

Effect of Ethanolic Leaf Extract of *Heinsia crinita* (Atama) on the haematological indices and lipid profile in Normoglycemic and Diabetic Albino Wistar Rats.

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ABSTRACT

The effect of ethanolic leaf extract of *Heinsia crinita* (HC) on the haematological indices and lipid profile of normal and alloxan-induced diabetic rats was studied. Graded doses of the extract (455 and 1365 mg/kg body weight (b.w.) and a reference drug Metformin (Met) were administered to normal rats, diabetic rats at different time points and diabetic rats continuously for 14 days. The antidiabetic activity of HC showed a significant reduction ($p > 0.05$) in blood glucose level in normal rats and alloxan-induced diabetic rats and corresponding diabetic rats treated with HC extract for 2 weeks. At the end, the total protein, aminotransferases (ALT and AST), alkaline phosphatase (ALP) were assayed in the blood serum. Total protein and albumin concentration was significantly increased ($p < 0.05$) in the normal rats, the diabetic rats when compared to the diabetic control rats was not significantly ($p > 0.05$) changed in all test groups after treatment. A general significant increase ($p < 0.05$) in AST, ALT, ALP was observed in the activities of the normal and diabetic test groups when compared to the subchronic diabetic control groups. Compared to the diabetic control there was significant increase in AST activities ($p < 0.05$) of diabetic rats administered 455mg/kg b. w. However at a test dose of 1365mg/kg body weight and 100mg/kg Metformin, ALT activities showed a significant difference ($p < 0.05$ for ALT; $p < 0.05$ for Met) relative to diabetic control and 455mg/kg group. The enzyme activity in the 1365mg/kg dose group was significantly different compared to the Met. group. Hence the ethanol leaf extract of *Heinsia crinita* leaf is not likely to cause liver pathology, and can provide alleviation and protection to the liver with a dose up to 1365 mg/kg b. w.

Key Words: Alloxan, diabetes, *Heinsia crinita*, liver enzymes, total proteins, liver function.

INTRODUCTION

Diabetes mellitus has become a problem of great magnitude globally. It is estimated that 10 - 12 % of the urban and 4 - 6 % of the rural population of people are now diabetic (Gupte, Ramchandran and Mutatkar, 2001). It has been predicted that worldwide, the prevalence of type 2 diabetes in adults will increase to 5.4 % by the year 2025 from the prevalence rate of 4 % in 1995. Consequently the number of adults with diabetes in the world would rise from 135 million in 1995 to 300 million in the year 2025 (King, Aubert and Herman (1998). Traditional plant remedies or herbal formulations exist from ancient times and are still widely used, despite all the controversy concerning their efficacy and safety (Huxtable 1990; Fugh-Berman 2000), to treat hypoglycemic and hyperglycemic conditions all over the world. The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity (Wild, Roglic, Green, Sicree and King, 2004).

Ethnopharmacological and ethnobotanical surveys indicate that more than 1200 plants are used

worldwide in traditional medicine to treat diabetes (Bnouham, Mekhfi, Legssyer and Ziyat, 2002; Tahraoui, Ei-Hilaly, Israili and Lyoussi, 2007). At least 80 % of Africans depend on plant medicine for their healthcare (Okigbo and Mmeka, 2006; Ajose, 2007; Okwu and Uchegbu, 2009). Today, medicinal plants are increasingly being used in most parts of the world as hypolipidemic (Ogbonna, Odimegwu and Enwuru, 2008); antihypertensive (Ojewole and Adewole, 2007) and hypoglycaemic agents (Ajao, Olayaki, Oshiba, Jimoh, Jimoh, Olawepo and Abioye, 2009). Hypoglycemic agents such as sulfonylurea and metformin have been used in the management of diabetes (type 2) especially where patients do not respond to diet, weight reduction and exercise (Higgins, 2014).

The liver helps maintain normal blood glucose concentration in the fasting and postprandial states. Mild chronic elevations of transaminases often reflect underlying insulin resistance. Diabetic hyperglycemia induces elevation of the levels of serum creatinine, urine total protein and urine albumin which are considered as significant markers of renal dysfunction (Bretzel, 1997).

