Research Journal of

Medicinal

Plant

Substitution of Combined Extracts of *Gongronema latifolium* and *Nauclea latifolia* with Insulin Requirement in the Management of Type I Diabetes

[1]Grace Sylvester Effiong, [2]Herbert O.C. Mbagwu, [1]Imoh E. Udoh, [2]Nsikan M. Udo, [3]Item J. Atangwho, [3]Patrick E. Ebong, [3]Ekpo N. Asuquo and [4]Lucy A. Wilson

[1]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo, Nigeria

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria

[3]Department of Biochemistry, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria

[4]Department of Chemistry, Akwa Ibom State College of Arts and Science, Nung Ukim Ikono, Nigeria

Corresponding Author:Grace Sylvester Effiong, Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo, Nigeria

ABSTRACT

Polyherbal therapy, sometimes called polyherbalism-a combination of herbs or phytochemicals from more than one source, has today become accepted as an effective therapeutic approach in sourcing medicament for degenerative ailments. The present study, investigated the effect of combined extracts from *Gongronema latifolium* (GL) and *Nauclea latifolia* (NL) on some hormonal indices of normal and diabetic rats. Type 1 diabetes was induced with streptozotocin (50 mg kg^{-1} b.w.) and the hormonal parameters were measured using standard methods. Measured c-peptide levels showed significant increase in diabetic rats treated with the combined extracts and only GL relative to non-diabetic control. Whereas there were significant increase ($p < 0.05$) in serum triiodothyronine (T_3) and tetraiodothyronine (T_4) concentrations of diabetic control when compared to normal control, administration of both combined extracts and insulin caused significant reduction ($p < 0.05$) in T_3 and T_4 relative to the diabetic control, although, this observation differed in the non diabetic test animals. Measured serum insulin level was significantly reduced ($p < 0.05$) in untreated diabetic rats relative to normal control, administration of only GL and its combination with NL extracts also reduced the level in a manner similar to insulin administration. On the contrary, normal rats that received similar treatments showed significant increase ($p < 0.05$) in serum insulin level. Therefore, combined extracts of *Gongronema latifolium* and *Nauclea latifolia* may mimic insulin in its antihyperglycemic action at least in rats.

Key words: *Gongronema latifolium*, *Nauclea latifolia*, diabetogenic hormones, insulin, diabetes

INTRODUCTION

The use of folk medicine in Africa and most Asian countries is prevalent, and the searches for herbal cures are the common practices especially among the low income groups and peasants. According to Okoli *et al.* (2007), in the past four decades, Africans had been using the traditional medicine for their health care before the introduction of orthodox medicine. Focho *et al.* (2009) reported on the usage of traditional medicine by 80% of developing countries for their health care. *Gongronema latifolium* (GL) (Asclepiadaceae) and *Nauclea latifolia* (NL) (Rubiaceae) are two

plants used traditionally in the management of diabetes in the African sub-region and Asia. *Gongronema latifolium* (Asclepiadaceae) is a perennial edible plant with soft and pliable stem. In the West African sub-region, GL is widely used for both medicinal and nutritional purposes (Nwanjo *et al.*, 2006). Apart from hypoglycemic activity, GL also possesses hepatoprotective and hypolipidaemic effects (Ugochukwu and Babady, 2003; Ugochukwu *et al.*, 2003; Nwanjo, 2005 and Nwanjo and Alumanah, 2005). On the other hand, *Nauclea latifolia* is from the family Rubiaceae. Traditional medicine practitioners profusely use NL, commonly known as Pin cushion tree. Gidado *et al.* (2005) reported the effect of *N. latifolia* on blood glucose levels in diabetic rats while the hypoglycemic activity is reported by Gidado *et al.* (2008).

Although, there are many investigations reported on GL and NL concerning antidiabetic action, few literatures are available on their mechanism(s) of action in hypoglycemia and antihyperglycemia. Moreover, the combined extracts of GL and NL had not undergone biochemical studies. Atangwho *et al.* (2010) reported on a recent pharmacological concept that has the advantage of producing maximum therapeutic efficacy with minimum side effects.

