

Full Length Research Paper

Effect of ethanolic root and twig extracts of *Gongronema latifolium* (utazi) on kidney function of streptozotocin induced hyperglycemic and normal wistar rats

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Electrolytes in the body fluid have important effects on the overall balance and are essential for normal functionality of cell and body organs. This study evaluated the effect of ethanolic root and twig extracts of *Gongronema latifolium* (GL) on kidney function of streptozotocin induced hyperglycemic and normal Wistar rats. A total of sixty (60) Wistar rats were divided into 10 groups of 6 rats each. Groups A and B served as normal (NC) and diabetic control (DC) respectively and received dimethylsulphoxide(DMSO). Groups C, D, E, F, G,H,I,J served as non-diabetic treated and diabetic treated receiving single dose(200mg/kg b.w) and double dose (400mg/kg b.w) of ethanolic twig and root extracts of GL respectively for 14days. Thereafter, the animals were sacrificed and blood collected for analyses of serum glucose, Na⁺, K⁺, Cl⁻, urea and creatinine. Our finding revealed a significant decrease (P<0.05) in serum glucose concentration for all the groups treated with both ethanolic extracts of GL compared to diabetic and normal control. The serum urea concentration showed a significant increase (P<0.05) in the SSDT-twig and DDDT-root compared to the diabetic and non-diabetic rats, while the DDNT-root showed a significant decrease (P<0.05) compared to the diabetic and non-diabetic rats. Whereas, a significant decrease (P<0.05) was observed in Cl⁻ level in DDNT-twig and SDDT-root respectively compared to diabetic and normal control. The creatinine concentrations in all treated groups showed a significant decrease (p<0.05) compared to the diabetic and non-diabetic Wistar rats. These results confirm the probable anti-hyperglycemic activity and kidney function potentials of the plant.

Key words: *Gongronema latifolium*, Kidney function, hyperglycemia, Wistar rats and Streptozotocin

INTRODUCTION

The phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics, with accelerated global efforts to harness and harvest those medicinal plants that bear a substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes-related complications (Tiwari and Rao, 2002).

Diabetes is a chronic metabolic disorder with impaired glucose tolerance and high risk of cardiovascular disease (Schenell and Standl, 2006). One of the major disorders apart from metabolic disorder is inappropriate hyperglycemia that is caused by an absolute or relative insulin deficiency (Rawi, 2007).

Most researchers have evaluated the efficiency of medicinal plants. They evaluate the efficiency of medicinal plants extracts on diabetes induced by both alloxan and streptozotocin in animal models (Atangwho *et al.*, 2008, Mfon *et al.*, 2011, Modi *et al.*, 2006). With respect to effect of diabetes and herbal treatment on serum, liver, tissue and histology of tissues, a decrease in serum glucose, pancreatic regeneration and

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and maintenance of liver tissue integrity has been documented in animal models (Atangwho *et al.*, 2008).

Gongronema latifolium commonly called 'utazi' and 'arokeke' in the South Western and South Eastern parts of Nigeria, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu *et al.*, 2003). Morebise *et al.*, (2002). The leaf extract is rich in proteins (27.2 %DM) which compared well with values reported for chickpea (24.0 %DM) and other protein rich plants (Glew *et al.*, 1997; Akwaowo *et al.*, 2000; Ajayi *et al.*, 2006; Iqbal *et al.*, 2006). Phytochemical analysis of leaf extract of *Gongronema latifolium* reveals the presence of essential oil, saponins, alkaloids, minerals with calcium, phosphorus, magnesium, copper and potassium (Morebise *et al.*, 2002; Schneider *et al.*, 2003; Eleyinmi and Bressler, 2007; Atangwho *et al.*, 2009). It is a tropical rainforest plant which has been traditionally used in the south eastern part of Nigeria for the management of diseases such as diabetes, high blood pressure (Ugochukwu *et al.*, 2003). Ugochukwu and Babady (2003), Ugochukwu *et al.*, (2003) and Ogundipe *et al.*, (2003) reported that aqueous and ethanolic extracts of *G. latifolium* exerts hypoglycemic, hypolipidemic and antioxidative properties while Morebise *et al.* (2002) and Akuodor *et al.* (2010) reported the anti-inflammatory and antimalaria actions respectively. Recent research findings on the root and twig of *Gongronema latifolium* from our laboratory reveals the presence of bioactive components and micronutrients (Egbung *et al.*, 2011).

