European Journal of Medicinal Plants
3(2): 297-309, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antioxidant Enzymes Activity and Hormonal Changes Following Administration of Ethanolic Leaves Extracts of *Nauclea latifolia* and *Gongronema latifolium* in Streptozotocin Induced-Diabetic Rats

Grace S Effiong[2*], Bob IA Mgbeje[1], Godwin O Igile[1], Justin I Atangwho[1], Eyong U Eyong[1] and Patrick E Ebong[1]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria.
[2]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, P.M.B 1017, Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author PEE project conception and design, coordination and interpretation of data. Author GSE experimentation and acquisition of data; preparation of draft manuscript. Author JIA experimentation and acquisition of data; preparation of draft manuscript. Author GOI extraction and fractionation methodologies, statistical analysis, interpretation of data & coordination. Author BIAM graphics, analysis and interpretation of data, preparation of final manuscript and coordination. Author EUE experimental design, protocols and interpretation of data. All authors read and approved the final manuscript

Research Article

Received 30th December 2012
Accepted 2nd March 2013
Published 12th April 2013

ABSTRACT

Aim of the Study: To evaluate the effects of ethanolic leaf extracts of *Gongronema latifolium* (G.L) and *Nauclea latifolia* (N.L) on antioxidant enzymes activity (GPx, SOD and CAT) and hormonal status (T3, T4, Insulin, c-peptide) in streptozotocin-induced diabetic Wistar rats.
Material and Methods: Thirty six (36) albino Wistar rats of both sexes weighing 150-250g were divided into 6 groups of 6 rats each. Groups 1, 2 and 3 received 400mg/kg body

**Corresponding author: Email: graceffiong2007@yahoo.com;*

weight (b.w) of G.L, N.L and 200mg/kg b.w each of G.L and N.L respectively while group 4 received 5 iu/kg b.w of insulin subcutaneously daily for 21 days, Groups 5 and 6 served as controls (diabetic and Normal) and received placebo. Fasting blood glucose was determined at the start of the experiment and thereafter at 72 hours interval and at the end of experimental period. The animals were sacrificed and sera preparations were used for antioxidant enzymes and hormonal assays.

Results: Blood glucose in diabetic animals decreased significantly (*P*=.05) by 66.34%, 18.12%, 67.73% and 86.62% of initial values upon treatment with G.l, N.l, G.I plus N.I and insulin respectively. There was only a 24.44% decrease in the diabetic control. A significant decrease (*P*=.05) in insulin and T3 levels was observed in the diabetes-induced rats (65 and 85% respectively) compared to NC. The levels of the hormones where however significantly increased (*P*=.05) on treatment of the diabetic animals with G.l, N.l, G.I plus N.I and insulin. Whereas a significant decrease (*P*=.05) was observed in T4 level of DC rats compared to the NC, treatment with the leaf extracts and insulin did not result in any elevation of the hormone relative to DC. The C-peptide levels for all groups were much lower than the corresponding insulin levels, suggesting a type 1 diabetes in the diabetes-induced rats. A significant decrease (*P*=.05) in activity was observed for GPx and SOD in the DC group relative to NC. A combination of G.l and N.l gave a much higher reversal in activity (*P*<.01) when compared to treatments with individual leaf extracts. There was a significant increase (*P*=.05) in CAT activity in the DC animals relative to NC. This was potentiated in all treatment groups with the combination group showing a synergy in its potentiating effect.

Conclusion: There was a reversal in the level of the hormones and the activity of the antioxidant enzymes towards normal control, and comparable to the reversals by treatment with insulin, on treatment of the diabetic animals with the leaves extracts especially in combination. The results taken together indicate a synergy that makes the combination of the two plants extracts a potent antidiabetic remedy.

Keywords: Diabetes mellitus; Gongronema latifolium; Nauclea latifolia; hormonal indices; antioxidant enzymes; hyperglycemia; combined extract.

ABBREVIATIONS

STZ: Streptozotocin; G.l.: Gongronema latifolium*; N.l.:* Nauclea latifolia*; NC: Normal control; DC: Diabetic control; CEP: C-peptide; Ins: Insulin; T3: Tri-iodothyronine; T4: Tetra-iodothyronine; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase.*

1. INTRODUCTION

Diabetes mellitus is an important health problem affecting major populations worldwide. The World Health Organization (WHO) has reported that the global prevalence of diabetes will increase, from 2.8% in 2000 to 4.4% in 2030 [1]. Diabetes mellitus is characterised by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism [2-3]. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia [4-6]. Persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) from glucose

auto-oxidation and protein glycosylation [7-10]. The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by non-enzymic antioxidants and enzymic antioxidants like catalase (CAT), glutathione peroxidase (GPx) and super oxide dismutase (SOD) [10]. Enhanced oxidative stress, resulting from an imbalance between radical-generating and radical-scavenging systems, is currently suggested as a mechanism underlying diabetes and diabetic complications [10-11].