Consequently, this present study investigated in part the mode by which extracts of *Gongronema latifolium* and *Nauclea latifolia* singly and in combination mediate or exert their anti-diabetic effects by evaluating some serum hormonal indices of diabetic rats given single and combined extracts from these plants.

MATERIALS AND METHODS

Collection and preparation of plant's extracts: Fresh but matured *Gongronema latifolium* leaves were collected from a cultivated land at Ibiaku Itam, Nigeria while *Nauclea latifolia* was collected in Calabar, Nigeria in March, 2012. They were authenticated by Dr. E.G. Amanke in the Department of Botany, University of Calabar, Nigeria and voucher specimen deposited in the Department of Botany herbarium, University of Calabar. The leaves were rinsed severally with clean tap water to remove particles and debris and thereafter allowed to completely drain. The ethanol extract was prepared using the wet method of extraction; one kilogramme of the fresh leaves of the plants were separately cut and chopped into pieces with a knife on a chopping board, blended in 1.5 L of ethanol (96%) with an electric blender and transferred into amber colored bottle and kept in cool (4°C) dark compartment for 72 h. These were then filtered using a cheese cloth and thereafter with Whatman No. 1 filter paper to obtain a homogenous filtrate. These filtrate were then concentrated *in vacuo* using a rotary evaporator at 37-40°C to about one tenth the original volume. The concentrates were allowed open in a water bath (40°C) to dryness yielding 30.00 and 78.95 g of greenish brown and brown oily substances of *G. latifolium* and *N. latifolia*, respectively. They were dried completely in a desiccator containing a self-indicating silica gel and then refrigerated at 2-8°C until used.

Animals and experimental design: Sixty male Albino Wistar rats of 150-200 g obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy of the University of Uyo, Nigeria were used. They were kept in clean cages (wooden bottom and wire mesh top), maintained under standard laboratory conditions (Temperature 25±5°C, Relative humidity 50-60% and a 12/12 h light/dark cycle) and were allowed free access to standard diet (Vital Feed from Grand Cereals and Oil Mills Limited, Jos, Plateau State of Nigeria) and water *ad libitum*. Animals were acclimatized for 14 days in the animal house of the Department of Biochemistry, University of Calabar, Nigeria, before each of the experiments. The 60 rats were divided into 5 parallel groups consisting of a diabetic and non-diabetic pair of 6 animals each (Table 1).

Table 1: Treatment schedule for diabetic and non diabetic rats

Diabetic rats			Non diabetic rats		
Group	No. of animals	Treatment	Group	No. of animals	Treatment
1	6	GL_D (400 mg kg^{-1} b.wt.)	1	6	GL_{ND} (400 mg kg^{-1} b.wt.)
2	6	NL_D (400 mg kg^{-1} b.wt.)	2	6	NL_{ND} (400 mg kg^{-1} b.wt.)
3	6	$(GL+NL)_D$ (400 mg kg^{-1} b.wt.)	3	6	$(GL+NL)_{ND}$ (400 mg kg^{-1} b.wt.)
4	6	$Insulin_D$ (5 unit kg^{-1} b.wt.)	4	6	$Insulin_{ND}$ (5 unit kg^{-1} b.wt.)
5	6	Placebo (Diabetic control (DC)	5	6	Placebo (Nondiabetic control (NC)

GL: *Gongronemal latifolium*, D: Diabetic, NL: *Naucela latifolia*, ND: Non-diabetic

Experimental induction of diabetes: Diabetes was induced in an overnight fasted animals by a single intraperitoneal injection of freshly prepared solution of streptozotocin (Sigma, USA) 50 mg kg^{-1} b.wt. in 0.1 M cold sodium citrate buffer pH 4.5 (Ghoraishian, 2006; Rao and Naidu, 2010). The animals were considered as being diabetic if the blood glucose value were >200 mg dL^{-1} on the third day after streptozotocin injection and were used in the experiment. This was estimated using One Touch Glucometer (Lifescan, inc 1995).