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Heinsia crinita commonly called 'atama' in Ibibio/Efik is a glossy leafy vegetable of the family Rubiaceae consumed mainly by the people of Southeastern Nigeria. The most common edible varieties include the White (Ibibio: "afia atama"), Black (Ibibio: "obubit atama"), and "Ekoi". It has been reported to have antimicrobial properties, justifying its use by native herbalists as a remedy for infections of the gastro-intestinal tract (Ekpa and Ebana, 1991).

Therefore, in the present study the efficacy of *Heinsia crinita* leaf (ethanolic extract) was evaluated in normoglycemic, diabetic and subchronic diabetic rats and analyzed chemically. Acute toxicity evaluation of the extract was also carried out in mice.

MATERIALS AND METHODS

All reagents and chemicals used in this work were of analytical grade.

Collection and preliminary preparation of plant materials

Fresh leaves of *Heinsia Crinita* were obtained from a garden in a house near the University of Uyo Campus, Akwa Ibom State, Nigeria in the month of April, 2011. The leaves was identified and authenticated by a botanist in Department of Botany, University of Uyo. The plants were washed and cut into tiny bits and consequently placed under shade. The ground leaf powder was stored in a glass bottle with a plastic screw cap and kept in a refrigerator (4°C) prior to extraction.

Preparation of plant extract

The grounded leaves of *Heinsia crinita* was weighed and immersed in ethanol for 72hr on a mixer for maximum extraction. The extract was filtered with a No 1 Whatmann filter paper and the filtrate was concentrated to dryness in vacuo on a rotary evaporator to obtain the crude ethanolic extract, the extract was stored in a refrigerator until required for use and the dried extract was also weighed.

Animal handling and treatment protocol

Sixty male and female wistar (albino) rats (200 - 260 g) were used for the study. They were housed in rat cages in well ventilated house and were allowed to acclimatize for three days before the experiment.

The animals were made diabetic by a single dose subcutaneous injection of freshly prepared alloxan monohydrate dissolved in normal saline to overnight fasted rats. Blood glucose level was

measured by using Roche Accu-check Active Glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats showing fasting blood glucose levels more than 250 mg/dl were considered to be diabetic and were selected for experimentation. The animals were divided into 3 sets of 20 rats (normal, diabetic and subchronic-diabetic) each.

Anti diabetic Studies:

The 20 rats were divided into four (4) groups of five (5) rats each forming the normal, diabetic, subchronic diabetic and control group. The studies were carried out on three (3) different models. The normoglycemic model which uses normoglycemic rats, diabetic model which uses alloxan induced diabetic rats and the subchronic diabetic model which involved the continuous administration of doses for a period of time to the diabetic rats. For each model, one group served as control group while the other three (3) groups served as the test groups. Varying concentrations (low and high) doses of the plant extract were administered to two of the test groups and a reference standard drug was given to the reference group.

Studies for Normoglycemic Model:

Studies for normoglycemic were carried out on overnight fasted normal rats, which were equally divided into four groups of five rats each. Normal control group received only the vehicle (distilled water), the reference drug group received the reference standard drug (Metformin) suspended in the vehicle (0.25ml/kg), while the remaining two groups were administered with 455 mg/kg and 1365 mg/kg of plant extract, respectively. Blood samples were collected from tail vein prior to dosing (0 hr) and then at regular intervals of 1, 2, 4 and 6 hours, respectively.

Study on Alloxan-induced Diabetic Rats

Overnight fasted diabetic rats were randomly divided into four groups (n=5) as follows:

Group-I: diabetic control rats that was administered with the vehicle (5 ml/kg distilled water;) only;

Group-II: Diabetic rats administered with 455 mg/kg *Heinsia crinita* extract.

Group-III: Diabetic rats administered with 1365 mg/kg *Heinsia crinita* extract;

Group-IV: Diabetic rats administered with metformin as reference standard drug respectively.

Blood samples were collected from tail vein prior to dosing (0 hr) and then at regular intervals of 1, 2, 4 and 6 hours respectively.