Current pharmacologic strategies in the treatment of Diabetes mellitus are aimed at management rather than prevention or cure. Leads provided by traditional medicine have indicated that natural products may be better treatments to ailments like diabetes than currently used conventional drugs [12]. Moreover, polyherbal therapies i.e. the combination of various types of agents from different plant sources, can be used to enhance efficacy given their synergistic, potentiative, agonistic/antagonistic pharmacological effects and minimum side effects [4,13]. It is in the light of this, with the hindsight of indigenous knowledge, that the current work on the antidiabetic properties of *Gongronema latifolium* and *Nauclea latifolia*, was undertaken.

Gongronema latifolium Benth. (*Asclepiadaceae*) is a perennial edible plant with soft and pliable stem. It is widely used in the West African sub-region for a number of medicinal and nutritional purposes [14]. In the Eastern states of Nigeria the plant, locally known as 'utazi', is a popular spice. The leaves have been reported to have antioxidant and antitussive properties [15], anti-ulcer, analgesic and antipyretic properties [16] and to affect some cardiac enzymes in diabetes-induced rats [17].

Nauclea latifolia Smith (syn. *Sarcocephalus latifolius*), a member of the family *Rubiaceae*, is commonly known as Pin cushion tree. It is a strangling shrub or small tree native to tropical Africa and Asia. *Nauclea latifolia* is used profusely by traditional medicine practitioners. The effect of leaf extract of N*auclea latifolia* on Serum electrolytes, lipid profile and cardiovascular activity have been reported [18]. The crude extract of the roots have been shown to have anti-hypertensive [19] and antidiabetic effects [20].

Despite the several investigations concerning the anti-diabetic action of these plants, report is scanty in the literature pertaining to their antihyperglycemic mechanisms of action and the effect of leave extracts on the activity of antioxidant enzymes and hormonal indices. The aim of this study was thus to evaluate the effect of ethanol extracts of *Gongronema latifolium* and *Nauclea latifolia,* singularly and in combination, on the activity of some antioxidant enzymes, hormone levels (serum insulin, c-peptide, tri [T3] and tetra-iodothyronine [T4]) and increased blood glucose level associated with diabetes.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh but matured *Gongronema latifolium* leaves were collected from a cultivated land at Ibiaku Itam in Akwa Ibom State, Nigeria, while *Nauclea latifolia* leaves was collected by the Industrial Training Fund (ITF) fence in Calabar, Nigeria. They were authenticated 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 dust particles and debris and thereafter allowed to completely drain. A portion of the leaves, about 1000g each, were taken for preparation of the plant extracts.

2.2 Preparation of Plant Extracts

The wet method of extraction as described in reference no. [13] was employed. One kilogramme of the fresh leaves of the plants, separately cut and chopped into pieces, was blended in 1.5 litres of ethanol (96%) with an electric blender. Homogenates were stored in amber coloured bottles in the dark at 4ºC for 72 hours. These were then filtered using a cheese material and thereafter with Whatman No 1 filter paper to obtain a homogenous filtrate. These filtrates were then concentrated *in vacuo* using a rotary evaporator at 37-40ºC to about one tenth the original volume. The concentrates were allowed open in a water bath at 40ºC to evaporate to dryness yielding 30.00g and 78.95g 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 2-8ºC until use.

2.3 Experimental Animals

Thirty six adult Wistar rats (150-200g) of either sex obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria, were used for the experiment. They were housed in polypropylene cages at room temperature under standard conditions (temperature 28±2ºC, relative humidity 50±5%) and maintained in a 12h light/dark cycle. The animals were allowed to acclimatize for two weeks.

2.4 Experimental Induction of Diabetes

Diabetes was induced in thirty of the rats, fasted overnight, by a single intraperitoneal injection of freshly prepared solution of streptozotocin (Sigma, USA) 50mgkg^{-1}b.w. in 0.1M cold sodium citrate buffer pH 4.5. The animals were considered as being diabetic if the blood glucose values, as estimated using One Touch Glucometer (Lifescan, California, USA) on blood obtained from the tail vein of the overnight fasted rats, were >200mg dL^{-1} on the third day after streptozotocin injection.

