusion, the z-test as a statistical test revealed a significant difference between the post induction fasting blood sugar level and post aqueous leaf extract administration ($p< 0.05$).

Introduction

Diabetes mellitus is one of the commonest tropical endocrine or metabolic diseases [1]. The incidence of diabetes mellitus is growing rapidly worldwide. For example, it is estimated that 135 million people worldwide are afflicted with the most common form of type II [2]. It is also estimated that about 10 million Nigerians are diabetic [3]. An estimated 20 million people in the United States of America or 6.3 percent of the population have diabetes, a serious life-threatening condition. Diabetes mellitus is a syndrome characterized by hyperglycemia caused by relative or absolute deficiency of insulin or peripheral resistance to insulin. It involves disturbance of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion or insulin action [4]. Diabetes mellitus is classified mainly into type I Diabetes Mellitus, Type II Diabetes Mellitus and Maturity-Onset Diabetes in Youth (MODY) or gestational diabetes. Type I diabetes mellitus formally called insulin dependent diabetes mellitus (IDDM) or juvenile diabetes is associated with profound insulin deficiency and accounts for only about 5-10% of those with diabetes mellitus. Type II diabetes mellitus formerly known as Non-insulin dependent diabetes mellitus (NIDDM) accounts for about 90-95% of those with diabetes. It is associated with insulin resistance. Management of this disease is basically lifestyle modification, diet, oral hypoglycemic agents and insulin therapy.

These selected tropical herbs used in this study are extensively applied by herbalists and taxonomists or several medical conditions such as diabetes mellitus. The major emphasis is on

their aqueous leaf extracts. Vernonia Amygdalina (bitter leaf) is a tropical shrub in the plant family of composite. The leaves are widely used as vegetables probably because of its therapeutic properties. Those who consumes, bitter leaf regularly stands a better chance of being prevented from developing diabetic and hypertensive complications [5]. The leaves of vernonia amygdalina are given with a characteristic odor and bitter taste attributed to anti-nutritional factors including alkaloids, saponins, tannins, glycosides, sesquiterpenes flavonoids as seen in phytochemical screening [6]. Strong anti-oxidant activities have been reported for flavonoids from vernonia amygdalina and its saponins have been reported to elicit anti-tumoral activities in leukemic cells [7].

Ocimum gratissimum

Ocimum gratissimum commonly called Scent leaf is an herbaceous perennial herb and wood at its base. It is commonly used in preparing foods owing to its spicy nature apart from its therapeutic importance. This herb is in the family of Labitae, and it is widely seen in Africa, east India and Brazil. It comprises of green leaves, stem and roots.The aqueous extract of ocimum gratissium has a hypoglycemic effect which is due to methanolic extract of the leaves which enhance its hypoglycemic activity. The extracts contain essential oil. The essential oil is anti-protozoan. It inhibits the growth of protozoan [8]. Antibiotic effects have been associated with the extract of ocimum gratissimum leaves. This is basically due to its essential oil. The essential oil in ocimum gratissimum has been found to inhibit staphylococcus. Aside the essential oil, the methanol extract has antibiotic properties and facilitates wound healing [9]. Phyllanthus amarus commonly called stone-breaker is a perennial herb grown in the tropical forest such as Africa. It is also seen in china and Asia. This herb has tiny green leaves and soft stems. It is in the family of Euphorbiaceous. The extract of phyllanthus amarus has been widely found and used in diabetic management by some herbalists probably due to its hypoglycemic effects. Aside its application in diabetic management, it has also been found useful in viral hepatitis including chronic hepatitis. Its extracts are said to be hepato-protective and it's used in the herbal treatment o primary hepatocellular carcinoma [10].

Materials and Methods

Experimental animals/grouping

Thirty-five guinea pigs of different sexes were randomly selected for this study and weighed with a weighing scale before administration of any substance or induction with alloxan. The average weight of the guinea pigs was 250grams body weight. These guinea pigs were grouped into five groups with five guinea pigs in each group. The first three groups were for the substances or extract used for the study. While the remaining two groups were for the negative and positive control groups with oral administration of 0.9% N/S (Physiological solution) and solution of Glibenclamide (Oral hypoglycemic) respectively. All the grouped guinea pigs were intraperitoneally induced with a single dose of 5% alloxan monohydrate dissolved in 0.9% Normal saline after determining their fasting blood sugar with a glucometer (Pre-induction fasting blood sugar).

