THE EFFECT OF HEINSIA *CRINATA EXTRACT* ON EXPERIMENTAL DIABETES AND HYPERLIPIDEMIA IN RATS

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Abstract

Aim: Objective of the study was to find out the effect of *Heinsia Crinata* extract (HC Et) on experimental diabetes, and hyperlipidemia in rats.

Method: Ethanolic extract of *Heinsia Crinata* administered orally and effect of different doses of the extract on blood glucose, glucose-6- phosphatase , serum and tissue lipids in rats with alloxan induced diabetes were studied. Chlopropamide was used as standard reference drug. Ethanolic extract of *Heinsia Crinata* (HC Et) at doses of 150,300,500, mg/kg body weight have been administered for present study.

Results: Results indicated that the aqueous extract of *HeinsiaCrinata* exhibited anti-diabetic activity. Studies results indicated ,the extract suppressed the elevated glucose and in anti-hyperlipidemic studies, lipid levels changed in diabetic rats treated with HC Et.

Conclusion: Our findings shows that extract possesed and anti-diabetic activities.

Key words: Anti-diabetic, Anti-hyperlipidemic, *Heinsia Crinata* (HC Et)and Alloxan.

Introduction:

Renewed interest on biological activities of medicinal plants emerged in early 1980's as the Council of Scientific and Industrial research have published the information on the screening of biological activities of many medicinal plants using experimental models. Recently the use of herbal preparations in remedies for various medical conditions have been rapidly increasing especially in India. It is believed that herbal preparations are safe although the ingredients have never been vigorously substantiated. Traditional medicine has many positive features viz[1] diversity and flexibility[2] accessibility and affordability in many parts of the world,[3] broad acceptance among many populations in developing countries,[4] increasing popularity in developed countries and[5] comparatively low cost, low level of technological input and growing economic importance.[6] comparatively less side effects. These can all be seen as opportunities to be maximized.

Heinsia Crinata (family- Rubiaceae.) is a shrub with woody stems and branches. It is cultivated in various parts of Africa for their nutritional values. The leaves juice are used to treat wounds and some gastro intestinal disorders. Two triterpenoid saponins have been isolated from the leaves of *Heinsia Crinata* Ajibesin et al., reported for *Heinsia Crinata* leaves used in anti microbial therapy and containing phyto constituents like saponins, tannins,cardiac glycosides,terpenes and alkaloids. Two new triterpenoid saponins isolated from the root bark of Heinsia crinata were characterized as heinsiagenin A-3 beta-O-(beta-D-glucopyranosyl-(1--)2)-beta-D-glucopyranosyl-(1-)6)-[alpha-L-rhamnopyranosyl-(1--)2)]-beta-D-glucopyranosyl-(1--)2)- beta-D -glucopyranoside) and heinsiagenin A-3 beta-O-(alpha-L-rhamnopyranosyl-(1--)2)-beta-D- glucopyranosyl-(1-)2)-beta-D-glucopyranoside). There is a growing intrest in co-relating phytochemical constituents of plants with its pharmacological activities. Scientists have started co-relating the botanical properties of plants with their pharmacological activities.

Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism. The liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and severely affected during diabetes, Baquer (1998). Liver participates in the uptake, oxidation , and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes a profound alteration in the concentration and composition of lipids occurs, Swanston et al (1990). Many traditional plant treatments for diabetes mellitus are used through out the world. A few of traditional plants for treatment for nocicepton, and diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrents attention, WHO(1980). The objective of the present investigation is to find out the effect of *Heinsia Crinata* on effect on blood glucose and hyperlipidemia.

Materials and Methods

Animals

Male Whistar rats (180-200 gm bw) of 8-9 weeks were used to carry out antidiabetic with antihyperlipidemic studies .The animals were maintained under standard laboratory conditions (light period of 12h/day and temperature 27 ^{o}C±2^{o}C),with access to food and water *ad* libitum. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations The experiment was performed in the morning according to the guidelines for the care oflaboratory animals.

Chemicals:

Alloxanmonohydrate, Chlorpropamide, were obtained from Himedia Laboratories, S.G Pharmaceuticals Ltd., respectively. All other reagents used were analytical grade.

