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THE HYPOGLYCEMIC EFFECTS OF AQUEOUS LEAF EXTRACTS OF GONGRONEMA LATIFOLUM, PIPER NIGRUM AND SOLANUM MELONGENA ON BLOOD GLUCOSE LEVEL OF ALLOXAN-INDUCED DIABETIC GUINEA PIGS

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ABSTRACT

The aim of the study was evaluate the aqueous leaf extract of Gongronema latifolium (Utazi), Piper nigrum (Uziza) and Solanum melongena (Gardern egg) on blood sugar level of alloxan induced diabetic guinea pigs. Thirty -five guinea pigs of body weight (250-300g) of both male and female were randomly selected and grouped in to five groups. Each guinea pig was made diabetic by induction with a single dose of 5% alloxan monohydrate dissolved in 0.9% normal saline at 300mg/kg body weight intraperitoneally. Prior to administration, concentration of alloxan used for diabetic induction in these guinea pigs was determined during the preliminary test. The positive and negative control groups had oral administration of glibenclamide solution (a know oral hypoglycemic agent) at 0.25mg/day and 0.9% NS (a physiological solution) at 15ml/day via canula for two weeks respectively. 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 Gongronema latifolium which was comparable to the fasting blood sugar level of positive control group (Glibernclamide solution), thereby showing an appreciable hypoglycemic effects. Similarly, the aqueous leaf extracts of Solanum melongena had a low percentage reduction which depicts mild hypoglycemic effects, while the group that had Piper nigrum showed a very insignificant percentage of reduction of mean fasting blood sugar which was comparable to the effect seen in negative control group. 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 ($p < 0.05$).

KEYWORDS: Gongronema latifolium (Utazi), Piper nigrum (Uziza) and Solanum melongena (Gardern egg).

INTRODUCTION

Diabetes is one of the most common metabolic disorders.[1] It has been reported that about 10million Nigerians are suffering from diabetes[2] while an estimation of 135 million people worldwide are inflicted with type 11 diabetes.[3] Diabetes mellitus is a syndrome characterized by hyperglycemia caused by absolute deficiency of insulin or peripheral resistance to insulin.[4] There are two main types of diabetes, type I Diabetes Mellitus and Type II Diabetes Mellitus. These selected tropical herbs used in this study are extensively applied by herbalists for several medical conditions such as in cases of diabetes mellitus. Gongronema latifolium is a rainforest tropical plant in the family of Asclepiadaceae. It is a herb with a climbing stem. It is called "Utazi" in Igbo or Ibibio, while in Yoruba it is called "Arokeke or Madumaru". It is used in food preparation as vegetable or as a herbal remedy in herbal practice. The aqueous or ethanolic extract of those leaves are used in herbal practice for management of diabetes because of its hypoglycemic (antiperglycemic) effects. The extracts of those leaves have anti-inflammatory and anti-oxidant effect. Owing to the above effects, they can be used to abort inflammation as anti-inflammatory extracts to reduce serum, cholesterol in cases of hyperlipidaemia and as anti-oxidant as to remove free radical or toxin in the body.[5,6]

Piper nigrum is a herb in the family of piperaceae. It is also called black pepper or "Uziza" in Igbo. It is a monoecious or dioecious climbing vine. The stout climbing stems are flexible. This herb is widely used as a homeopathis remedy, so its therapeutic values are more appreciated in the homeopathic or herbal practice.[7] It is also used as spice in food preparation especially, in tropics.