Procedure

Those for the preliminary test were induced with a single dose of alloxan intraperitoneally at concentration of 100ml/dl, 150ml/dl and 200mg/dl for the respective groups as to determine the optimal concentration of alloxan that will bring about a significant increase in fasting blood sugar three days post alloxan induction in three consecutive readings. The three main groups of guinea pigs were induced with a single dose of alloxan intraperitoneally with 200mg/kg body weight with a 2ml syringe to make them diabetic after blood sugar level pre-alloxan induction had been taken by the glucometer. These guinea pigs were fed with elephant grasses and fasted for 9 hours in each reading.

Aqueous leaf extract of the fresh leaves of these tropical herbs were obtained by squeezing one kg each bunch of fresh leaves in 280 mls of water in different washing basin and thereafter received in different labeled bottles after sieving. Prior to extraction, the leaves were bought from the local market aside the phyllanthus amarus which was gotten from the bush. These leaves were identified by staff of department of Plant Science University of Port Harcourt. The aqueous leaf extracts were given per oral with a canula at 300mg/kg body weight to the grouped alloxan induced diabetic guinea pigs. Solution of glibenclamide at 0.25mg/day orally was given via canula to the positive control group post alloxan induction. In the same manner, 0.9% normal saline was also administered orally via canula to the diabetic induced negative control group. The fasting blood sugar level was determined after three consecutive days for two weeks and then the mean values determined. The aqueous leaf extracts were taken for phytochemical analysis at the department of Biochemistry, Macdonald University Elele, and Rivers State.

Phytochemical screening

The phytochemical screening (test) was done in the department of Biochemistry Macdonald University Elele, Rivers State. Phytochemical screening of the six aqueous leaf extracts under study was carried out on the crude methanolic extract and the weakly acidic fraction using standard procedure and reagent outlined by Harbourine [11]. In general, test for the presence or absence of phytochemical compounds using the above method involving the addition of an appropriate chemical reagent to the tests sample in a test tube. The presence or absence of the phytochemical compounds in each extract was established. These phytochemical compounds are saponins, flavonoids, alkaloids, tannins, carbohydrates such as glucosides (reducing sugars), proteins, resins, oil, steroids and terpenes. All the extracts were tested for these above phytochemical compounds.

Discussion

In the preliminary test, single dose alloxan induction of the guinea pigs given intraperitoneally at different concentrations of 100mg/kg, 150mg/kg and 200mg/kg body weight was seen in the Table 1 above. This Table 1 shows the mean fasting blood sugar level after a single dose alloxan induction of the five guinea pigs in three different subgroups. Induction with 200mg/kg body weight revealed an appreciable increase in the mean fasting blood sugar level of 1.6mmol/L which was greater than the readings obtained from the other two concentrations. Alloxan induction with 100mg/kg and 150mg/kg revealed an increase in the mean fasting blood sugar of 0.4mmol/L and 0.8mmol/L respectively. These mean values were less than 1.0mmol/L. Table 2 shows the percentage reduction of the mean fasting blood sugar using aqueous leaf extracts of the three tropical herbs on alloxan induced diabetic guinea pigs when administered singly with the glibenclamide solution (oral hypoglycemic drug) as positive control and 0.9% Normal saline as a negative control. In Table 3, the percentage reduction of blood sugar level in different alloxan induced diabetic guinea pig groups were compared with that of the control groups. The percentage reduction of blood sugar level of these diabetic guinea pigs was seen to be highest for the groups of guinea pigs that had oral administration of aqueous leaf extract of Phyllanthus amarus and Vernonia amygdalina which were comparable with the positive control groups that had oral administration of glibenclamide solution. This indicates an appreciable hypoglycemic effect.

Table 1: The preliminary tests showing differential increase in mean fasting blood sugar level after alloxan induction at concentration of 100mg/kg and 200ml/kg.