Plant material

Heinsia Crinata plant leaves were collected freshly from uyo market of Nigeria. Leaves dried under shade, made into coarse powder by grinding. Plant was identified and authenticated at the Botany department in University of Nigeria.

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Preparation of plant extract:

Ethanolic Extract

To 20 g of dried plant powder form, 500 ml ethanol were added and contents of flask were mixed thoroughly by gentle shaking Flask were kept for four days with frequent shaking. After the completion of maceration process the filtrates were obtained and ethanol evaporated to get the dried extract.(evaporation by keeping flasks in electric mantle at 80 ^{0}C). The residual extract was dissolved in water and used in the studies, Pari et al (2002).

Induction of experimental diabetes

To induce diabetes in rats alloxan monohydrate (150 mg/Kg)was administered intraperitoneally to a group of rats . The alloxan was freshly prepared as 5 % weight/ volume solution in distilled water. After two days, blood samples were taken from these animals after two hours of oral dosing of HC extract, chlorpropamide(400mg/kg) and equivalent amount of 2% weight/volume aqueous accacia solution as (control).Blood sugar levels of the animals were determined by the O-toluidine method. Rats with moderate diabetes having glycosuria and hyperglycemia(that is with a blood glucose of 200-300mg /dl) were used for the experiments, Siddiq et al (1987).

Experimental procedure for Anti-diabetic and Anti-hyperlipidemic activites

In the experiment, a total of 36 rats (30 diabetic surviving rats and six normal rats) were used . The rats were divided into six groups of six animals in each group.The treatment continued for consecutive 30 days. The rats from gr 2 to gr 6 were fed with 1 ml of aqueous solution (with the mentioned dose and material) using an intragastric tube.

Group 1: Normal untreated rats
Group 2: Diabetic control rats .
Group 3: Diabetic rats given HC Et(150 mg/ Kg body weight).
Group 4: Diabetic rats given HC Et(300 mg/ Kg body weight).
Group 5: Diabetic rats given HC Et(500 mg/ Kg body weight) .
Group 6: Diabetic rats given Chlorpropamide(400 mg/ kg body weight) .

At the end of 30 days, the animals were deprived of food overnight and sacrificed by the decapitation. Blood samples were collected in two different tubes (ie.) one with anti-coagulant - potassium oxalate and sodium fluoride for plasma and another without anti-coagulant for serum preparation. Plasma and serum were separated by centrifugation. Liver was immediately dissected out, washed in ice cold saline and patted,dry and weighed.

Analytical procedure

Fasting blood glucose was estimated by O-toludine method.Sasaki et al(1972).Plasma insulin level was assayed by enzyme linked immunosorbent assay(Rat Insulin ELISA) kit. Haemoglobin was estimated by the method of Drabkin et al (1992) and glycosylated haemoglobin by the method of Sudhakar et al (1981). Lipids were extracted from serum and tissue by the method of Folch et al(1957). Total cholesterol and triglycerides were estimated by the method of Zlatkis et al(1950)and Folch et al (1973) respectively. Free fatty acids and phospholipids were analysed by the method of Falholt et al(1973) and Zilversmit et al(1950). Hexokinase and glucose-6 phosphatase were assayed by the method of Brandstrup et al(1957) and Koida et al (1959).

Statistical analysis anti-diabetic/antihyperlipidemic activities

Numerical result are expresses as mean ± SD, unless otherwise stated. one way analysis of variance(ANOVA) was used for statistical comparison; $P<0.05$ being the criterion for statistical significance. The significant treatment means were further subjected to Duncan multiple post test, Hosseinzadeh et al (2000). for anti-diabetic activity/antihyperlipidemic, all values were expressed as the mean obtained from number of experiments (n). Data from all the tables of normal animals, diabetic control animals, reference drug treated nd HC Et treated animals were compared by ANOVA followed by Duncans multiples range test(DMRT), Benet et al (1978).

