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RESEARCH ARTICLE

EFFECT OF AQUEOUS LEAF EXTRACT OF *HEINSIA CRINATA* ON BLOOD GLUCOSE LEVELS IN NON-DIABETIC ALBINO RATS

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ABSTRACT

This research was designed to study the effect of aqueous extract of *Heinsia crinata* commonly called "atama" on blood glucose level in non-diabetic rats. The extract and reference drug (glibenclamide) were administered intraperitoneally in dosage of 120,180,240 and 300mg per kilogramme body weight of rats, which were observed for 24hrs. Glucose estimation using the one touch glucometer at an internal of 2 hours into the eight hour and then at the 24 hour was done. The result showed a decrease in glucose level which was significant statistically at $p< 0.05$.The greatest reduction in blood glucose levels occurred between the 8th and 24th hours respectively and became noticeable after the 4th hour and more pronounced in the 120mg/Kg body weight which shows a decrease from 2.55±0.20Mmol/L to1.67±0.58 after the 24th hour. It also showed that with increase in dosage, there was a stimulatory effect of the extract which was not significant statistically at the $p<0.05$ level. This suggests that the hypoglycaemic effect of *Heinsia crinata* was not concentration but time dependent. The phytochemical analysis of the plant leaf extract both qualitatively and quantitatively revealled the presence of akaloid, saponnis, tannins flavonoid, terpenoids, carotenoids and anthraquinones. The dosage applied in the study did not show any toxic effect on the rats. The result therefore suggests that aqueous leaf extract of *Heinsia crinata* possess hypoglycaemic activity and so can be useful in the treatment of diabetes even as it is been used as food.

INTRODUCTION

Diabetes is a metabolic disorder that results to the increase in blood of glucose levels, a condition generally referred to as hyperglycaemia. The resultant effect is the excretion of glucose in urine of affected persons. It is a pathological condition which along with other clinical complications like cardiovascular disease has been managed over time with drug like insulin and exercise with dietary control. Most of these drugs have some side effects which makes them become a burden even to the patient. Consequently there has been the need for an alternative medication with a safer and efficient curative potency. The search has been turn on nature's endowment of plant, most of which had found place in folklore as food as well as therapeutic agents. A number of these medicinal herbs have been reportedly used to treat diabetics (Ivorra *et al.,* 1981; Bailey and Day, 1989; Marles and Fransworth, 1995) and its complications (Grover *et al.,* 2001). This has therefore arisen much research into the use of herbal remedy for the treatment of diabetes (Alberti and Zimmet, 1998; Gupta *et al*., 2005; Kesari *et al.,* 2005) with promising result of cure. Most of these herbs are also consumed as food both in its cooked or raw state and this is the practice among African locals and particularly Nigerians. One of such herbs is *Heinsia crinata*. *Heinsia crinata* (Rubicceae) is known commonly among English speaking people as "bush apple" and by the Yorubas as "Tonoposho" (Abo *et al*., 2011). The Effiks calls it "Atama" (Okokon *et al.,* 2009) while the Ogonians call it "Etaabasi". These all use the leaves for soup and though an originally wild plant, it is now domesticated by the users. It grows as a scrambling shrub with persistent and well visible leaf calyx-lobes and produces greenish fruits which are very acidic when unripe but when ripe are either yellow or red and sweet to the taste. (Abo *et al.,* 2011). It has been reportedly to be used in the treatment of hypertension and abscesses (Ajibesin *et al.,* 2008); as antimicrobial and antifungal agent (Abo *et al.,* 2011); as antiplamodial and antidiabetics (Okokon *et al.,* 2009). It is casually classified into dark and white varieties which are used depending on the choice of the consumer. The difference in the two is that the dark variety is bitter than the white and this is due to the presence of more alkaloids in the dark than in the white, which contains more of saponnis (Okokon *et al.,* 2009). The white variety is widely used for soup by the Ogoni people of the Niger Delta area of Nigeria. Okokon *et al.,* (2009) reported on the hypoglycaemic and antidiabetic potential of the ethanolic extract of *Heinsia crinata*. However, its consumption as leaf for cooking soup is in the aqueous form. Therefore it became interesting to investigate if its antidiabetic activity could also be expressed in the aqueous form and so the research was designed with this in mind.