Study on Subchronic Diabetic Rats

Overnight fasted diabetic rats divided into four groups (n=5) the Group-I: diabetic control received 5 ml/kg distilled water while test Group-II, III, IV received 455 mg/kg, 1365 mg/kg *Heinsia crinita* extract and 100mg/kg metformin as reference standard drug respectively. Both feed and water were given *ad libitum* and treatment lasted for 14 days. The plasma glucose was estimated by withdrawing blood samples from tail vein prior to dosing (day 0) and then at regular intervals of day 1, 2, 6, 10 and 15, respectively for all groups of animals.

Preparation of sample

At the end of 14th day, all the rats were euthanized by chloroform and sacrificed by cervical dislocation. Blood sample were withdrawn from abdominal aorta into centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain serum. Serum samples were stored at -20 °C until utilized for further biochemical estimations.

Biochemical assays

Estimation of Liver Function Enzymes:

The three enzymes assayed in the study were Alanine amino transferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (AP) based on UV- Kinetic method. All kits were obtained from Teco Diagnostic, Lakeview avenue Anaheim, United states of America and were of analytical grade. The absorbances were measured using Optima Spectrophotometer.

Statistical analysis

Results were expressed as means \pm standard deviations (SD) and subjected to statistical analysis using one-way analysis of variance (ANOVA) and the turkey's test. The significance level considered was $p < 0.05$.

RESULTS

The result of the anti-diabetic activities on the normoglycemic model are shown in table 4.1 while that of diabetic model and subchronic diabetic model are as shown in table 4.2 and 4.3. The results of liver protein levels and enzyme activities of normoglycemic rats are shown in table 4.1 while that of that diabetic rats and subchronic diabetic rats are shown in table 4.2.

Table 4.1: Effect of *Heinsia crinita* (455 and 1365 mg/kg) and standard drug Metformin on blood glucose level in normoglycemic rats.

Group	0hr	1hr	2hr	4hr	6hr
I	90.8 \pm 10.4	92.4 \pm 21.6	94.0 \pm 17.0	82.0 \pm 17.0	79.0 \pm 17.2
II	83.2 \pm 12	82.6 \pm 8.2	81.6 \pm 19.9	73.2 \pm 19.1	64.4 \pm 7.0
III	84.4 \pm 4.3	89.4 \pm 6.0	88.8 \pm 56.2	71.8 \pm 8.6	44.6 \pm 24.6
IV	86.2 \pm 21.8	82.2 \pm 33.6	77.6 \pm 45.4	68.0 \pm 42.0	57.0 \pm 26.8

Fasting blood sugar of normoglycemic animals treated with;

Group I- 5 ml/kg distilled water. Group II-455 mg/kg body weight of extract.

Group III-1365 mg/kg body weight of extract. Group IV- 100 mg/kg body weight of Metformin

Table 4.2: Effect of *Heinsia Crinita* (455 and 1365 mg/kg) and standard drug Metformin on blood glucose level in diabetic rats.

Group	0hr	1hr	2hr	4hr	6hr
I	288.6 \pm 22.2	286.8 \pm 21.8	283.0 \pm 26.8	276.6 \pm 32.0	274.8 \pm 27.8
II	276 \pm 74.2	248.6 \pm 72.0	175.6. \pm 60.5	162 \pm 54.6	144.2 \pm 64.5
III	256.6 \pm 84.3	215.2 \pm 72.8	143.0 \pm 46.5	128.2 \pm 38.0	116.0 \pm 41.0
IV	310.0 \pm 132.7	239.4 \pm 89.0	163.6 \pm 133.0	117.4 \pm 81.0	117.6 \pm 89

Table 4.3: Effect of *Heinsia crinita* leaf extract (455 and 1365 mg/kg), standard drug metformin on the fasting blood sugar in alloxan-induced diabetic rats in a period of 14 days.