Drawing from the above findings, the present study was designed to evaluate the efficacy of ethanolic root and twig extract of *Gongronema latifolium* on the kidney function of normal and streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Sample collection and preparation

G. latifolium plant was identified and authenticated by a Botanist (Dr Mike Eko) of the Department of Botany, University of Calabar. Fresh roots were excavated and stem of the plant harvested from Akpabuyo Local Government Area of Cross River State, Nigeria. The roots and stems were thoroughly washed to remove debris and the earth remains. From these the barks were divested and thereafter chopped into bits and allowed to dry under shade. They were blended into fine powder using a Q-link electrical blender Model QBL-18L40. Three hundred and ten point eight (310.8g) of the blended stem bark and Three hundred and sixty (360g) of the blended root barks were separately soaked in 1200ml of ethyl alcohol (80% BDH) each, it was vigorously agitated then allowed to stay in refrigerator for 48 hours at 4°C. The mixtures were first filtered with cheese cloth, then with

Whatman No 1 filter paper (24cm). The filtrates were then separately concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 37°C - 40°C and subsequently concentrated to complete dryness in water bath, yielding 37.1g (11.96%) of stem bark and 49.1g (13.6%) of root bark extracts. The extracts were stored in a refrigerator from where aliquots were used for the administration.

Animal handling

Sixty albino Wistar rats of both sexes weighing 120-180g were used for this study. The rats were obtained from the animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar. They were acclimatized for one week then reweighed and housed in wooden cages with wire-mesh top and kept under controlled environmental conditions of temperature (28±2°C), relative humidity (50±5%) and a 12 hour light/dark cycle. The animal facility was adequately ventilated and the animals maintained regularly on the commercial rat chow. Tap water and food were provided *ad libitum* throughout the experimental period.

Induction of experimental diabetes

Diabetes was induced by intra-peritoneal injection of 65mg/kg b.w of streptozotocin (STZ), Batch No. U925 obtained from Sigma St. Louis, M.O. USA. Prior to diabetes induction, the animals were fasted for 12 hours. Confirmation of diabetes was done four days after induction using One Touch glucometer (Lifescan Inc 1995 Milpas, California, USA). Blood sample for the fasting blood sugar (FBS) determination was obtained from tail puncture of the rats, and animals with FBS ≥ 200mg/dl were considered diabetic and included in the study as diabetic animals.

Experimental Design

The design for animal groupings and treatments (see table 1). The dosages of plant extracts used were according to the methods of Ebong *et al.*, (2008). Plant extracts were administered twice daily via orogastric intubation for 14 days (at 6.00 am and 6.00pm). At the end of the 14 days, feed was withdrawn from the rats and they were fasted overnight but with free access to water. They were then anaesthetized under chloroform vapors and sacrificed. Whole blood was collected via cardiac puncture using sterile needles and syringes into plain tubes and allowed for about two hours to clot. The clotted blood was centrifuged at 3000rpm for 10mins for serum collection meant for biochemical assays.

Table 1. Experimental design

Grouping	Number of rats	Treatment	Dosage
NC	6	DMSO	0.2ml
DC	6	DMSO	"
SDDT-TWIG	6	GL TWI	200mg/kg
DDDT- TWIG	6	"	400mg/kg
SDNT-TWIG	6	"	200mg/kg
DDNT-TWIG	6	"	400mg/kg
SDDT-ROOT	6	"	200mg/kg
DDDT-ROOT	6	GL ROOT	400mg/kg
SDNT-TWIG	6	"	200mg/kg
DDNT-TWIG	6	"	400mg/kg

Key: NC= normal control, DC= diabetic control, SDDT= single dose diabetic treated group, DDDT= double dose diabetic treated group, SNDT= single dose normal treated, DDNT= double dose normal treated

Glucose Estimation

Randox-assay kit (CHOD-PAP) method based on Barham and Trinder (1972) was used. The principle involves the enzymatic oxidation of glucose in sample by the enzyme Glucose oxidase which generates hydrogen peroxide and gluconic acid. The concentration of H₂O₂ released is proportional to initial amount of glucose in the sample and it reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to form a red violet quinoneimine dye whose colour intensity reflects the concentration of glucose in the sample.

Electrolyte Estimation

Sodium

Principle: Sodium is estimated by colometric method based on modified Maruna and Trinders (1951) method. Using Agape diagnostic kit. Sodium and Protein are precipitated together by Magnesium uranyl acetate as Uranyl magnesium sodium acetate salt. Excess of uranyl salt reacts with potassium ferrocyanide to produce a brownish color. The intensity of the color is inversely proportional to the sodium concentration in the specimen and is measured photometric ally at 530nm (500-546 nm).

