Anti diabetic effect of aqueous leaf extract of *Heinsia crinata* on key glycolytic enzymes and glycogen in streptozotocin induced diabetic rats

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**ABSTRACT**

The anti-diabetic effect of aqueous leaf extract of *Heinsia crinata* on the activities of hexokinase, glucokinase and phosphofructokinase, and hepatic glycogen levels in streptozotocin induced diabetic rats was studied. The extract potency was compared with that of a standard anti diabetic drug, glibenclamide. The test dosage (120 mg/kg body weight) administered caused significant reduction (p<0.05) in blood glucose levels 168.66±0.52 mg/dl on day zero to 82.67 ±3.05 mg/dl on the 21st day of the experiment against the rise of glucose levels in the diabetic control (188.06 ± 1.21 to 198.00±0.04 mg/dl). The activities of hexokinase, glucokinase, and phosphofructokinase were significantly reduced in diabetic control as compared to the normal control while the extract and glibenclamide resulted to significant recovery of activities for hexokinase (twofold), glucokinase (more than a 100 fold and phosphofructokinase (half of the normal control) without a corresponding increase in glycogen level (P < 0.05) respectively. Phytochemical result of the extract revealed the presence of alkaloid, flavonoid, tannins, terpenoids, and anthraquinones in trace amount and saponins in a moderate amount. It appears that the anti diabetic potential of the extract is mediated prominently by its saponin content which acts by stimulating the activities of the key glycolytic enzymes towards an increased flux of glucose for cellular energy utilization rather than glycogen production. In conclusion, aqueous extract of *H. crinata* is suitable for management of diabetics.

**Key words:** *Heinsia crinata*, glycolytic enzyme, saponins, carbohydrate metabolism, antidiabetic, glycogen.

**INTRODUCTION**

The metabolism of glucose for energy generation is made possible by its entry into cells by the glycolytic processes. The pathway is made up of both irreversible reversible reactions with those points of irreversibility serving as regulatory points. These are the points catalyzed by hexokinase, glucokinase and phosphofructokinase. The first two enzymes regulate at the same point, all depending on the substrate available. Hexokinase acts on hexose sugars including glucose whereas glucokinase is specific for glucose. These irreversible points are known as regulatory points and so enzymes catalyzing those points are considered key enzymes of carbohydrate metabolism (Iwueke et al., 2008).

In diabetic condition, these enzymes being modulated by insulin are impaired in their activities (Iwueke et al., 2008; Panneerselvan and Govindoswamy, 2002). Treatment of the ailment or a claim of such should play a substantive role on the activities of these enzymes. *Heinis crinata* (AF2) G. Taylor (Rubiaceae) is popularly called "atama" by the local tribes (Abo et al., 2011; Okokon...
et al., 2009) and “Etaabasi” among the Ogoni indigenes of southern Nigeria, both in the Niger delta area of Nigeria. The plant is domesticated mostly because of its leaves which are used in preparation of soup.

Although literature on this plant is still scanty (Abo et al., 2011), the antidiabetic and antiplasmodial activities of its ethanolic leaf extract in alloxan induced diabetic animals have been reported (Okokon et al., 2009). The hypoglycaemic activity of the aqueous leaf extract in non-diabetic rats have also been reported (Miikue-Yobe et al., 2013).

These reports did not present any finding of the leaf extract on glucose metabolizing enzymes. In this paper therefore, we report on the antidiabetic effect of aqueous leaf extract of *Heinsia crinata* and its effect on key glycolytic enzymes and glycogen in streptozotocin induced diabetic rats as a means of understanding its probable mechanism of antidiabetic action.

**MATERIALS AND METHODS**

**Materials**

The fresh leaves of *Heinsia crinata* were collected in June 2011 from Kpean community in Ken Khana Local Government Area of Rivers State, Nigeria. The plant was identified and authenticated by Dr. Wisdom N. Barade, a taxonomist with the Science Laboratory Technology Department of Rivers State Polytechnic, Bori where the voucher specimen (SLT 2011/001TF) was deposited.

**Extract preparation**

The leaves were destalk, washed with distilled water and spread out on a tray in the laboratory for water to drain off it. It was placed in a laboratory enamel mortar and ground. The coarse product was weighed (50 g) and this was cold extracted by placing in 500 ml of distilled water, agitated by steering with a glass rod. It was properly covered and left standing on the laboratory bench for 24 h. With the aid of a Muslin cloth, the mixture was filtered. The filtrate was freeze dried and a green brown substance of weight 0.3 g was obtained. A mass of 120 mg/kg body weight of this substance was weighed and dissolved in 100 ml of distilled water (this dosage was determined from a preliminary study as the effective dosage that can cause the least reduction in blood glucose level under the experimental condition). A volume of 1.0 ml of this extract was administered by intraperitoneal injection on the rats once a day for 21 days.