2.5 Experimental Design

Thirty six rats were divided into six groups of 6 rats each consisting of a diabetic (DC) and non-diabetic (NC) control group, three groups for treatment with each of the two plant extracts (G.l and N.l) and a combination of the two plants (G.I + N.I), and one group for treatment with insulin. Before use, the extracts were reconstituted in distilled water (vehicle) and administered orally via gastric intubations, at a dose of 400mgkg^{-1}b.w. for single extract treatment and 200mgkg^{-1}b.w. each in combined extracts treatment, twice daily (7.00 am: 7.00 pm). The dosage of the extract was determined from preliminary studies in our laboratory. Insulin was administered at a dose of 5Ukg^{-1}b.w.s.c once per day to simulate human regimens [21]. The controls received distilled water (placebo). The animals were maintained on pelletized Growers Feed obtained from Vital Feeds, Jos, Nigeria, and tap water. Both the feed and water were provided *ad libitum* and treatment lasted for 21 days. At the end of the 21 day period, the animals were fasted for 12h, then anaesthetized under chloroform vapour and dissected. Whole blood was obtained by cardiac puncture into sterile

plain tubes and was allowed to clot for about 2h and thereafter centrifuged (3,000g for 10min) to remove cells and recover serum which was used for the Biochemical assays.

2.6 Biochemical Investigations

Elisa kits used for c-peptide, tri- and tetra-iodothyronine (T3 and T4) assay were purchased from Monobind Inc. California, U.S.A. Kits for insulin assay was from DRG Instrument Germany. Triiodothyronine (T_3), Tetraiodothyronine (T_4) and the connecting-peptides (C – peptides) were determined using the ELISA method [22]. Measurement of serum levels of the oxidative stress enzymes, Glutathione (GPx), Super oxide Dismutase (SOD) and Catalase (CAT) was as described by Tietz [23].

2.7 Statistical Analysis

The results were analysed 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 and P values = 0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Effect of Treatment on Blood Sugar Level

Three days after Streptozotocin treatment, blood glucose of diabetic rats was significantly raised by 7-9 times the value of the normal control rats. However, at the end of 21-day treatment, blood glucose in diabetic animals decreased significantly (*P =.05*) by 66.34%, 18.12%, 67.73% and 86.62% of initial values upon treatment with *G.latifolium* (G.I), *N.latifolia* (N.I), *G.latifolium* and *N.latifolia* (G.I + N.I) and insulin respectively. There was only a 24.44% decrease in the diabetic control (Table 1). Thus, hyperglycaemia was ameliorated by treatment with *G.latifolium* and insulin, as measured by the percentage decrease in glucose concentration. The percentage decrease in blood sugar levels in *N. Latifolia* (18%) was lower than that of the diabetic control suggesting that the amelioration in the G.I plus N.I treatment (68%) may be due to the G.I (66%) effect alone.

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion or action or both [2,10,24,25] as is manifested in decreased serum insulin levels [26,27]. Loss of insulin effect leads to glycogenolysis, increase in glucose production and decreased cell utilization of glucose [24,28-29]. The reduction in the serum insulin levels in the STZ treated rats might be attributed to the reduced secretion of the hormone due to the damage of the beta cells of endocrine pancreas; STZ selectively destroys the pancreatic cells and induce hyperglycemia [30-33]. Glycemic control is important for the management of diabetes [2,34]. The antiglycemic activity of *G. latifolium*, comparable to that of insulin, makes it a good candidate for management of diabetes.

Table 1. Effect of treatment with *Gongronema latifolium*(G.l.), *Nauclea latifolia*(N.l.), a combination of the two plant extracts (G.l. + N.l.) and Insulin on blood glucose levels of diabetic rats (*P* =.05)

Treatment	Glucose concentration(mg/dl)		Percentage change in glucose concentration %
	Initial	Final	
G.l	504.00±30.54*, [a]	169.67±16.62[a]	66.34
N.l	406.67±27.68*, [a, c]	333.00±23.08*, [a, c]	18.12
G.l+N.l	502.67±2.76*, [a]	164.33±32.76[a]	67.73
Insulin(SD)	578.00±6.34*	77.33±10.36*	86.62
DC	292.00±21.05	214.67±14.42	26.43

**P=.05 vs DC; a = (P=.05) vs insulin; c = (P=.05) vs G.l+N.l.*
Each symbol and bar indicates the mean ± S.E.M. of six experimental rats in each group.