Chloride

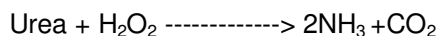
Agape diagnostic kit method by Schonfeld (1964). Principle: in an acid medium chloride ions and mercury-II-thiocyanate form thiocyanate ions. These ions react with HNO and Fe³⁺ and give a red color. The intensity of the color is directly proportional to the concentration of chloride ions.

Urea

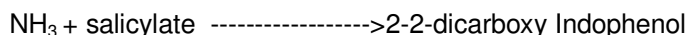
Agape diagnostic kit method by Bertherlot (1959) and Tobacco *et al.* (1979)

Principle: Enzymatic determination of Urea according to the following reaction.

Urease



Nitroprusside



Hypochlorite

Creatinine

Agape diagnostic kit method by Allen (1982) and Haeckel *et al.* (1981).

Principle: Creatinine reacts with picric acid to produce a colored compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration.

Potassium

Centronic GmbH kit using turbidimetric determination method by Tietz (1976) and Hillmann *et al.*, (1967). The potassium liquid test is based on the turbidimetric analysis, where potassium ions (without deproteinization) from a stable suspension. The turbidity which has been formed is proportional to the potassium concentration.

Statistical Analysis

Glucose and electrolyte measurements are presented as mean \pm SE. One way Analysis of Variance (ANOVA) and

Table 1a. Effect of 14 days administration of ethanolic twig extract on kidney function of streptozotocin induced diabetic and non-diabetic albino wistar rat

Grouping	GLUCOSE (mg/dl)	Chloride (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)	Urea (mg/dl)	Creatinine (mg/dl)
NC	97.82±9.45	88.12±8.60	80.33±48.27	5.21±0.83	41.30±0.66	17.47±0.13
DC	207.23±122.73*	108.54±11.13	45.45±34.38	7.44±2.76	58.99±19.86	17.42±0.29*
SDDT-TWIG	93.67±19.24 ^a	85.52±3.32	56.42±27.24	10.83±1.97	92.84±8.34* ^a	2.33±0.07* ^a
DDDT-TWIG	116.75±34.75	65.67±13.62	43.52±21.99	7.54±0.03	43.24±7.80	3.24±0.99* ^a
SDNT-TWIG	108.24±26.20 ^a	68.32±2.75	26.62±9.68	5.69±0.73	47.97±10.01	2.66±0.17* ^a
DDNT-TWIG	91.92±7.66 ^a	68.21±13.70* ^a	28.71±7.44	11.24±4.35	40.05±4.87	3.01±0.38* ^a

Values are expressed as Mean ± SD, n=6, *P<0.05 vs NC; a = P<0.05 vs DC

NC= normal control, DC= diabetic control, SDDT= single dose diabetic treated group, DDDT= double dose diabetic treated group, SDNT= single dose normal treated, DDNT= double dose normal treated

Table 1b. Effect of 14 days administration of ethanolic root extract on kidney function of streptozotocin induced diabetic and non-diabetic albino wistar rat

Grouping	GLUCOSE (mg/dl)	Chloride (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)	Urea (mg/dl)	Creatinine (mg/dl)
NC	97.82±9.45	88.12±8.60	80.33±48.27	5.21±0.83	41.30±0.66	17.47±0.13
DC	207.23±122.73*	108.54±11.13	45.45±34.38	7.44±2.76	58.99±19.86	17.42±0.29*
SDDT-ROOT	105.81±0.00 ^a	53.97±0.00 ^a	99.18±0.00	6.42±0.00	60.55±0.00	2.65±0.00* ^a
DDDT-ROOT	71.84±16.57 ^a	98.38±13.78	94.20±12.42	10.16±0.87	87.13±9.39* ^a	3.18±0.83* ^a
SDNT-ROOT	58.71±8.94 ^a	113.38±2.83	37.24±3.62	23.57±6.19* ^a	46.21±2.66	8.54±3.00* ^a
DDNT-ROOT	126.79±8.21 ^a	141.33±19.74	28.98±3.41	6.93±2.25	33.56±4.30 ^a	14.74±2.57

Values are expressed as Mean ± SD, n=6, *P<0.05 vs NC; a = P<0.05 vs DC

NC= normal control, DC= diabetic control, SDDT= single dose diabetic treated group, DDDT= double dose diabetic treated group, SDNT= single dose normal treated, DDNT= double dose normal treated

the LSD post hoc test were used to analyse the data (p<0.05).

RESULTS

Results presented in two tables above viz: 1a for twig extract of *Gongronema latifolium* and 1b for root extract of *Gongronema latifolium*.