**Animals**

Wistar rats of both sexes were purchased from the animal house of the Biochemistry Department, University of Port Harcourt and transported in a plastic cage to the Biology Laboratory of the Department of Science Laboratory Technology, Bori-Ogoni for housing. They were kept in good condition and given standard food pellets and water *ad libitum*. All the animals were allowed to acclimatize for two weeks and maintained at normal conditions of light (12/24 h) and temperature (27±1°C) before been used in the research. The ethical Committee of the Department of Biochemistry, University of Port Harcourt approved the study.

**Chemicals used**

All the chemicals used were obtained from Sigma-Aldrich, USA. The reference drug (Daonil, a broad of glibendanamide) was purchased commercially from a local store. The entire chemicals used were of analytical grade.

**Phytochemical screening**

Qualitative phytochemical screening was done on the extract using standard procedures and reagents. The compounds were screened for the presence of saponins, flavonoids, tannins, alkaloids etc by the addition of appropriate reagent(s) to the extract in separate test tubes. The mixture was shaken and/or heated as appropriate (Trease and Evans, 1978; Soforawo, 1984).

**Induction of diabetes in experimental rats**

Rats were fasted for 18 h and allowed free access to water only and made diabetic by an intraperitoneal injection of 1 ml of streptozotocin (Sigma-Aldrich, U.S.A), 70 mg/kg body weight; in 0.9% cold normal saline and observed for 5 days with daily monitoring of blood glucose level using the one touch glucometer. Animals were allowed free access to food and water. After the 5 days duration, rats with fasting blood sugar level ≥ 150 mg/dl were considered diabetic and included in the study.

**Treatment of experimental rats**

Table 1 shows the schedule of treatment adopted for the experimental rats. Rats were treated for 21 days and before each treatment, they were allowed to fast for 18 h. A volume of 1 ml each of extract, reference drug and distilled water was administered to the rats in their respective groups of 5 rats per group.

At the end of the treatment days, the rats were fasted and their blood glucose levels were determined again. They were then euthanized using chloroform and their livers were
surgically removed, washed in cold KCl (1.15%) and refrigerated. The liver tissues were used for the glycolytic enzyme assay.

**Glycolytic enzyme assay**

**Hepatic glucokinase (EC 2.7.1.3) activity**

One gram of liver tissue was homogenized in 10 ml volume of homogenizing buffer (75 mM Tris HCl, pH 9.0). The enzyme activity was measured according to the Method of Goward et al. (1986). In a 3.0 ml reaction mixture containing 60 mM Tris, 80 mM MgCl₂ 4.0 mM ATP, 12.0 mM glucose, 0.9 mM D-NADP, 10 units of glucose-6-dehydrogenase and 50 µl of supernatant. Enzyme activity was measured at 340 nm for 5 min interval and then the change in activity per minute was obtained.

**Hepatic hexokinase (EC 2.7.1.2) activity**

One gram of liver tissue was homogenized in 10 ml of homogenizing buffer (50 mM Tris HCl, pH 8.0, 100 mM MgCl₂). Enzyme activity was followed in a 3.0 ml reaction mixture containing 39 mM Tris, 216 mM D-Glucose, 0.75 mM ATP, 7.8 mM MgCl₂, 1.1 mM NADP, 2.5 units glucose-6-phosphate and 50 µl of supernatant as reported by Bergmeyer et al. (1983). Enzyme activity was monitored at 340 nm for 4 min interval and then change in activity per minute was obtained.

**Phosphofructokinase (EC 2.7.1.11) activity**

One gram of liver tissue was homogenized in homogenizing buffer (60 mM Tris Tris HCl, pH 8.0, 250 mM MgCl₂). The enzyme activity was estimated in a 3.0 ml reaction mixture containing 50 mM Tris, 1 mM D-Dithiothreitol (DTT), 2 mM ATP, 0.2 mM NAOH, 5 mM MgCl₂ 2 units of aldolase, 1 unit glyceraldehyde dehydrogenase, 8 unit triosesphosphate isomerase, 4 mM fructose-6-phosphate and 50 µl supernatant. Activity was monitored at 340 nm for 5 min interval, and the change in activity per minute was obtained (Massey and Deal, 1973).