	Pre-test	Day 1	Day 8	Day 12	Day 15
Group I	288.6±22.2	279.8±29.5	270.0±34.0	264.2±29.2	241.4±39.2
Group II	267 ± 21.5	125.4±44.0	114.8± 35.2	107±34.0	92.0±43.4
Group III	248.6±29.4	105.8±51.1	100.8±39.7	88.6±23.9	70.8±7.7
Group IV	259.8±15.7	115.0±72.4	105.2±57.9	89.2±46.2	76.2±17.5

Table 4.4 Effect of ethanol extract of the leaf of *Heinsia crinita* on serum total protein and Albumin in Wistar albino rats.

GROUPS	TOTAL PROTEIN (g/L)	ALBUMIN (g/dl)
I	8.8 ± 1.09	3.27 ± 0.95
II	9.78 ± 3.78	3.30 ± 0.32
III	10.98 ± 1.47	3.83 ± 0.53
IV	11.79 ± 1.52	3.92 ± 0.70

Values are expressed as Mean ± S.D, n = 5

Table 4.5 Effect of ethanol extract of the leaf of *Heinsia crinita* on liver enzymes in albino wistar rats

GROUPS	ALP (U/L)	AST (U/L)	ALT (U/L)
I	69.51 ± 1.82	96.17 ± 2.68	32.18±4.74
II	83.11 ± 11.98 ^a	143.92 ± 24.13 ^a	50.57±3.87 ^a
III	89.23 ± 5.87 ^a	117.39 ± 8.71 ^b	51.27±4.33 ^a
IV	81.98 ± 7.99	113.86 ± 2.68 ^b	75.67±12.40 ^{a,b,c}

Values are expressed as Mean ± S.D, n = 5. a = p < 0.05 (comparing with test group I).
b = p < 0.05 (comparing with test group II). c = p < 0.05 (comparing with III).

Results of Fasting Blood Sugar

The results of the fasting blood sugar determined in three different models of the experimental design are as shown in the Table 4.1- 4.4. Figure 1 which describes the **Normoglycemic Model**, illustrates the effect of *Heinsia crinita* leaf extract (455 and 1365 mg/kg), standard drug metformin on the fasting blood sugar in the normoglycemic rats at different time point. The result showed that there was a significant (p<0.05) decrease in fasting blood glucose of experimental animals in all treated groups compared to those in the normal control group (test group I). The result also showed that the extract (at high dose of 1365 mg/kg body weight and low dose of 455 mg/kg bd. wt.) was most effective in the reduction of blood sugar level in normoglycemic animals as compared with those experimental animals treated with distilled water (test group I) and the standard drug, metformin (test group IV).

Diabetic Model: Table 4.2 illustrates the effect of *Heinsia crinita* leaf extract (455 and 1365 mg/kg), standard drug metformin on the fasting blood sugar in the normoglycemic rats at different time point. The result showed the trend in alteration of blood sugar level in diabetic animals subjected to different treatment within 0 hr to 6 hr. It also showed a significant (p< 0.05) decrease in blood glucose level in all treated groups compared to test group I. For all the treatments, the result showed that there was a time dependent reduction in fasting blood sugar level.

Subchronic Diabetic Model: Table 4.3 illustrates the effect of *Heinsia crinita* leaf extract (455 and 1365 mg/kg), standard drug metformin on the fasting blood sugar in alloxan-induced diabetic rats in a period of 14 days. The results of fasting blood sugar level of experimental animals in subchronic diabetic model showed that for all the treatments,

the values of fasting blood sugar obtained were decreasing at each day interval. The result also showed that treatment with extract (at high and low dose) was as effective in the reduction of fasting blood sugar as treatment with the standard drug, metformin. It further reveals that for all the treatments, the values of the fasting blood sugar were highest at day 1 and lowest at day 15.

Effect of ethanol extract of the leaf of *Heinsia crinita* on serum total protein and albumin in albino wistar rats:

The results of the effect of the ethanol extract of the leaf of *Heinsia crinita* on serum total protein and albumin levels of experimental rats shown in Table 4.4 reveals that there were no significant ($p > 0.05$) changes. However, increase in total protein and albumin levels of experimental animals in all test groups compared to those in the diabetic control group, was observed.