3.2 Effect of Treatment on Hormonal Level

Diabetic induction caused a significant decrease (*P =.05*) of insulin and T3 by 64.66% and 81.08% in diabetic control compared to the non-diabetic control (Fig. 1). Intervention with individual extract of G.l and N.l significantly increased (*P =.05*) the level of these indices by 25% and 145% respectively for G.l and 103% and 111% for N.l relative to diabetic control. However, the combined extract produced 62% and 98% increases in insulin and T3 levels relative to diabetic control. The increases in the insulin treated groups were 15% and 181% respectively relative to the DC group. The T4 value in the diabetic control was 23% less than the non diabetic control but comparable to the value after treatment with N.l and a combination of G.l and N.l. There was, however, a 20% and 10% decrease in T4 values in the G.l and insulin treatment groups compared with the diabetic control. Except for the G.l group with a 16% increase on the DC value, the C-peptide value was significantly lower (*P=.05*) in the other treatment groups, including the non diabetic control than the diabetic control. However the C-peptide levels for all groups were much lower than the corresponding insulin levels.

The increment of serum insulin levels in the treatment groups might be due to increased secretion of the hormone, inferring a possible 'repair' of the damaged insulin-secreting beta cells of the pancreas due to STZ. The decreases in T3 and T4 levels observed in the diabetic control is consistent with earlier report [35] were mean plasma T3 and T4 levels were significantly different between untreated diabetics and normal control subjects. Other workers [34,36] while showing T3 levels to be lower in non insulin dependent diabetes mellitus compared with the non diabetics reported no significant change in T4 levels between the two groups. Altered thyroid hormone level is a common feature in uncontrolled diabetes patients [34,37]. Endogenous production of glucose is thus further affected by the combination of hypothyroidism and diabetes [37-41].

A type 1 model of diabetes such as was induced by STZ in this experiment is usually characterised by decreased circulatory insulin whose concentration does not parallel that of c-peptide. A low level of c-peptide with a high blood glucose level is consistent with type 1 diabetes [42]. This is shown in our present results.