Results

Blood glucose and plasma insulin as shown in table 1 indicates levels of blood glucose, plasma insulin , total haemoglobin, glycosylated haemoglobin , changes in body weight and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose and glycosylated haemoglobin levels, while the plasma insulin and total haemoglobin levels decreased significantly in alloxan induced diabetic rats when compared with normal rats.

Administration of HC Et and Chlorpropamide tends to bring parameters significantly towards the normal. The effect of HC Et at a dose of 500mg/kg bodyweight was more highly significant than 150 mg, & 300 mg mg/kg body weight and therefore the dose was used for further biochemical studies . In diabetic rats , the urine sugar was (+++) but in the case of HC Et treated rats at a dose of 150 mg and 300 mg/kg body weight showed decreased urine sugar(++) and (+) respectively. Urinary sugar was nil by the effect of HC Et at a dose of 500mg /kg b.w as seen in normal rats. These effects were compared with Chlorpropamide.

Hexokinase & Glucose -6- phosphatase activities of carbohydrate enzymes are represented in table 2. Treatment with HC Et in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase ativity.

The effect of HC Et on serum and tissue lipids of normal and experimental rats were summerised in tables 3 and 4 respectively. A marked increase in the frequency of cholesterol, free fatty acids, triglycerides, and phospholipids were observed in diabetic control rats.

Treatment with HC Et reduced the lipid levels significantly

Table1. Changes in body weight, Blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, and urine sugar of normal and experimental animals as follows

	Groups	Body wt.g	Fasting blood glucose mg/dl	Plasma insulin (µu/ml)	Haemoglobin g/dl	Glycosylated Hb.(mg/g Hb	Urine sugar[A]
Normal	194± 11.38	202± 8.78	99.48± 9.03 [a]	14.03± 0.11 [a]	13.83± 1.74 [a]	0.24± 0.01 [a]	Nil
Diabetic control	202± 16.70	152± 14.66*	233± 16.40 [b]	5.35± 1.95 [b]	5.60± 0.45 [b]	1.81± 1.07 [b]	+++
Diabetic+HC Et (150mg/kg)	188± 17.36	193± 16.29*	208± 20.48[b]	5.76± 1.38 [b]	8.12± 1.52 [c]	0.61± 0.02 [c]	++
Diabetic+HC Et (300mg/kg)	198± 19.08	208± 10.26*	160± 15.20 [c]	8.11± 1. 80 [c]	9.22± 0.90 [d]	0.50± 0.05 [d]	+
Diabetic+HC Et (500mg/kg)	200± 20.60	210± 14.08*	115.2± 11.34 [d]	13.80± 0.62[d]	11.7± 1.87[e]	0.31± 0 .04[e]	Nil
Diabetic+Chlorpropamide400 mg//kg)	192± 11.78	207± 13.43*	125.6± 11.32[d]	12.70± 0.65[e]	11.36± 2.01[d]	0.47± 0.04[d]	Trace

- Values are given as mean ± SD for six rats in each group.
- Duncan procedure, Range for the level 2.88, 3.02,3.14,3.21,3. 24.
- Diabetic control was compared with normal , *p<0.001
- Experimental groups were compared with diabetic control *p<0.001.
- Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
- A- indicates 0.25 % sugar and (+++) indicates >1% sugar

Table2. Changes in activities of hexokinase and glucose-6-phosphatase in liver of normal and experimental animals.

Groups	Hexokinase(units[A]/g protein)	Glucose-6-phosphatase(units[B]/gm protein)
Normal	146.66±6.09 [a]	0.169±0.014 [a]
Diabetic control	106.48±4.74 [b]	0.242±0.023 [b]
Diabetic+HC Et500mg/kg	128.61±10.38 [c]	0.183±0.010 [ac]
Diabetic+Chlorpropamide 400mg/kg	124.20±6.40 [c]	0.200±0.008 [c]

- Values are given as mean ±S.D for six rats in each group.
- Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
- Duncan procedure ,Range for the level 2.96, 3.08,3.21.
- A-µ moles of glucose phosphosylated/ min.& B- µ moles of pi liberated /min.

Table3. Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in liver of normal and experimental animals.

