

Anti-diabetic and Anti-hyperlipedemic Properties of Ethanolic Root Extract of *Gongronema Latifolium* (Utazi) on Streptozotocin (STZ) Induced Diabetic Rats

*A. E. Robert, U. O. Luke, E. O. Udosen, S.U. Ufot, A E Effiong, V.S. Ekam

Department of Biochemistry, University of Calabar, P.M.B. 1115, Calabar, Nigeria

Department of Science Laboratory Technology, Heritage polytechnic, Eket, Akwa Ibom State Nigeria

*anietieyo@yahoo.com

ABSTRACT

Diabetes mellitus is associated with alteration in carbohydrate and lipid metabolism. The present study was designed to investigate the hypoglycaemic and hypolipedemic activities of ethanolic root extract of *Gongronema latifolium* (GL) in Streptozotocin (STZ) diabetic rats. Diabetes was induced by intraperitoneal injection of STZ (65mg/kg b.w) after a 24hour fast. After being confirmed as diabetic, the animals were treated with 200mg/kg b.w and 400mg/kg b.w of ethanolic root extract of GL for 14days. Induction of diabetes with STZ in experimental rats showed significant ($P<0.05$) increase in serum glucose, triacylglycerol (TG), total cholesterol (TC), very low density lipoprotein (VLDL) and significant ($P<0.05$) reduction in high density lipoprotein cholesterol (HDL-C) in the diabetic control group compared with the normal control. Treatment with extract (at these doses) significantly ($p<0.05$) reduced serum glucose, TG, TC, VLDL but increased HDL in diabetic rats. There was no significant change on low density lipoprotein (LDL) in serum. The result from this study suggests that root extract of GL has ant diabetic and hypolipidemic properties in experimental diabetes mellitus rats model and therefore could be used for the management of diabetes mellitus (DM) in human.

Keywords: *Diabetes mellitus, streptozotocin, ant diabetic, antihyperlipidemic.*

1. INTRODUCTION

Research on medicinal plants has increased all over the world and a large body of evidence show the potential of medicinal plants used in various traditional systems [1].The plant kingdom is a vast storehouse of phytochemicals, and plants have been used for centuries as remedies for human diseases [19]. There has been growing interest in exploiting the biological activities of flora and fauna owing to their natural origin, cost effectiveness and lesser side effects [17]. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seed, etc [5]. In the South Eastern State of Nigeria, the plant is known locally as "utazi" and used primarily as staple vegetable/spice [24, 10, 23]. GL has a very bitter taste and its photochemical composition indicates that it contain saponins [10]. In some African cultures it is used as a spice to support the pancreas [14] and in the United States it is also a constituent of a DM Tea blend marketed for the maintenance of healthy blood glucose. Since diabetes is increasing and global prevalence was estimated to be 2.8% in 2000 and is projected to rise to 4.4% in 2030 [25]. In Nigeria, the prevalence o diabetes according to National survey reveals that 2.2 %; that is 2-3 in every 100 person are suffering from the diseases [11]. Cheaper sources of anti diabetic plant extract may be the answer, hence the choice of GL root for this study

2. MATERIALS AND METHODS

2.1 Plant Materials

The roots of GL were collected from a farm settlement in Biase local government, Cross River State, Nigeria and authenticated in the Department of Botany, University of Calabar. The roots were chopped into small

pieces and dried, blended with an electric blender into powder. One kilogram (1kg) of the powder was soaked in 80% ethanol at the ratio of 1:4(w/v) of powder to ethanol respectively. The mixture was agitated using an electric blender and cooled at (0-8°C) for two days. The mixture was doubly filtered using first a cheese cloth and then whatman No.1 filter paper. The filtrates were concentrated in vacuum at a temperature of (40-45°C) to about one tenth of the original volume using a rotary evaporator. The concentrate was kept in a water bath (40°C) for complete dryness and it yielded 18.3% of brown oily substance of GL. The extract was refrigerated at 2.8°C until used.