Fig. 1A

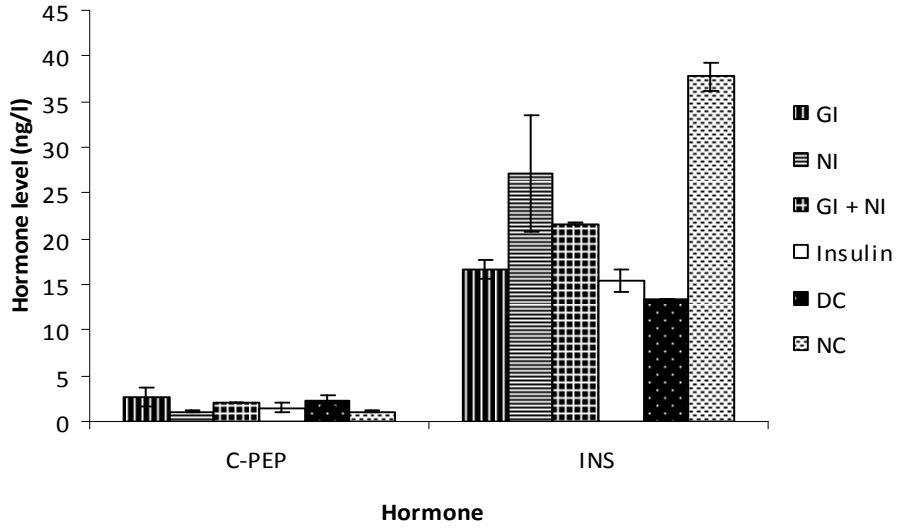

Fig. 1B

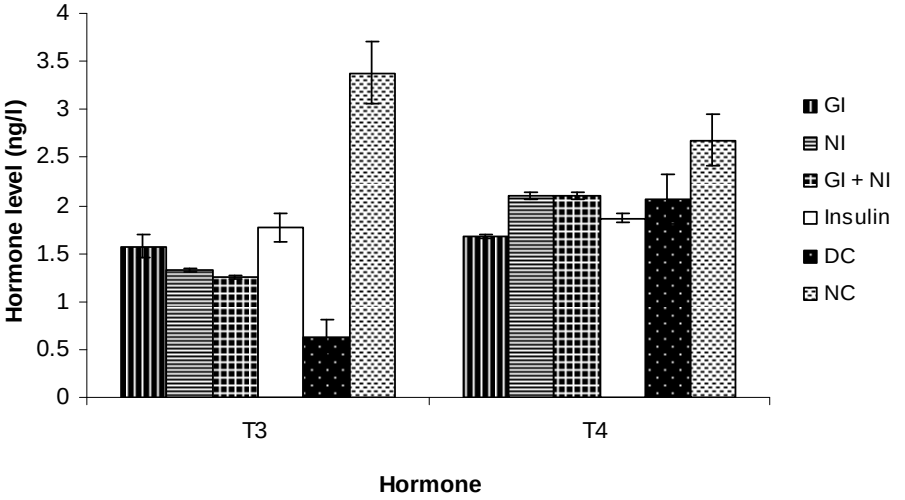

Fig. 1. Effect of treatment with *Gongronema latifolium* (G.l.), *Nauclea latifolia* (N.l.), a combination of the two plant extracts (G.l. + N.l.) and Insulin on hormonal levels of diabetic rats. (A) Insulin and C-peptide; (B) T3 and T4. Each symbol and bar indicates the mean ± S.E.M. of six experimental rats in each group (*P*=.05)

3.3 Effect of Treatment on Antioxidant Enzymes

The effect of treatment on antioxidant enzymes is shown in Fig. 2. The Glutathione peroxidase (GPx) activity in the DC group decreased by 66% relative to the non diabetic control. The activity level did not change significantly on treatment with G.l and insulin but increased significantly by 22% and 64% in the N.l and combination (N.l + G.l) groups respectively. The Super oxide dismutase (SOD) values were markedly depressed in all the

treatment groups relative to the non diabetic control and except for the N.l group that had a comparable value to the diabetic control, SOD values in the other treatment groups showed an 11-18% increase on the DC group. The Catalase (CAT) activity in the DC group was 34% more than the non diabetic group. Treatment with the plant extracts G.l, N.l and a combination of the two extracts resulted in 20%, 7.5%, and 78% increases respectively on the DC value. Treatment with insulin showed a 100% increase in CAT activity relative to DC.

Thus while the two antioxidant enzymes, Glutathione peroxidase (GPx) and super oxide dismutase were very much depressed in the diabetic groups relative to the non diabetic group, the activity of the catalase enzyme was elevated in the diabetic group relative to the non diabetic control.

An interesting observation to note is that while G.l treatment did not affect the level of GPx in the diabetic animal and increased by only 22% in the Nl treatment, a combination of the two plant extract potentiated the level of the antioxidant enzyme by as much as 64%, far in excess of the value for any of the individual treatment. In the same vein, while catalase activity in diabetic rats was increased only slightly by N.l treatment (7.5%) and G.l (20%), a combination of the two extracts increased the catalase activity by 78% almost comparable to the effect of insulin treatment (100%). *Clearly combinations of the two plant extract provide a synergy that has potent insulin mimetic action*. Thus a combination of the two plant extracts can be used as an alternative to insulin in reversing the depressed antioxidant enzyme activity in the diabetic rat.

While our studies show a decrease in GPx and SOD activities in DC animals compared to NC, other studies on the erythrocyte antioxidant enzymes [10,43] showed an increase in the two enzymes in DC relative to NC. The study by Taheri *et al*. [25] showed increased GPx activity but a reduced SOD activity in diabetic patient relative to normal patients. Other studies have shown both increase and decreases in SOD activity in erythrocytes in diabetic patients [25]. However antidiabetic therapy should enhance the levels of these enzymes [44] as was observed in our study. Oxidative stress is currently suggested as a mechanism underlying diabetes and diabetic complication [10,11,45-46]. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems i.e. increased free radical production or reduced activity of antioxidant defenses or both [10,47]. The activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals [48]. An increase in the activity of these enzymes after diabetic induction may be a compensatory response to oxidative stress whereas a decrease could be explained by a reduced activity of antioxidant defenses. There is a considerable body of evidence indicating that hyperglycemia may interfere with natural defense of the antioxidant system, in addition to increasing the production of free radicals [25,49]. Since SOD enzyme is part of the first line of defense against free radicals, it is expected that the activity of this enzyme may be affected by oxidative stress before the other antioxidant enzymes [44].There is evidence to show that hyperglycemia is accompanied by the loss of Cu^{2+}, which is an essential cofactor in SOD activity, and SOD is inactivated by glycosylation in erythrocytes [44].