Experimental protocol: Diabetic and non-diabetic animals were grouped as shown in Table 1 and accordingly, treated with extracts and/or insulin. The dosages of the plant extracts were as determined from preliminary work in the laboratory whereas insulin dose, NPH (5 U kg^{-1} b.wt.) was as previously used by Atangwho (2008) and also chosen to simulate human regimen. The plant extracts were administered via gastric intubation, twice per day (6:00 a.m.: 6:00 p.m.) and insulin once per day post prandial (6:00 p.m.). The animals were treated for 21 days.

Collection of samples for analysis: At the end of the 21 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 thereafter centrifuged at 3,000 rpm for 10 min to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until used for hormonal analysis.

Estimation of serum hormonal indices: Elisa kits for hormonal assays including c-peptide, tri- and tetra-iodothyronine were purchased from Monobind Inc. Lake Forest, CA 92630. U.S.A. and insulin kit from DRG Instruments, GmbH, Germany, Division of DRG International, Inc Frouenbergergstr.18, D-35039 Marburg. Serum was used for the estimation of various hormonal parameters: c-peptide based on Eastham (1985), (T_3) based on Gharib *et al.* (1971), (T_4) based on DIALAB Method and insulin based on Starr *et al.* (1978).

Statistical analysis: The results were analyzed for statistical significance by one way ANOVA using the SPSS statistical program and *Post Hoc* Test (LSD) between groups using MS excel program. All data were expressed as Mean±SEM. p-values<0.05 were considered significant.

RESULTS
Effect of treatment with GL, NL, GL and NL combined and insulin on serum hormonal indices including C-peptide, triiodothyronine (T_3), tetraiodothyronine (T_4) and insulin of non-diabetic and diabetic experimental animals are shown on Table 2. GL and NL combined treated rats (2.10±0.04)

Table 2: Effect of treatment on some selected hormonal indices in non diabetic and diabetic rats

	C-PEP (ng L^{-1})	INS (U mL^{-1})	T$_3$ (ng mL^{-1})	T$_4$ (ng mL^{-1})
GL$_D$	2.64±0.75*	16.63±2.95*	1.57±0.12*,a	1.67±0.02*,c
GL$_{ND}$	3.20±0.06*,a,b,c	32.85±1.45	1.33±0.48*,c	3.30±0.06*,a
NL$_D$	1.13±0.11	27.10±6.37a	1.33±0.02*,a,b	2.10±0.04
NL$_{ND}$	2.57±0.42*,a,c	29.55±1.51*,a	1.42±0.61*,c	3.30±0.10*,a
GL+NL$_D$	2.10±0.04*	21.65±0.16*,a	1.25±0.02*,a,b	2.10±0.04
GL+NL$_{ND}$	2.40±0.38*,c	33.45±1.63	1.27±0.61*,b,c	3.00±0.29a
Insulin$_D$	1.51±0.54	15.37±1.28*	1.77±0.15*,a	1.87±0.04*
Insulin$_{ND}$	1.10±0.11	35.24±1.85	3.02±0.51	3.13±0.32a
DC	2.28±0.63	13.33±0.02*	6.63±0.18*	8.07±0.26*
NC	1.13±0.10	37.72±1.51	3.38±0.32	2.68±0.27

*$p<0.05$ vs. NC, a: $p<0.05$ vs. DC, b: $p<0.05$ vs. Insulin, c: $p<0.05$ vs. GL+NL, Mean ± SE, n: 6, D: Diabetic rats, ND: Non-diabetic rats, DC: Diabetic control, NC: Normal control