Glucose

The serum glucose concentration showed a significant decrease (P<0.05) in all groups treated with ethanolic twig and root extract respectively compared to the diabetic and non-diabetic groups. There was a significant increase (P<0.05) in the serum glucose of the diabetic groups compared to the non-diabetic group.

Urea

The serum urea concentration was significantly increased (P<0.05) in the SDDT-twig (92.84±8.34) and DDDT-root

(87.13±9.39) compared to the diabetic and non-diabetic group. Also the DDNT-root group (87.13±6.25) showed a significant decrease compared to the diabetic group. SDNT-twig, DDDT-twig, SDDT-root, SDNT-root showed a comparable change but not significant to diabetic and non-diabetic groups.

Creatinine

In the groups treated with twig extracts, the result showed a significant decrease (P<0.05) in all the treated groups compared to diabetic and non-diabetic control groups, except for the DDNT-root groups that showed no significant change at P>0.05.

Electrolyte

The serum Na⁺ concentration showed no significant change (P>0.05) compared to diabetic and non-diabetic group. Also, the serum K⁺ concentration showed no significant change (P>0.05) in all treated group compared to the diabetic and non-diabetic group, except for the SDNT-root group that showed a significant increase

($P < 0.05$) compared to the diabetic and non-diabetic subjects (23.57 ± 6.17). Moreover, a significant decrease ($P < 0.05$) was observed in the serum Cl^- concentration in the DDNT-twig and SDDT-roots (68.21 ± 13.70) and (53.97 ± 0.00) respectively compared to diabetic and non-diabetic group.

DISCUSSION

It is now well established that streptozotocin selectively destroys the pancreatic cells and produce hyperglycemia. The hyperglycemia induced by streptozotocin mimics insulin dependent diabetes mellitus (IDDM) in adult animals and also induces a variety of metabolic abnormalities (Soling *et al.*, 1976). In view of this, streptozotocin induced diabetic rat models is ideal for screening of anti-hyperglycaemic/anti-diabetic activity of various phytochemicals and synthetic products. In the present study, the anti-hyperglycemic activity of 14 days administration of graded doses of ethanolic twig and root extract of *Gongronema latifolium* was evaluated. From the result, graded doses of ethanolic twig and root extract of *Gongronema latifolium* respectively showed a significant reduction ($P < 0.05$) in the serum glucose compared to diabetic and non-diabetic control, these anti-hyperglycemic activity of these plant parts may be due to the presence of significant concentrations of bioactive components reported in our previous work. Furthermore, there is also possibility that this extract might increase the uptake of glucose by peripheral tissue and thereby presumably decreased the serum glucose level.

The function of the kidneys is to regulate endocrine processes such as RBC synthesis, Vitamin D secretion and blood pressure maintenance. The renal system plays a primary role in the regulation of electrolyte/fluid balance, the PH buffer system and in the elimination of waste products. When kidney function declines, obviously these processes become impaired. Overtime, individuals with diabetes can develop a condition called diabetic nephropathy. The nephropathy causes sclerotic changes in the kidney structure. These structural changes lead to the development of proteinuria and declining glomerular filtration rates in the kidney. Creatinine and urea are markers alongside electrolyte balance used in the assessment of liver functions. The results of 14 days administration of graded doses of ethanolic twig and root extracts of *Gongronema latifolium* in our study show a significant decrease ($P < 0.05$) in creatinine level compared to diabetic and non-diabetic control group, while the urea level shows no significant change ($P < 0.05$) compared to diabetic and non-diabetic group, while result of change in the selected serum electrolyte concentrations viz: Sodium (Na^+), Chloride (Cl^-) and Potassium (K^+). There was no significant change ($P > 0.05$) in serum Na^+ comparable to diabetic and non-diabetic

control group. However, the serum K^+ concentration showed no significant change ($P > 0.05$) in all treated group compared to the diabetic and non-diabetic control groups, except for the SDNT-root group that showed a significant increase ($P < 0.05$) compared to the diabetic and non-diabetic controls. Moreover, a significant decrease ($P < 0.05$) was observed in the serum Cl^- concentration in the DDNT-twig and SDDT-roots respectively compared to diabetic and non-diabetic groups. The effect of extract on electrolytes of diabetic treated animals agree with report by Atangwho *et al.*, 2007b on effect of *Vernonia amygdalina* on diabetic animal but Sharma *et al.*, 2001 reported that there are no actual conditions that can directly cause electrolyte imbalance. However decrease in serum creatinine level indicates that this plant may have an effect in reducing the creatinine level during diabetic condition and as well help in the maintenance of the kidney function.

CONCLUSION

Our findings indicated probable anti-hyperglycemic and kidney function potentials of *Gongronema latifolium* potentials.

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