Fig. 2A

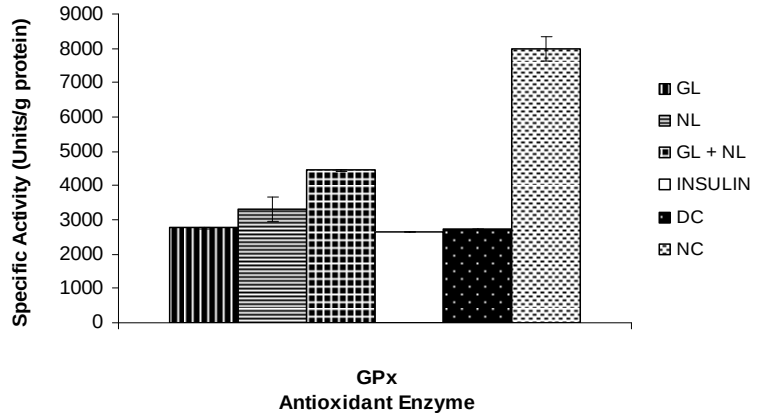

Fig. 2B

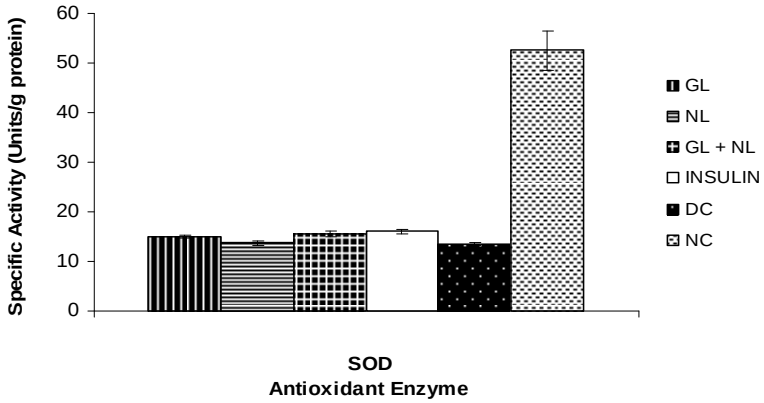

Fig. 2C

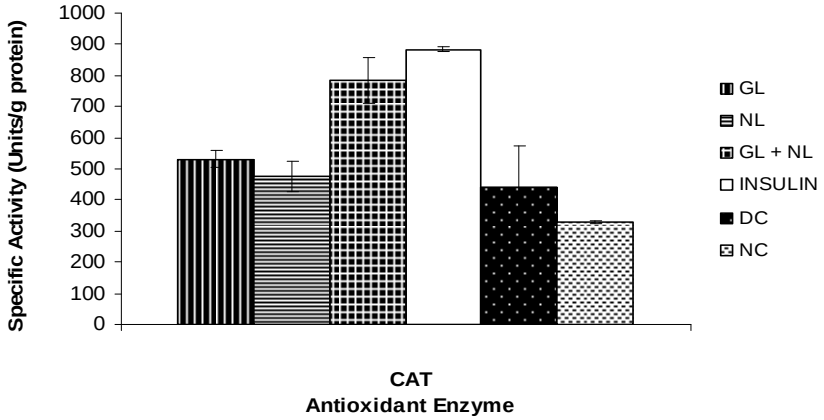

Fig. 2. Effect of treatment with *Gongronema latifolium* (G.l.), *Nauclea latifolia* (N.l.), a combination of the two plant extracts (G.l. + N.l.) and Insulin on Antioxidant enzyme activity. (A) GPx (B) SOD (C) CAT.

Each symbol and bar indicates the mean ± S.E.M. of six experimental rats in each group (P=.05)

4. CONCLUSION

In conclusion, the superior antiglycemic action of G.l., the far superior reversal of insulin level by N.l and the much higher activities of the antioxidant enzymes when the combination of the extracts are administered offer a synergy that make the combination of the two extracts a potent antidiabetic remedy and a possible alternative to current drugs used for the management of diabetes.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that the research has been determined exempt from review by the University animal research or ethics review committee and that the principles of laboratory animal care were followed.

ACKNOWLEDGEMENT

The Authors acknowledge support from the Science and Technology Education Post-Basic (STEP-B) Project CR:UN:UNI 4304.

COMPETING INTERESTS

The authors affirm that there is no conflict of interest in the publication of this article.