Groups	Cholesterol (mg/100gwet tissue)	Free fatty acids (mg/100gwet tissue)	Triglycerides (mg/100gwet tissue)	Phospholipids (mg/100gwet tissue)
Normal	329.04 ± 2.88[a]	608.70± 31.68 [a]	348.88±14.07 [a]	2.66± 0.11 [a]
Diabetic control	513.70± 6.88 [b]	914.22± 49.27 [b]	622.35±9.40[b]	2.54± 0.08 [b]
Diabetic+HCEt (500mg/kg)	420.14± 5.30 [c]	773.09± 47.82 [c]	440.99±12.06[c]	2.03± 0.06 [c]
Diabetic+Chlor propamide. (400 mg/kg)	442.98± 6.36 [d]	807.67± 26.30 [c]	530.19±11.7 [d]	2.29± 0.10 [c]

- Values are given as mean ±S.D for six rats in each group.
- Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
- Duncan procedure , range for the level 2.94, 3.08,3.21

Table 4. Changes in level of cholesterol, free fatty acids, triglycerides and phospholipids in serum of normal and experimental animals.

Groups	Cholesterol (mg/100ml)	Free fatty acids (mg/100ml)	Triglycerides (mg/100ml)	Phospholipids (mg/100ml)
Normal	74.00 ±1.49[a]	70.43±5.06 [a]	44.53±3.36 [a]	80.25±1.57 [a]
Diabetic control	99.66±5.03 [b]	83.86±6.67 [b]	63.83±2.50 [b]	98.75±4.28 [b]
Diabetic+HC Et500mg/kg	83.44±3.16 [c]	74.58±1.56 [c]	53.98±3.68 [c]	84.50±1.82 [c]
Diabetic+Chlorpropamide 400 mg/kg	91.26±2.37 [d]	78.51±0.87 [d]	59.46±2.70 [d]	89.00±1.12 [d]

Values are given as mean ±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
Duncan procedure , Range for the level 2.94, 3.07,3.22.

Discussion

Our findings show that HC Et possesed anti-diabetic and anti-hyperlipidemic activities. The phyto compounds may be present in the crude extract of HC Et, that may account for the anti-diabetic and anti-hyperlipidemic activities.

Alloxan is well known for its selective pancreatic islets ß–cells cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interfere with cellular metabolic oxidative mechanisms, Papaccio et al (2000) Intraperitoneal administration of alloxan 150 mg/kg effectively induced diabetes in normal rats as reflected by

glycosuria, hyperglycemia, polyphagia, polydipsia and body weight loss when compared with normal rats. In our present study we have observed that an ethanolic extract of HC can reverse these effects. The possible mechanism by which HC Et brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from B-cells of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with CA Et .

In the case of anti-diabetic activity level of blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin , changes in body weight and urine sugar of normal and experimental rats showed in table 2. There was a significant elevation in blood glucose and glycosylated haemoglobin level, while the plasma insulin and total haemoglobin level decrease significantly in alloxan induced diabetic rats when compared with normal rats. Administration of HC Et and Chlorpropamide tends to bring parameters significantly towards the normal. The effect of CAEt at dose of 500mg/kg bodyweight was more highly significant than 150 and 300 mg mg/kg body weight and therefore the dose was used for further biochemical studies. In diabetic rats , the urine sugar was (+++) but in the case of HC Et treated rat at a dose of 150 and 300 mgkg body weight showed decrease in urine sugar(++) and (+) respectively. HC Et at a dose of 500mg /kg body weight, showed urine sugar as seen in normal rats. These effects were comparable with Chlorpropamide.

Activity of hexokinase in liver decreased markedly while the glucose-6-phosphatase activity increased significantly in diabetese conrol rats . Treatment with HC Et in diabetic rats increase the hexokinase activity and decreased the glucose-6-phosphatase activity.

HC Et may be capable of oxidizing NADPH Enhanced hexokinase ctivity in HC Et treated rats suggests greater uptake of glucose from blood by the liver cells .It can be concluded from the data that HC Et significantly reduces the level of plasma insulin and hexokinase activity. Of course the above informations from the present study prove that *Heinsia Crinata* extract posseses anti-diabetic and anti-hyperlipidemic activities.

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