MATERIALS AND METHODS

Plants / Materials

The leaves of *Heinsia crinata* were collected from Kpean village in Bori-ogoni of Rivers State. The plant specimen was identified by Dr Barade, Wisdom. N, a taxonomist with the Department of Science Laboratory Technology of the Rivers State Polytechnic, Bori-Ogoni where a voucher specimen is been deposited.

Extract Preparation

The leaves were destalk, washed with distilled water and spread out in a tray to allow water to drain off. It was then pulverized using laboratory motor and pestle and about 50g of the coarse form was cold extracted by placing it in 500ml of distilled water and left standing on the laboratory bench for 24hrs. The extract was filter using a muslin cloth and the filtrate was concentrated by freeze drying. A greenish brown substance of weight 0.3g was obtained. This was red is solved in 100ml of distilled water and it was completely soluble.

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Chemicals used

All the chemicals used were obtained from Sigma Alderich, USA. The reference drug (Daonil, a brand of glibenclamide) was obtained commercially. All these were of analytical grade.

Animals

Wistar albino rats (n=40) of both sexes with ages about 8 months and weighing 150-180g were purchased from the animal house of the Biochemistry Department, University of Port Harcourt and transported in a cage to the Biology Laboratory of the Department of Science Laboratory Technology, Rivers State Polytechnic, Bori-Ogoni for housing. They were kept in good condition and given standard food pellets and water *ad labitum*. All of the animals were maintained at normal conditions of light (12/24h) and temperature (27±1 °C). Their use and experimental protocols used in the study was approved by the Ethical Committee of the Department of Biochemistry, University of Port Harcourt.

Phytochemical Screening

Qualitative photochemical screening was done for tannins, Alkaloids and saponnis according to the method of Sofowora (1984); Flavonoids by the method of Cuilel(1982), anthraquinones by the method of Trease and Evans (1978); Terpenoids by the method of Salkowoski as reported by Edeaga *et al.*, (2005) and Carotenoids by the AOAC method (1975). Quantitative analysis was done for Alkaloids, Flavonoids, Saponnins, Carotenoids and Terpenoids by the AOAC method (1975), Tannins by the method of Porter *et al.*, (1986), and anthraquinones by the method of ASEAN (1993).

Experimental Design

The non-diabetic rats were randomly assigned into seven groups of five rats each as follows:

Group 1 (control) received 2.0ml distilled water daily
Group 2 (reference) received 0.2ml glibenclamide (10mg/ml) daily.
Group 3 received 2.0ml aqueous extract of 60mg/kg/body weight ip
Group4 received 2.0ml aqueous extract of120mg/kg /body weight ip
Group5 received 2.0ml aqueous extract of 180mg/kg body weight ip
Group 6 received 2.0ml aqueous extract of 240mg/kg body weight ip
Group7 received 2.0ml aqueous extract of 300mg/kg body weight ip.

Blood glucose determination

All blood samples were collected by cutting the tail-tip of the rats. The blood samples were collected at intervals of 0,2,6,8 and 24hours. Estimation of the blood glucose level was done by the oxidase principle using the one touch glucometer. Results were displayed in mg/dl but converted to mmol/L .

STATISTICALLY ANALYSIS

Blood glucose levels were expressed as mean ± SEM mmol/l. The data was statistically analyzed using the two ways ANOVA with multiple comparisons against the groups. The values were considered significant at P<0.05 (Duncan *et al.*, 1997).

RESULT

Photochemical analysis

Freshly prepare extract were subjected to photochemical screening both qualitatively and quantitatively. The result is as shown in Table1. Table 2, shows the result of the effect of the aqueous leaf extract on the glucose levels in the non-diabetic rats. The result showed that 2 hours after the administration of the extract, there was no significant change in the glucose levels of the treated rats. However, 4 hours after, the change became noticed and continued in the 6, 8 and 24hours. This change was significant at the P<0.05level. The result also showed that the least values were obtained with the 120mg/kg body weight concentration.

Table 1. Qualitative and quantitative result of the Phytochemicals in *Heinsia crinata*

Phytochemicals	qualitative analysis	quantitative analysis (mg/g)
Alkaloids	+	3.47 ±0.01
Anthraquinones	+	0.97 ±0.80
Carotenoids	+	2.28 ±0.50
Flavonoids	+	2.83 ±0.20
Saponnins	+	7.36 ±1.00
Tannins	+	1.94 ± 0.38
Terpenoids	+	1.62 ± 1.20

In the qualitative analysis "+" represents "Present", "++" represents "moderately Present? The quantitative result values are present as mean ± SEM mg/g.