REFERENCES

1. Wild SH, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(10):2569.
2. ADA (American Diabetes Association). Diagnosis and classification of diabetes mellitus. Diabetes Care 2005;28(suppl. 1):S37–S43.
3. Pasupathi P, Bakthavathsalam G, Saravanan G, Sundaramoorthi, R. Screening for Thyroid Dysfunction in Diabetic/ Non-Diabetic Population. Thyroid Science. 2008;3(8):1-6.
4. Tiwari AK, Rao M. Diabetes mellitus and multiple therapeutic approaches of Phytochemicals; Present status and future prospects. Current Science. 2002;83:30-38.
5. Saravanan G, Pari L. Hypoglycaemic and Antihyperglycaemic effect of *Syzygium cumini* bark in streptozotocin-induced diabetic rats. Journal of Pharmacology and Toxicology. 2008;3:1-10.
6. Bobb A, Gale D, Manmohan S, Mohammed A, Seetathal F, Small P, Mungrue K. The Impact of the chronic disease assistance plan (CDAP) on the control of type 2 diabetes in Trinidad. Diabetes Research in Clinical Practice. 2008;80:360-364.
7. Aragno M, Tamagno E, Gato V, Brignardello E, Parola S, Danni O, Boccuzzi G. Dehydroepiandrosterone protects tissues of streptozotocin-treated rats against oxidative stress. Free Radic. Biol. Med. 1999;26(11/12):1467–1474.

8. Bonnefont Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab. 2000;26:163–176.
9. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J. Biol. Chem. 2004;279(41**)**:42351–42354.
10. Moussa SA. Oxidative stress in diabetes Mellitus. Romanian J. Biophys. 2008;18(3):225-236.
11. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine, 2nd edition. Oxford: Clarendon Press; 1989.
12. Rates SM. Plants as source of drugs. Toxicon. 2001;39(5):603-613.
13. Ebong PE, Atangwho JI, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss)(Neem) and *Vernonia amygdalina* (Del.)(African bitter leaf). American Journal of Biochemistry and Biotechnology. 2008;4(3):239-244.
14. Nwanjo HU, Okafor MC, Oze GO. Anti-lipid peroxidative activity of *Gongronema latifolium* in Streptozotocin-induced Diabetic Rats. Nigerian Journal of Physiological Sciences. 2006;21(1-2):61-65.
15. Essien JP, Ebong, GA, Akpan, EJ. Antioxidant and Antitussive Properties of Gongronema latifolium leaves used Locally for the treatment of fowl Cough in Nigeria. Journal of Applied Sciences in Environmental Management. 2007;11(4):47-50.
16. Akuodor GL, Idris-Usman MS, Mbah CC, Megwas UA, Akpan JL, Ugwu TC, Okoroafor DO, Osunkwo, UA. Studies on anti-ulcer, analgesic and antipyretic properties of the ethanolic leaf extract of *Gongronema latifolium* in rodents. African Journal of Biotechnology. 2010;2316-2321. DOI: 10.5897/AJB10.1563
17. Edet EE, Akpanabiatu MI, Eno AE, Umoh IB, Itam EH. Effect of *Gongronema latifolium* crude leaf extract on some cardiac enzymes of alloxan-induced diabetic rats. African Journal of Biochemistry Research. 2009;3 (11):366-369.
18. Akpanabiatu MI, Umoh IB, Udosen EO, Udoh AE, Edet EE. Rat serum electrolytes, lipid profile and cardiovascular activity on *Nauclea latifola* leaf extract administration. Indian Journal of Clinical Biochemistry. 2005;20(2):29-34.
19. Nworgu ZA, Ejerakeya AE, Onwukaeme DN, Afolayan AJ, Ameachina FC, Ayinde BA. Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. African Journal of Pharmacy and Pharmacology. 2008;2(2):37-41.
20. Gidado AI, Danladi AA, Sunday EA, Sani II. Antidiabetic effect of *Nauclea latifolia* ethanolic leaf extract in STZ-induced diabetic Rats. Pharmacology Research. 2009;1:392-395.
21. Sonia B, Scrinivasan BP. Investigations into the anti-diabetic activity of *Azadirachta indica*. Journal of Pharmacology. 1999;31:138-141.
22. Ashby, J and Frier B. Circulating C-peptide: measurement and clinical applications. Annals of clinical Biochemistry. 1981;18(3):125-30.
23. Tietz, NW. Fundamentals of clinical chemistry, 3rd edition. Philadelphia: W.B Saunders Company; 1987:448-481, 517-532, 614-704, 729-761.
24. Harris EH. Elevated liver function tests in type 2 diabetes. Clinical Diabetes. 2005;23(3):115-119.
25. Taheri T, Djalali M, Saedisomeolia A, Moghadam AM, Djazayeri A, Qorbani M. The relationship between the activates of antioxidant enzymes in red blood cells and body mass index in Iranian type 2 diabetes and healthy subjects. Journal of Diabetes & Metabolic Disorders. 2012;11:3. doi:10.1186/2251-6581-11-3.
26. Mohammad Ali E, Razeih Y. Hypoglycemic effect of *Teucrium polium* studies with rat pancreatic islets. Journal of Ethnopharmacology. 2004;95:27-30.