showed a significant increase ($p<0.05$) in C-peptide concentration compared to NC (1.13±0.10). However, treatment with extracts of only GL, NL or insulin increased non significantly the C-peptide levels in diabetic rats compared to NC. The extracts treated non-diabetic rats showed a significant increases ($p<0.05$) in C-peptide concentration (GL$_{ND}$ = 3.20±0.06; NL$_{ND}$ = 2.57±0.42; (GL+NL)$_{ND}$ = 2.40±0.38: Where ND = Non diabetic rats) in comparison with NC while insulin treated rats indicated a non significant difference ($p>0.05$) compared to NC. Serum triiodothyronine (T$_3$) decreased significantly in treated diabetic (GL$_D$ = 1.57±0.12; NL$_D$ = 1.33±0.02; (GL+NL)$_D$ = 1.25±0.02 Where, D = Diabetic rats) and non-diabetic (GL$_{ND}$ = 1.33±0.48; NL$_{ND}$ = 1.42±0.61; (GL+NL)$_{ND}$ = 1.27±0.61) rats in this study with the exception of insulin treated non-diabetic rats which showed a non significant decrease compared to NC (3.38±0.32). The diabetic rats serum tetraiodothyronine (T$_4$) recorded a significant increase ($p<0.05$) in DC (8.07±0.26), while a non significant difference ($p>0.05$) was observed in treatment with NL singly and when combined with GL but GL alone (1.67±0.02) and insulin (1.87±0.04) treated rats indicated a significant decrease ($p<0.05$) in comparison with NC (2.68±0.27). In the non-diabetic rats, T$_4$ showed a significant increase in the individual treated extracts (GL$_{ND}$ = 3.30±0.06; NL$_{ND}$ = 3.30±0.10) while the combined extract group and insulin treated rats recorded a non significant increase compared to NC. There were significant decreases in serum insulin level of diabetic rats compared to NC (37.72±1.51) in both test (GL$_D$ = 16.63±2.95; NL$_D$ = 27.10±6.37; (GL+NL)$_D$ = 21.65±0.16) and control (DC = 13.33±0.02) groups but NL showed a non significant decrease, also there were non significant decreases in serum insulin of treated non-diabetic rats compared to NC but NL individual treatment (29.55±1.51) indicated a significant decrease ($p<0.05$) when compared to NC (37.72±1.51).

DISCUSSION

A type 1 model of diabetes such as was induced by STZ in this experiment is usually characterised by decreased circulatory insulin whose concentration parallels that of c-peptide. This was in consonance with this work; it is well established that c-peptide has a longer half-life of about 3-4 times that of insulin (Starr *et al.*, 1978; Champe *et al.*, 2005) and so can be retained in blood in higher concentrations compared to insulin. Infact, according to Karam and Forsham (1994), 100 pmol L^{-1} of c-peptide can still be found in humans plasma after an overnight fast despite the degrading of insulin by insulinase.

No significant changes in serum C-peptide levels were observed in both test and control animals, however, treatment with the combined extracts of GL and NL increased significantly ($p < 0.05$) the C-peptide levels both in diabetic and non-diabetic rats compared to their respective controls. The NL treatment reduced the diabetic increased C-peptide levels similar to NC, suggesting that NL may therefore contain some C-peptide mimetic phytochemicals, T_3 and T_4 concentration in serum of untreated diabetic rats were increased significantly. Often, T_3 and T_4 which are diabetogenic hormones increased in diabetes thereby sustaining and exerbating hyperglycemia (Brandenburg, 2008; Mayes, 2000) and usually, this is antagonistic to insulin. This probably may explain the significantly decreased T_3 and T_4 levels in insulin treated diabetic rats indicated in this study. This observation was similar to the report of Atangwho *et al.* (2010).

Treatment with extracts of GL in diabetic rats caused significant decrease in serum insulin similar to insulin treatment in diabetic rats as opposed to non significant increase in only NL treated diabetic and non-diabetic rats. These two extracts when combined reduced insulin levels compared to NC. GL may therefore contain some insulin mimetic phytochemicals, whose action was complemented when combined with extracts of NL and moreso further strengthens our proposition that GL and its combination with NL may have potent insulin mimetic action, since in these groups, T_3 concentration was significantly reduced just like insulin. Also, this was in consonance with the work of Atangwho *et al.* (2010). It also confirms the principle that polyherbal therapy is said to have the advantage of producing maximum therapeutic efficacy.

CONCLUSION

Treatment with combined extracts of GL and NL may therefore have the potentials to be substituted for insulin requirement in the management of type I diabetes.

REFERENCES

Atangwho, I.J., 2008. Biochemical impact of combined administration of extracts of Vernonia amygdalina and Azadirachta indica leaves on STZ diabetic rat models. Ph.D. Thesis, University of Calabar, Calabar.