Sub groups	Mean fasting blood sugar level before alloxan induction (mmol/L)	Mean fasting blood sugar level after alloxan induction (mmol/L)		
		100 mg/kg	150 mg/kg	200 mg/kg
1 (n=5)	5.0	5.4	-	-
2 (n=5)	5.0	-	5.8	-
3 (n=5)	5.1	-	-	6.7
Differential increase in mean fasting blood sugar level after alloxan induction		0.4	0.8	1.6

Table 2: The percentage reduction of mean fasting blood sugar level readings obtained from induced groups of guinea pigs with alloxan (200mg/kg) after administration of aqueous leaf extracts using 0.9% normal saline and Globenclamide solution as negative and positive control groups respectively.

Substances Administered	Mean weight of 70 Guinea pigs(g) (250g)	Mean fasting blood sugar level before alloxan induction (mmol/L)	Mean fasting blood sugar level after alloxan induction (mmol/L)	Mean fasting blood sugar level after substance administration (mmol/L)	Actual rate of reduction	Percentage reduction of mean fasting blood sugar level of alloxan induced diabetic guinea pigs after substance administration (%)
0.9% Normal Saline (n=5)		4.98	6.97	7.05	1.98	-
Glibenclamide (n=5)		5.02	7.0	5.02	1.83	20
Phyllanthus amarus (Stone breaker) (n=5)		4.98	6.95	5.02	1.93	20
Vernonia amygdalina (Bitter leaf) (n=5)		5.02	7.0	5.07	1.9	19
Ocimum gratissimum (Scent leaf) (n=5)		5.07	6.67	5.42	1.3	13

Ocimum gratissimum aqueous leaf extracts also showed moderate percentage reduction of 13% respectively which were less than the readings from positive control group of 20% reduction in mean fasting blood sugar level after those extracts. The negative control group of diabetic guinea pigs that had 0.9% Normal saline which is a physiological solution revealed no percentage reduction of blood sugar level. Using ANOVA as the statistical analytic methods, mean fasting blood sugar level of different groups as shown in this Table 3 before alloxan induction could be compared with fasting blood sugar level after alloxan induction. This Table 3 indicates that there was a significant difference between the mean fasting blood level before and after alloxan induction in all the groups ($p<0.05$). Also, in Table 4, Using Z-Test to compare the mean fasting blood sugar level of these guinea pigs after alloxan induction and mean fasting blood sugar after substance administration, a significant difference was seen in groups of guinea pigs that had glibenclamide

solution and those that had aqueous leaf extracts of phyllanthus amarus, vernonia amygdalina and ocimum gratissimum (P<0.05). This indicates that the mean fasting blood sugar level of the groups of guinea pigs that had these aqueous leaf extracts above is comparable to the mean fasting blood sugar level of the positive control group that had glibenclamide (oral hypoglycemic agent).

Table 3: The mean fasting blood sugar level of the grouped guinea pigs before and after alloxan induction using ANOVA as the statistical analytic method as seen in the table above.

Substances	Fasting Blood Sugar before Alloxan induction (mmol/L)	Fasting Blood Sugar after Alloxan Induction (mmol/L)	Fasting Blood Sugar after substance administration (mmo/L)	ANOVA
0.9% N/S (n=5)	4.98±0.08	6.97±0.04	7.05±0.05	0.01
	4.88-5.10	(6.9-7.0)	(7.0-7.10)	
Glibenclamide (n=5)	5.02±0.12	7.0±0.12	5.02±0.12	0.02
	4.90-5.13	(6.8-7.1)	(4.83-5.10)	
Phyllanthus Amarus	4.98±0.07	6.95±0.42	5.02±0.02	0.01
	4.90-5.10	(6.9-7.0)	(5.0-5.04)	
Vermonia Amygdalina (n=5)	5.02±0.03	7.0±0.10	5.07±0.04	0.01
	(4.98-5.05)	(6.6-6.9)	(5.03-5.10)	
Ocimum Gratissium	5.07±0.48	6.67±0.10	5.42±0.36	0.03
	5.0-5.10	(6.5-6.8)	(5.38-5.53)	

Table 4: Comparison between the mean fasting blood sugar level of the grouped guinea pigs after alloxan induction and after substance administration using Z-Test.