27. Gohil T, Pathak N, Jivani N, Devmurari V, Patel J. Treatment with extracts of *Eugenia Jambolana* seed and *Aegle marmelos* leaf extracts prevents hyperglycemia and hyperlipidemia in alloxan induced diabetic rats. African Journal of Pharmacy and Pharmacology. 2010;4(5):270-275.
28. Briscoe VJ, Davis SN. Hypoglycemia in type 1 and type 2 diabetes: Physiology, pathophysiology and management. Clin Diabetes. 2006;24:115-21.
29. Guyton AC, Hall JE. Textbook of Medical Physiology, 11th edition. New Delhi: Elsevier. 2006:972-7.
30. Yoon JW, Ray UR. Perspectives on the role of viruses in insulin-dependent diabetes. Diabetes Care. 1985;1:39-44.
31. Gilman AG, Rall TW, Nles, AS, Tayer, P. The pharmacological basis of therapeutics,8th edition. New York: Pergamon press. 1990:1317-1322,1463-1495.
32. Jarvenin YH. Role of insulin resistance in the pathogenesis of NIDDM. Diabetologia. 1995;38:1378-1388.
33. Kurup S, Bhonde RR. Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatictomized adult BALB/C mice. Hormone Metabolism Research. 2003;32(8):330-334.
34. Islam S, Yesmine S, Khan SA, Alam NH, Islam S. A comparative study of thyroid hormone levels in diabetic and non-diabetic patients. The Southeast Asian Journal of Tropical Medicine and Public Health. 2008;39(5):913-916.
35. Saunders J, Hall SHE, Sonksen PH. Thyroid hormones in insulin requiring diabetes before and after treatment. Diabetologia. 1978;15:29-32.
36. Schlienger JL, Anceau A, Ghabrier G, North ML, Stephan F. Effect of diabetic control on the level of circulating thyroid hormones. Diabetologia. 1982;22:486-8.
37. Wu P. Thyroid disease and diabetes. Clin Diabetes. 2000;18:38.
38. Bernal J, Refetoff F. The action of thyroid hormone. Clinical Endocrinol. 1977;6:227-249.
39. Nortabartolo A, Rini G, Licarta G, Scaglione R, Di Fede G,Averna MR, Montalto G, Butturini U. Correlation between different degree and duration of metabolic control and thyroid hormone levels in type 1 and type 2 diabetes. Acta Diabetol. 1983;20:341-6.
40. Karam JH, Forham PH. Pancreatic hormones and diabetes mellitus. In: Greenspan FS, Baxter, JD, editors. Basic and Clinical Endocrinology, 4th edition. USA: Appleton and Lange. 1994:571-634.
41. Mayes PA. Glucogenesis and control of the blood glucose. In Harper's Biochemistry, 25th edition. New York: McGraw-Hill. 2003:208-218.
42. Argoud GW, Schade DS, Eaton RP, Sterling WA. C-peptide suppression test and recurrent insulinemia. Amer J Med. 1989;86(3):335-7.
43. Pasaoglu H, Sancak B, Bukan N. Lipid peroxidation resistance to oxidation in patients with type 2 diabetes mellitus. Tohoku J Exp Med. 2004;203:211 -218.
44. Hamden K, Carreau S, Jamoussi K, Miladi S, Lajmi S, Aloulou D, Ayadi, F, Elfeki, A. 1 Alpha, 25 dihydroxyvitamin D3: therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in alloxan-induced diabetes in rats. J Nutr Sci Vitaminol. 2009;55(3):215–222.
45. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991;40:405–412.
46. Ceriello A. Oxidative stress and glycemic regulation. Metabolism. 2000;49:27–29.
47. Strain JJ. Disturbances of micronutrient and antioxidant status in diabetes. Proceedings of the Nutrition Society. 1991;50:591–604.
48. Soto C, Recoba R, Barron H, Alvarez C, Favari L. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comparative Biochemistry and Physiology. 2003;136:205–212.

49. Karasu C. Glycoxidative stress and cardiovascular complications in experimentally induced diabetes: effects of antioxidant treatment. Open Cardiovasc Med J. 2010;4:240.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=195&id=13&aid=1252