2.2 Animals and Experimental Design

Thirty six (36) albino rats of Wistar strain weighing about 140-180g were obtained from the animal house of the Department of Pharmacy, University of Uyo, Uyo. The animals were allowed to acclimatize for three weeks in the animal house of the Department of Biochemistry, University of Calabar, and Calabar. The animals were housed in well ventilated cages (wooden bottom and wire mesh to p) and kept under controlled environmental conditions of temperature ($25\pm 5^{\circ}\text{C}$), relative humidity ($50 \pm 5\%$) and 12 h our light / dark cycle. The 36 rats divided into 3 parallel groups consisting of a diabetic and non-diabetic pair of 6 animals each (table 1).

2.3 Induction of Experimental Animals

Prior to diabetes induction, the rats were subjected to a 12 hour fast, then diabetes was induced by intraperitoneal injection of 65mg/kg b.w. of streptozotocin (STZ) (Sigma St. Louis, N10, USA) reconstituted in normal saline [23]. Control animals received normal

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saline only. A week after injection of STZ, diabetes was confirmed in STZ treated rats with fasting blood glucose levels above 200mg/dl, estimated by using Glucometer (Acon Laboratories, Inc 4108 Sorrento Valley Boulevard, San Diego, USA). Blood was obtained from the tail vein of the rats.

2.4 Experimental Protocol

Diabetic and non diabetic animals were grouped as shown in table 1. The doses used were based on the

predetermined after LD 50 values from preliminaries studies [9]. The extracts 200mg/kg bw and 400mg/kg bw were given via oral gastric intubation twice per day (6.00am; 6.00pm). Treatment lasted for 14 days. Blood sugar was determined using glucometer. The animals were maintained on pellets prepared from growers feed from Jos, Plateau State, Nigeria and tap water given ad libitum.

Table 1: Experimental design

| Diabetic rat | | | Non Diabetic rats | | |
|--------------|----------------|-----------------------------|-------------------|----------------|-----------------------------|
| Group | No. of Animals | Treatment | Group | No. of Animals | Treatment |
| 1 | 6 | Placebo(Diabetic Control) | 1 | 6 | Placebo(Diabetic Control) |
| 2 | 6 | Root extract (200mg/kg b.w) | 2 | 6 | Root extract (200mg/kg b.w) |
| 3 | 6 | Root extract (20mg/kg b.w) | 3 | 6 | Root extract (20mg/kg b.w) |

2.5 Collection of Samples for Analysis

At the end of the 14 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles, emptied into plain tubes and allowed to clot for about two hours. The clotted blood was there after centrifuged at 3,000rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until used for lipid profile analysis.

2.6 Estimation of Serum Glucose and lipids

The lipid profiles were determined using kits manufactured by RANDOX kits reagent (Randox Laboratories, UK). Serum Total cholesterol (TC) and high density lipoprotein(HDL) was determined based on NCEP (2001) while Triacylglycerol(TG) was determined by the method of Tietz (1990).The Lipoproteins, VLDL and LDL was estimated from relationship established by Friedewald et al.(1972). Analysis of serum glucose was carried out using assay kits obtained from DIALAB produktion, Gessellschaft m.b.H A-1160 Wien-panikengasse, Austria .The method was based on the principles of Trinder (1972).

2.7 Statistical Analysis

Data obtained was expressed as Mean \pm Standard Deviation and analysed using the Analysis of Variance (ANOVA) or students' test where applicable. Values $P <$

0.05 were regarded as significant in comparison to appropriate controls.

3. RESULTS

In table 2, the mean value of serum glucose in diabetic rats significantly increased when compared with the control and significantly ($p < 0.05$) reduced in a dose dependent manner in diabetic rats treated with root extract of GL.

The mean value of TG, TC And VLDL significantly ($p < 0.05$) higher in diabetic control rats compared with the normal control were all decreased in diabetic treatment groups. However, these decreases were only significant ($p < 0.05$) for diabetic groups that receives 400mg/kg b.w of root extract compared with diabetic control. HDL cholesterol level of diabetic rats treated with ethanolic root extracts significantly ($p < 0.05$) increase as the dose of the extract increases. This increases compared well with non diabetic treatment group. No significant change was observed in LDL concentration in all diabetic and non diabetic treated groups.