Substances	Mean Fasting Blood Sugar Level after Alloxan Induction (mmol/L)	Mean Fasting Blood Sugar after substance administration (mmol/L)	Z-Test
0.9% N/S (n=5)	6.97	7.05	0.08(No)
Glibenclamide (n=5)	7	5.02	0.03(yes)
Phyllanthus Amaraus (n=5)	6.95	5.02	0.01(Yes)
Vernonia Amygdalina (n=5)	7	5.07	0.01(Yes)
Ocimum Gratssimum (n=5)	6.67	5.42	0.01(Yes)

Table 5 represented above showed the quantity of the phytochemical constituents in each aqueous leaf extract of these tropical herbs during the phytochemical analysis. The presence of these phytochemical constituents is related to the hypoglycemic potency. In Table 6, the quantity of phytochemical constituents of these aqueous leaf extracts was expressed in percentages. The result obtained from the phytochemical screening of these aqueous leaf extracts as shown in Table 5 & 6 indicated that Vernonia amygdalina contains moderate levels of Alkaloids, Saponnine, Flavinoids at 100% composition and glycosides, tannins, Steroids at 66.6% composition with mild levels of Resins, terpenes and Proteins at 33.3% composition. In other words, there are more alkaloids, flavonoids, saponnins than other phytochemical compounds in vernonia amygdalina. This extract contains no carbohydrates or reducing sugars. The presence of many of these phytochemical constituents in appreciable quantities up to 100% and the absence of carbohydrate including reducing sugars may be responsible for its high hypoglycemic potency [12-25].

Table 5: The quantitative analysis obtained from phytochemical screening of the aqueous leaf extracts.

Phytoconstituent	Vernonia amygdalina	Ocimum gratissimum	Phyllanthus amarus
Alkaloids	+++	-	+
Glycosides	++	++	++
Carbohydrates	-	++	-
Reducing sugar	-	++	+
Flavinoids	+++	+++	-
Resins	+	-	+
Tannins	++	+	++
Steroids	++	+	-

Saponnins	+++	++	+
Terpenes	+	+	+
Proteins	+	+	-

= absence of the phytochemical compound

= = presence of the phytochemical compound.

Table 6: The percentage composition of the phytochemical constituents found in phytochemical screening of the aqueous leaf extracts of these three tropical herbs used for the study.

Phytoconstituent	Vernonia amygdalina (%)	Ocimum gratissimum (%)	Phyllanthus amarus (%)
Alkaloids	100	0	0
Glycosides	66.6	66.6	66.6
Carbohydrates	0	66.6	0
Reducing sugar	0	66.6	33.3
Flavinoids	100	100	0
Resins	33.3	0	33.3
Tannins	66.6	33.3	66.6
Steroids	66.6	33.3	0
Saponnins	100	66.6	33.3
Terpenes	33.3	33.3	33.3
Proteins	33.3	33.3	0

% = percentage composition of Phytochemical constituents in extracts.

Phyllanthus amarus was found to have moderate levels of glycosides and tannins at 66.6% each, with mild level of reducing sugars, resins, saponins and terpenes at 33.3% composition. It was also found to contain no alkaloids, carbohydrates aside reducing sugars, flavonoids, steroids and proteins. The aqueous leaf extracts of this herb contain more of glycosides and tannins than any other phytochemical compounds. The saponins in this extract is far less than that of vernonia amygdalina but contains the same quantity of tannins and resins. The absence for its hypoglycemic effect. It has been noted that steroids are diabatagenic. Ocimum gratissimum contained moderate levels of flavonoids, glycosides and carbohydrates, reducing sugars and saponins at 66.6% each during phytochemical screening. The glycosides and terpenes were found to be of the same quantity as in the above two extracts. The saponins in this leaf extracts are less than the quantity obtained from vernonia amygdalina but contained the same quantity of flavonoids at 100% composition. However, flavonoids as a phytochemical constituent has antioxidation effect while saponins and peptides have antitumoral effects as stated in the early part of this work.

Conclusion

The results obtained from this study have revealed that aqueous leaf extract of phylianthus amarus, vernonia amygdalina and ocimum gratissimum have appreciable hypoglycemic effect owing to the presence of mild level of terpenes and can be termed oral hypoglycemic agents. These agents are associated with significant reduction in blood sugar level which was comparable with the effect seen in glibenclamide (positive control) and hence showed no significant difference with the effects seen in glibenclamide ($p>0.05$). However, using a statistical test such as Z-Test, a significant difference was seen between the post-alloxan induction and post-extract administration of these above three tropical leaves ($P<0.05$).

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