Solanum melongena. These are the tropical economic flowering plants belonging to the family of solanaceace. Solanum species are popularly known as Garden egg plants. They are widely grown in farms of tropical and temperate region. In Igbo, it is called "Anara" or

"Afufa"; "guata" in Hausa, "Igbaaja", in Yoruba, "Nya", in Ibibio "Anyara", in Efik. It is also called bitter tomatoes. The leaves and fruits of this tropical plant are consumable. The leaves are used for preparing soups as vegetables aside its therapeutic value. There are two major botanical species namely, Solanum aergiopicum and solanum melongena. In this study, solanum melangena leaf extract was used. In herbal practice, the aqueous leaf extracts are used for treatment of diabetes mellitus due to its mild hypoglucemic effects. The aqueous leaf extracts of this herb is popularly used by many herbalist in the management of chronic anaemia. Solanum melongena may be used by patients suffering room raised intraocular pressure (Glaucoma) and convergence insufficiency.[8] The aqueous leaf extracts of these tropical herbs may be obtained by squeezing the green leaves with a given quantity of water. The aqueous leaf extract should be taken when fresh; otherwise storage of the extract in the refrigerators or deep freezer reduces the potency to nil.[9,10]

MATERIALS AND METHODS

Thirty five guinea pigs of different sexes were randomly selected for this study weighing between 250-300g. These guinea pigs were grouped into five groups with five guinea pigs in each group. The first three groups were for the extract used for the study. While the remaining groups one and two were 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).

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.

RESULTS

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)		
		100mg/kg	150mg/kg	200mg/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
Gongronema latifolium (Utazi leaf) (n=5)		4.94	6.95	5.23	1.8	18
Solanum melongena (Garden egg)		4.97	7.04	6.02	1.0	10
Piper nigrum (Uziza) (n=5)		5.03	6.96	6.85	0.1	1

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)	
Gongronema Latifolium (n=5)	4.94±0.05	6.95±0.12	5.23±0.07	0.02
	4.88-5.0	(6.8-7.1)	(5.23-5.40)	
Solanum Melongena (n=5)	4.97±0.57	7.04±0.05	6.02±0.25	0.01
	4.88-5.03	(7.0-7.1)	(5.73-6.40)	
Piper Nigrum (n=5)	5.03±0.09	6.96±0.67	6.85±0.05	0.01
	4.90-5013	(6.9-7.0)	(6.80-6.90)	

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)
Gongronema Latifolium (n=5)	6.95	5.23	0.04(Yes)
Solanum Molengena (n=5)	7.04	6.02	0.02(Yes)
Piper Nigrum (n=5)	6.96	6.85	0.279(No)

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

- = absence of the phytochemical compound
- = = presence of the phytochemical compound.

Phytoconstituent	Piper nigrum	Solanum melongena	Gongronema latifolium
Alkaloids	-	++	++
Glycosides	-	++	-
Carbohydrates	+	+	+
Reducing sugar	-	+	-
Flavinoids	+++	+	+
Resins	+	+	+
Tannins	++	++	++
Steroids	-	-	+
Saponnins	+	+	++
Terpenes	-	-	+
Proteins	-	-	++

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

Phytoconstituent	Piper nigrum (%)	Solanum melongena (%)	Gongronema latifolium (%)
Alkaloids	0	66.6	66.6
Glycosides	0	66.6	0
Carbohydrates	33.3	33.3	33.3
Reducing sugar	0	33.3	0
Flavinoids	100	33.3	33.3
Resins	33.3	33.3	33.3
Tannins	66.6	0	66.6
Steroids	0	0	33.3
Saponnins	33.3	33.3	66.6
Terpenes	0	0	33.3
Proteins	0	0	66.6

% = percentage composition of Phytochmical constituents in extracts.

DISCUSSION

In this 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 above. The table 1 above showed the mean fasting blood sugar level after a single dose alloxan induction of the five guinea pigs in three different groups at different concentration of 100mg/kg, 150mg/kg and 200mg/kg which showed that there was an appreciable increase in the mean fasting blood sugar level of 1.6mmol/L when compared to the other two concentrations. Alloxan induction with 100mg/kg and 150mg/kg showed an increase in the mean fasting blood sugar of 0.4mmol/L and 0.8mmol/L respectively, which 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. Table 3 showed that there was a significant difference between the mean fasting blood level before and after alloxan induction in all the groups ($p<0.05$), using ANOVA as an analytical method. 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 gongronema latifolium and solanum melongena. This indicates that the mean fasting blood sugar level of the group of guinea pigs that had these aqueous leaf extracts are comparable to the mean fasting blood sugar level of the positive control group that had glibenclamide. Similarly, there was no significant difference in the negative control group of guinea pigs that had oral administration of 0.9% Normal Saline (physiological solution) and the group that had piper nigrum ($P>0.05$).