Table 2. Effect of aqueous extract of *heinsia crinata* on blood glucose levels in non-diabetic rats in mMol/l

Treat group	O hr	2hrs	4hrs	6hrs	8hrs	24hrs
Control	4.01 ±	4.00±	3.79±	3.15±	3.12±	1.87±
	0.10^{a}	0.62^{b}	$0.28^{b,a}$	$0.32^{b,a}$	0.83^{c}	0.83^{c}
Reference drug	2.30±	2.30±	1.67±	1.67±	1.75±	1.78±
(Glibenclamide)	$0.56^{a,c}$	$056^{a,c}$	0.39^{b}	0.39^{b}	$0.35^{b,c}$	$0.32^{b,c}$
60mg 1kg BW	3.85 ±	3.83±	3.23±	2.95±	2.00±	1.22±
	$0.10^{a,b}$	$0.16^{a,b}$	$0.40^{a,b}$	0.39^{b}	0.90^{b}	0.08^{c}
120mg/kg BW	2.55±	2.53±	2.14±	2.11±	1.67±	1.22±
	$0.20^{a,c}$	$1.22^{a,c}$	$1.54^{a,c}$	1.00^{a}	$0.58^{b,c}$	$0.58^{b,c}$
180mg/kg BW	4.55±	4.47±	4.52±	2.78±	2.64±	2.33±
	0.16^{a}	1.18^{a}	0.25^{a}	0.10^{b}	0.44_{b}	$0.61a^{,b}$
240mg/kg BW	3.85±	3.80±	2.86±	2.87±	2.81±	2.67±
	$0.50^{a,b}$	$0.35^{a,b}$	$0.04^{b,c}$	0.12^{b}	0.50^{b}	$0.23^{b,a}$
300mg/kg BW	3.56±	3.56±	3.17±	3.03±	2.78±	2.35±
	$0.07^{a,b}$	$0.08^{a,b}$	$0.39^{a,b}$	0.59a,	0.09^{b}	$0.39^{b,a}$

Values are presented as mean SEM for n=5 rats in each group. Experimental groups are compared with the normal control and reference drug. Values with Different superscripts in the same row are significantly different at p<0.05.

DISCUSSION

There is a current rise of interest in the discovery of alternative hypoglycaemic agents other than the biguanides, sulphonylurea, diphenylalanine, thiazolidinedione, sulphonyl derivatives and insulin due to their known side effects (thirunavukkarasu *et al*., 2003). Plant materials seem to be favaourable as most of them had already been in use for treatment in ayurvedic and traditional medicine practice. Most of these herbs served both as medicine and food. These plant materials- leaves, roots, bark- are known to contain phytochemicals which were considered as waste product of the plant but have been found to exert therapeutic properties in animals. Since they are nature endowed, they serve as a cheap means of treatment as they are been consumed as food. *Heinsia crinata* is a vegetable that is known to serve both as medicinal and nutritive purposes, being mostly consumed as vegetables in soup by the locals. We report in this paper its effect on blood glucose level. In relation to normal control, the aqueous extract of *Heinsia crinata* shows significant (p<0.05) reduction in blood glucose levels in all the concentrations and within the time interval. The greatest reduction in blood glucose was observed between the 8th and 24th hours of administration of the dosage of 120mg/kg body weight of aqueous extract. The significant reductions were observed after 4hours of extracted administration, while the first 2 hrs of treatment did not show any significant change in blood glucose levels. In relation to the reference drug, it was

observed that the reference drug, glibenclamide, caused a greater reduction in blood glucose level than the aqueous extract of *Heinsia crinata;* except at the 8 and 24hrs of the extract dosage of 120mg/kg body weight. Secondly, it was observed that the reduction is blood glucose level were not concentration dependent over a period of time. The photochemical analysis of the extract of *Heinsia crinata* revealed the presence, alkaloids, saponins, tannins, carotenoids, flavonoids, anthraquinone and terpenoid. Saponins was shown to occur in a greater amount (7.36± 1.00mg/g) as compared to the others; an indication that its hypoglycaemic activity could have been mediated, predorminately by its saponin contents. It can therefore be concluded that aqueous leaf extract of *Heinsia crinata* based on our study and results presented above, possesses antihyperglyceamic potential and so can play a significant role as antidiabetic while been eaten as food vegetables. Furthermore, the extract shows moderate presence of saponins, a characteristic of the white variety of the leaf, implying that saponins may be its most active ingredient.

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