Table 2: shows the effect of ethanolic root extract of GL on serum glucose and lipids

| Group treatment | Glucose mg/dl | TG mg/dl | TC mg/dl | VLDL mg/dl | LDL mg/dl | HDL mg/dl |
|-------------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|--------------|-----------------------------|
| Diabetic control (DC) | 207.23 ± 22.73* | 80.37 ± 3.21 | 103.47 ± 10.47 | 72.16 ± 13.1 | 78.89 ± 5.87 | 14.19 ± 3.85 |
| Normal control(NC) | 97.82 ± 9.45 | 64.75 ± 6.53* | 88.78 ± 3.43* | 45.22 ± 0.24* | 67.81 ± 8.48 | 23.51 ± 3.12 |
| Root extract (200mg/kg bw) D | 185.81 ± 0.00 ^a | 42.77 ± 0.0 | 83.5 ± 0.0 | 46.58 ± 2.40 | 37.95 ± 0.0 | 18.00 ± 0.00* ^a |
| Root extract (200mg/kg bw) ND | 58.71 ± 8.94 ^a | 45.75 ± 6.53 | 85.51 ± 6.25 | 44.06 ± 4.81 | 44.06 ± 2.40 | 20.19 ± 10.08 |
| Root extract (400mg/kg bw) D | 171.84 ± 16.57 ^a | 59.83 ± 2.70 ^a | 79.64 ± 4.23 ^a | 47.65 ± 7.80 ^a | 47.65 ± 7.80 | 22.50 ± 10.97* ^a |
| Root extract (400mg/kg bw)ND | 126.79 ± 8.21 | 65.77 ± 6.02 | 95.45 ± 7.34 | 45.32 ± 0.81 | 45.32 ± 0.81 | 23.97 ± 4.70 |

*P < 0.05 vs NC; a=P < 0.05 vs DC D = Diabetic, ND = Non diabetic, NC = Normal Control, DC= Diabetic Control

levels. The observe significant reduction in the serum concentration of

4. DISSCUSION

Diabetes mellitus is a complex disorder that is characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipid and protein metabolism [18]. It is the fastest growing metabolic diseases in the world and as knowledge of the disease increase so does the need for challenging and appropriate therapies [24].The treatment strategies of diabetes mellitus include nutritional therapy, insulin injection, treatment with various classes of oral hypoglycaemic agent which could be synthetic or of herbal origin [26]. However in African management of diabetes mellitus, varieties of medicinal plants are employed, some of which have been investigated and documented [15,19]. Some of these plant include leaves and roots of gongronema latifolium [12,2]. Aqueous and ethanolic root culture of vernonia amygdalina (Khallafalla et al, 2009), cacrica papaya soak with stem bark extracts of enantia chlorantia in water (Olapade,1995), chloroform eluted fraction of the petroleum ether extract of root bark of balacia oblonga wall (pokoranti) demonstrated hypoglycemic tendency [3], to list just a few. According to Ojiako and Nwanjo, all part of a plant are pharmacologically useful [12]

In this present study, the hypoglycaemic and hypolipidemic effect of ethanolic root of GL was investigated in diabetic rats after 14 days administration. The study showed that oral treatment with 200mg/kg b.w and 400mg/kg b.w of root extract induced significantly progressive hypoglycaemic and hypolipidemia.it may appears root extract exert or enhances peripheral glucose utilisation. Lipid profile, which is altering in the serum of diabetic patients [16], appears to be a significant factor in the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol

TG,TC, cholesterol fraction and increase in HDL could be due to depressed hepatic gluconeogenesis by root extract. However, this claim remains speculative and is subject to further scientific validation by the key enzymes regulating this pathway. A positive relation between gluconeogenesis and lipogenesis is has been well documented in literature [6].Any drug that interferes with gluconeogenesis has been reported to also interfere with lipogenesis [3]. From the foregoing, it may be possible for the root extract to be inducing its hypoglycaemic and hypolipidemic effects via this common pathway. Further studies will be needed to purify the bioactive compounds in the extract, and use the purified compounds for bio assay directed experiments.

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