Effect of ethanol extract of the leaf of *Heinsia crinita* on liver enzymes in albino wistar rats:

The effect of the ethanol extract of the leaf of *Heinsia crinita* on AST, ALT and ALP levels of experimental rats are shown in Table 4.5. The results showed increase in AST, ALT and ALP level of all treated groups compared to those in the diabetic control group. ALP level of experimental animals in test group II, III and IV was significantly ($p < 0.05$) higher than the observed level in the diabetic control group. AST level in experimental animals in the treated groups was only significantly ($p < 0.05$) increased in test group II when compared to those in the diabetic control group. Changes in ALT was only significantly ($p < 0.05$) different in experimental animals of test groups III and IV when compared to those in test group I. ALT levels in test group III and IV was significantly ($p < 0.05$) different from the observed level in test group II. The enzyme level in experimental animals of test group IV was significantly ($p < 0.05$) different from those in test group III.

DISCUSSION

The present study on the antidiabetic activity of *Heinsia crinita* on normoglycemic rats, diabetic rats and continuous post-treatment for 14 days with the *Heinsia crinita* on diabetic rats showed potential antidiabetic activity in terms of significant hypoglycaemic, hypolipidemic and haematological activity. It is clear from the results of this animal study that the tested leaf extract *Heinsia crinita* induced significant dose-dependent reductions ($p < 0.05$) in blood glucose concentrations of normoglycemic, acute alloxan induced diabetic rats

and subchronic alloxan-induced diabetic rats treated for 14 days. The fact that this extract caused significant reductions in blood glucose levels in alloxan-induced diabetic rats suggests that *Heinsia crinita* may act in yet undetermined ways apart from stimulating insulin production from the pancreatic islets since these would have been severely damaged by alloxan. However, stimulation of the undamaged or residual pancreatic islets to produce insulin cannot be ruled out since the reference drug metformin caused significant reductions in blood glucose levels in alloxan-induced diabetic rats. In addition, significant reductions in blood glucose levels in alloxan induced diabetic rats by the extract may suggest that *Heinsia crinita* could, at least in part, stimulate insulin production and glucose utilization, like metformin, to bring its hypoglycemic effect in the mammalian experimental model used.

Elevated level of biomarker enzymes ALP, AST and ASP in the 455 mg/kg and 1365 mg/kg dose group and reference drug group were recorded in circulation of diabetic rats when compared with diabetic control rats reflecting hepatocellular damage and/or indicative of liver mitochondrial injury (Rathod, Raghuveer, Chitme and Chandra, 2009). During diabetes the insulin deficiency contributes to increased serum level of transaminase enzymes due to increased availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes. *Heinsia crinita* extract did not show any ability to restore the normal functional status of the damaged liver.

During diabetes, there is increased protein catabolism with inflow of amino acids to liver, which feed gluconeogenesis and accelerate ureagenesis, resulting in hypoproteinemia and hypoalbuminemia (Bhavpriya and Govindasamy, 2000). Diabetic hyperglycemia induces elevation of the levels of serum creatinine, urine total protein and urine albumin which are considered as significant markers of renal dysfunction (Bretzel, 1997).

In the present study, diabetic animals treated with *Heinsia crinita* showed reduction in protein urea and albumin urea and also showed improvement in the serum total protein and albumin level. These results indicate that *Heinsia crinita* attenuates the progression of renal damage in alloxan induced diabetic rats.

CONCLUSION

In general, these results suggest further that the leaf extract of *Heinsia crinita* (455 and 1365 mg/kg) is likely to be effective in the reduction of the blood sugar in normal, acute alloxan-induced diabetic rats and subchronic alloxan-induced diabetic rats compared with the standard drug metformin. The leaf extract of *Heinsia crinita* is not likely to cause liver pathology in experimental animals particularly at doses below 4500mg/kg b.w as the synthetic ability of the liver was also maintained judging from total protein and albumin values. Generally, the leaf extract of *Heinsia crinita* was shown to suppress protein and albumin level while elevating liver biomarker enzymes (ALP, AST and ASP).

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