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. Table 6, showed 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 Gongronema latifolium which was comparable with the positive control groups that had oral administration of glibenclamide solution. This indicates an appreciable hypoglycemic effect. Solanum melongena aqueous leaf extracts also showed moderate percentage reduction 10% which were less than the readings from positive control group of 20% reduction in mean fasting blood sugar level after those extracts. The aqueous leaf extracts of Piper nigrum showed a minimal percentage reduction of 1% which was as good as no reduction of the mean fasting blood sugar level of the diabetic guinea pigs in this group. 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.

The results obtained from the phytochemical screening of these aqueous leaf extracts as shown in table 5 and 6 indicated that Gongronema latifolium aqueous leaf extracts showed moderate level of tannins at 66.6% composition as seen in all the aqueous leaf extracts used in the study. They also contained moderate level of proteins at 66.6% composition which was more than the quantity seen in both vernonia amygdalina and ocimum gratissmum. Moderate level of saponnins which was less than the quantity seen in vernonia amygdalina was observed during the screening. It was found that these aqueous leaf extracts contained mild levels of alkaloids, carbohydrates, resins, flavinoids and terpenes at 33.3%. However, gongronema latifolium aqueous leaf extracts contained no glycosides and reducing sugars. The presence of carbohydrates in this extract may be responsible for the decreased hypoglycemic effect while

the absence of reducing sugars may account for its hypoglycemic effect. The aqueous leaf extracts of solanum melongena was found to contain moderate level of alkaloids, glycosides and tannins at 66.6% composition with mild levels of carbohydrate, reducing sugars, flavinoids, resins and sapronnins at 33.3% quantity of saponnins seen in this extract was less than the quantity seen in vernonia amygdalina 100% ocimum gratissimum (66.6%) and in gongronema latifolium (66.6%). Nevertheless, there were no steroids, terpenes and proteins seen during phytochemical screening of this aqueous leaf extract. The presence of both carbohydrates and reducing sugars may be responsible for its decreased hypoglycemic effects. The aqueous leaf extracts of piper nigrum contained moderate levels of flavinoids (100%) and tannins (66.6%) with mild levels of carbohydrates, resins and saponnins at 33.3% each. There were no presence of alkaloids, glycosides, steroids, terpenes and proteins during the phytochemical screening of this aqueous leaf extracts. The absence of many of these phytochemical constituents such as terpenes, alkaloid, proteins etc may account for its lack of hypoglycemic effect.

CONCLUSION

The results obtained from this study have revealed that aqueous leaf extract gongronema latifolium have appreciable hypoglycemic effect owing to the presence of mild level of terpenes and can be termed oral hypoglycemic agents. Solanum melongena revealed mild hypoglycemic effects probably due to presence of carbohydrates and reducing sugars which are diabetogenic and hence it could be termed mild hypoglycemic agent. Therefore the above extracts may have similar mechanism of action as the other groups of oral hypoglycemic drugs.

RECOMMENDATION

Having reviewed significant hypoglycemic effects of aqueous leaf extracts of Gongronema latifolium and Solanum melongena in this study, formulation of these as supplements may be useful for diabetic patients. Considering the fact that most of these leaves have nutritive values, they may be recommended in our daily food delicacies as useful nutritive ingredients. The aqueous extracts of these tropical herbs may be recommended to persons who are at risk of having type II diabetes mellitus such as those with family history of diabetes in the first degree relative older age (more than 45 years).

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Conflict of interest

No conflict of interest.

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