Atangwho, I.J., P.E. Ebong, G.E. Egbung and A.U. Obi, 2010. Extract of *Vernonia amygdalina* del. (African bitter leaf) can reverse pancreatic cellular lesion after alloxan damage in the rat. Aust. J. Basic Applied Sci., 4: 711-716.

Brandenburg, D., 2008. History and diagnostic significance of C-peptide. Exp. Diabetes Res., Vol. 2008. 10.1155/2008/576862

Champe, P.C., R.A. Harvey and D.R. Ferrier, 2005. Biochemistry. In: Lippincott's Illustrated Reviews, Harvey, R.A. and P.C. Champe (Eds.). Lippincott Williams and Wilkins, USA.

Eastham, R.O., 1985. Biochemical Values in Clinical Medicine. 7th Edn., Wright Bristol, USA.

Focho, D.A., W.T. Ndam and B.A. Fonge, 2009. Medicinal plants of Aguamb-Bamumbu in the Lebialem highlands, South West Province of Cameroon. Afr. J. Pharmacy Pharmacol., 3: 001-013.

Gharib, H., R.J. Ryan, W.E. Mayberry and T. Hockett, 1971. Radioimmunoassay for triiodothyronine (T_3): Affinity and specificity of antibody for T_3. J. Clin. Endocrinol., 33: 509-516.

Ghoraishian, S.M., 2006. The effect of hazel-leaf decoction on blood glucose reduction in the diabetic rats. World J. Med. Sci., 1: 144-146.

Gidado, A., D.A. Ameh and S.E. Atawodi, 2005. Effects of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr. J. Biotechnol., 4: 91-93.

Gidado, A., Ameh, D.A., S.E. Atawodi and S. Ibrahim, 2008. Hypoglycaemic activity of *Nauclea latifolia* Sm. (Rubiaceae) in experimental animals. Afr. J. Tradition Complement Altern. Med., 5: 201-208.

Karam, J.H. and P.H. Forsham, 1994. Pancreatic Hormones and Diabetic Mellitus. In: Basic and Clinical Endocrinology, Greenspan, F.S. and J.D. Baxter (Eds.). Appleton and Lange, East Norwalk, Conn, USA .

Mayes, P.A., 2000. Gluconeogenesis and Control of the Blood Glucose. In: Harper's Biochemistry, Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell (Eds.). McGraw-Hill, New York, USA.

Nwanjo, H.U., 2005. Effect of aqueous extract of *Gongronema latifolium* leaf on blood glucose level in rats. Alvana J. Sci., 1: 84-89.

Nwanjo, H.U. and E.O. Alumanah, 2005. Effect of aqueous extract of *Gongronema latifolium* leaf on some indices of liver function in rats. Global J. Med. Sci., 4: 29-32.

Nwanjo, H.U., M.C. Okafor and G.U. Oze, 2006. Anti-lipid peroxidative activity of *Gongronema latifolium* in streptozotocin-induced rats. Nig. J. Physiol. Sci., 21: 61-65.

Okoli, R.I., O. Aigbe, J.O. Ohaju-Obodo and J.K. Mensah, 2007. Medicinal herbs used for managing some common ailments among esan people of edo state, Nigeria. Pak. J. Nutr., 6: 490-496.

Rao, P.V. and M.D. Naidu, 2010. Anti diabetic effect of *Rhinacanthusnasutus* leaf extract in streptozotocin induced diabetic rats. Libyan Agric. Res. Center J. Int., 1: 310-312.

Starr, J.I., M.E. Mako, D. John and A.H. Rubenstein, 1978. Measurement of serum proinsulin-like material: Cross-reactivity of porcine and human proinsulin in the insulin radioimmunoassay. J. Lab. Clin. Med., 91: 691-692.

Ugochukwu, N.H. and N.E. Babady, 2003. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Sci., 73: 1925-1938.

Ugochukwu, N.H., N.E. Babady, M. Cobourne and S.R. Gasset, 2003. The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. J Biosci., 28: 1-5.