**Table 1.** The schedule of treatment adopted for the experimental rats.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Group</th>
<th>Number of rats</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>5</td>
<td>Distilled water (1 ml/kg)</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>5</td>
<td>Distilled water (1 ml/kg)</td>
</tr>
<tr>
<td>3</td>
<td>Diabetic extract</td>
<td>5</td>
<td>Extract (120 mg/kg)</td>
</tr>
<tr>
<td>4</td>
<td>Glibenclamide</td>
<td>5</td>
<td>Drug in Distilled water (10 mg/ml/kg)</td>
</tr>
</tbody>
</table>

**Hepatic glycogen**

Glycogen content of liver was estimated according to the method reported by Ong and Khoo. One gram of tissue was homogenized in 30% (w/v) ice cold KOH (10 ml) and set to boil at 100°C for 30 min. The solution was treated with ethanol to precipitate glycogen which was pelletted, washed and redissolved in distilled water. Absorbance was measured at 625 nm using anthrone reagent (Iwueke et al., 2008).

**Hepatic protein**

The protein content of liver was estimated by the Biuret method using Bovine serum albumin as standard.

**Statistical analysis**

The result of the study is presented as mean ± SEM for 3 rats in each group. The test of statistical significance was done using the one way ANOVA and further subjected to multiple comparisons. The values were considered statistically significant at the P<0.05 level (Duncan et al., 1997).

**RESULTS**

**Phytochemical screening**

Biological active ingredients present in the aqueous leaf extract were screened. The result showed the presence of alkaloids, saponins, flavonoid, tannins, terpenoids, anthraquinones, and carotenoids as shown in Table 2.

**Anti-hyperglycaemic effect of Heinsia crinata**

The effect of the aqueous leave extract of H. crinata on blood glucose level of streptozotocin induced diabetic rats is shown in Table 3. Baseline glucose level of the animal is shown to be low which has also contributed to the overall value of hyperglycemia to be on the low side as well.
Table 2. Phytochemical constituent of the extract.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Heinsia crinata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
</tbody>
</table>

++ = positive, + = trace.

Table 3. Effect of aqueous extract of *Heinsia crinata* on glucose levels in streptozotocin induced diabetic rats in mg/dl concentration.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Base line</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>84.66±0.57</td>
<td>86.33±2.51</td>
<td>87.33±0.38</td>
<td>92.33±0.58</td>
<td>98.67±1.15</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>85.05±0.01</td>
<td>188.06±1.21</td>
<td>170.02±0.28</td>
<td>198.04±1.10</td>
<td>198.00±0.04</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>88.33±0.57</td>
<td>187.66±0.52</td>
<td>123.67±3.21</td>
<td>94.33±0.57</td>
<td>88.00±0.01</td>
</tr>
<tr>
<td>Heinsia crinata</td>
<td>85.66±0.57</td>
<td>1.68.66±0.52</td>
<td>113.00±2.65</td>
<td>95.33±4.04</td>
<td>82.67±3.05</td>
</tr>
</tbody>
</table>

Result is presented as mean ± SEM (n =5). Values with different superscript in the row are significantly different at the P < 0.05 level.

Table 4. Effect of aqueous extract of *Heinsia crinata* on key glycolytic enzymes and hepatic glycogen in streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hexokinase (unit/ml enzyme)</th>
<th>Glucokinase (unit/ml enzyme)</th>
<th>Phosphofructokinase (unit/ml enzyme)</th>
<th>Hepatic glycogen (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.096±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.023±0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.054±0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.75±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.016±0.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.019±0.000&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.014±0.009&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.55±2.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.078±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.035±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.034±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.58±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract treatment</td>
<td>0.094±0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.037±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.025±0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.41±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Result is presented as mean ± SEM for n=5. Values with different superscript in the same column are significantly different at the P<0.05 level.

However, significant reduction (P < 0.05) in value occurred in the extract treatment when compared to the normal control and the reference drug, glibenclamide. These changes in concentration of glucose level at P<0.05 could be observed in the Diabetic controls that represents the untreated case as it could be seen to have risen steadily as compared to the normal control. The extract and reference drug cases which represented the treatment group showed a steady decrease in glucose level as the duration of drug administration was increased.

**Effect on activities of key glycolytic enzymes and glycogen**

Table 4 shows the result of the effect of the aqueous leaf extract, glibenclamide and distilled water on the activities of hexokinase, glucokinase and phosphofructokinase enzymes. As compared to the normal control activity levels of hexokinase, glucokinase and phosphofructokinase activities decreased in the diabetic control. Treatment with extract and glibenclamide resulted to a rise in activities for hexokinase, glucokinase and phosphofructokinase enzymes respectively. This rise in activity was significant at the P < 0.05.

**Hepatic glycogen**

The result of the hepatic glycogen in the diabetic rats treated with extract, glibenclamide and distilled water for 21 days is low as shown in Table 4. The result indicated that
there was no significant change in the diabetic control when compared to the normal Control. Treatment also resulted to a more decrease in value for both the extract and glibenclamide treated rats when compared to the normal and diabetic control. This decrease was significant at the P < 0.05 level.

DISCUSSION

The anti-diabetic activity of ethanolic extract of *H. crinata* on allaxan but not streptozotocin induced diabetic rats has been reported (Okokon et al., 2009). *H. crinata* being a vegetable used for soup, an aqueous mixture of several ingredients, we therefore investigated its anti-diabetic properties in the aqueous form using the streptozotocin model. The result as shown in Table 3 indicated a progressive reduction in glucose level in the extract and glibenclamide treated rats as compared to the diabetic control. This implies that the aqueous extract of *H. crinata* demonstrated antidiabetic property, a finding which is consistent with the reports of other researchers (George and Joseph, 2008; Okokon et al., 2009) in respect to the antidiabetic potential of the leaves of *H. crinata*. This also agrees with our earlier finding of the hypoglycaemic effect of the aqueous leaf extract on non-diabetic rats (Miikue-Yobe et al., 2013).

Phytochemical screening of the aqueous extract shows the presence of alkaloid, flavanoids, tannins and saponins, with the later in greater quantity. It is therefore suggestive that the anti-diabetic activity of this leaf is a combination of the rich phytochemical constituent but majorly that of the saponins content. The antidiabetic potentials of saponins have also been reported both in Types 1 and 2 diabetes milletus (Ugochukwu and Babady, 2003; Zheng et al., 2012). It has been noted earlier that saponins exert stimulatory effect on insulin producing organs (Zheng et al., 2012).

The mechanism of its action was also investigated by looking at the effect of the extract on the key glycolytic enzymes. It is reported that in Diabetic mellitus due to insulin defect, there occurs a decrease in the activities of hexokinase, glucokinase and phosphofructokinase enzymes (Iwueke et al., 2008; Panneerselvan and Govindoswamy, 2002). The result of our investigation as shown in Table 4 indicated a significant reduction (P < 0.05) in the activities of these enzymes in the diabetic control as compared to the normal control. Treatment with extract as well as glibenclamide restored the activity of hexokinase almost 100 fold, that of glucokinase more than normal while that of phosphofructokinase was half of the normal control. These finding is consistent with other reports of restoration of activity of key glycolytic enzymes by anti hyperglycaemic agents. (Iwueke et al., 2008; Panneerselvan and Govindoswamy, 2002; Gupta et al., 1999; Stanley et al., 2000). By this increase in activity of the enzymes, it appears probable that aqueous extract stimulated the entry of glucose into the cells and so reduces its concentration in the blood. It therefore implies that the extract might have caused increased production of insulin by available pancreatic cells or an increase sensitivity of glucose receptor cells, a confirmation of which is subject to further testing. However, the result obtained for the glycogen levels present in the liver did not correlate this position as there occurred a decrease in the hepatic glycogen concentration in both the extract and glibenclamide treated as against the normal and diabetic controls (Chattopadhyay, 1998). The implication is that glucose is not channeled into glycogen synthesis and storage in peripheral tissues. It therefore implies that the extract might have caused increased production of insulin by available pancreatic cells or an increase in the number of islet cells and a sensitivity of glucose receptor cells to glucose. The position agrees with the finding of Koneri et al. (2009). They reported that saponins were capable of restoring the regeneration of pancreatic cells which resulted to increased insulin secretion.

It is therefore our conclusion that aqueous extract of *H. crinata* exerted anti diabetic effect as shown in this study. This activity seems to be potentiated by its saponin fraction in combination with other bioactive substances present in the leaf. The mechanism of the activity is thought to be through the increased flux of glucose into the cells for consumption and not for storage. This is brought about by the stimulation of glycolytic enzymes particularly hexokinase, glucokinase and phosphofructokinase enzymes which catalyze irreversible reactions and regulatory points of glycolysis. Their stimulated activities as shown in this work did not encourage glycogen synthesis but glucose utilization for cellular energy production. This is consistent with earlier findings in which it was asserted that there occurred a decrease in the treated than in the control (Grover et al., 2000; Welihinda and Karunanayake, 1998). There is also another view that glycogen levels in the diabetic control are significantly reduced compared to the normal control animals (Bollen et al., 1998; Hikino et al., 1998; Mahmoud et al., 2003). Therefore, based on the results obtained in this research, it can be concluded that as the leaves of *H. crinata* has been enjoyed as soup ingredient, it also plays a role in regulating blood glucose levels. Treatment of diabetes also requires diet modification, so the leaves of *H. crinata* will serve as a good leafy vegetable food for the diabetics.

ACKNOWLEDGEMENT

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