Full Length Research Paper

Biochemical effects of leaf extracts of *Gongronema latifolium* and selenium supplementation in alloxan induced diabetic rats

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This study evaluated the effect of selenium and extracts of *Gongronema latifolium* on some biochemical parameters in alloxan-induced diabetic rats. Forty male albino rats equally divided into eight experimental groups were used. Two groups served as normal and diabetic control and received placebo treatment. Four diabetic test groups were treated with *G. latifolium* extract, glibenclamide, combination of *G. latifolium* extract and selenium, and selenium, respectively. Two non-diabetic test groups were treated with *G. latifolium* extracts and selenium, respectively. Treatment lasted for 28 days after which the rats were sacrificed and blood collected for biochemical evaluation. Variations in animal weights were also measured within the period of study. The results showed that treatment with combination of *G. latifolium* extract and selenium significantly reduced (P < 0.05) the weight, aspartate transaminase activity and glucose levels and increased superoxide dismutase activity, glutathione-S-transferase activity, albumin and protein levels. Treatment with selenium alone significantly increased catalase activity. There were no significant differences in alanine aminotransferase, alkaline phosphatase and lipid peroxidation.

Key words: Diabetes mellitus, *Gongronema latifolium*, selenium, alloxan.

INTRODUCTION

Diabetes mellitus is a non-communicable metabolic disorder characterized by hyperglycaemia due to overproduction and underutilization of glucose (Ugochukwu et al., 2003; Das and Elbein, 2006; Dharmeshkumar et al., 2008; Srinivasan et al., 2008). Diabetes tends to damage cell membranes which results in elevated production of reactive oxygen species (ROS). The generation of ROS appears to play a critical role in the pathogenesis of diabetes mellitus (Harnett et al., 2000). Hyperglycemia associated with diabetes also increases the production of ROS and affects antioxidant enzymes and reactions (Uchimura et al., 1999; Kowluru et al., 2000; Haskins et al., 2004).

Plants are sources of potential therapeutic agents against various diseasess due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites (Farombi, 2003). The use of medicinal plants in management of diseases is as old as mankind and is still an important alternative therapy widely employed in developing countries. Several

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investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes (Roja and Rao, 2000; Jung et al., 2006; Malviya et al., 2010; Rao et al., 2010). The anti-diabetic properties of several plants including cinnamon used in treatment and management of diabetes are generally due to various phytochemicals such as polyphenols, catechins, saponins and flavonoids (Bnouham et al., 2006; Khan et al., 2003). Studies have also revealed enhanced efficacy and potency of combination of various anti-diabetic agents in treatment of diabetes (Ebong et al., 2011).

Tropical plants elaborate diverse phytochemicals that are medicinally useful especially in the management of diabetes (Iweala and Okeke, 2005; Pandhare et al., 2012). Amongst them is *Gongronema latifolium* whose individual and synergistic anti-diabetic effects have been reported (Atangwho et al., 2010). *G. latifolium* is a perennial edible shrub of the family, Asclepiadaceae widely employed in Nigeria for various medicinal and nutritional purposes (Ugochukwu et al., 2003; Morebise et al., 2002). Scientific studies have established the hypoglycaemic, cardio-protective, hypolipidaemic, anti-inflammatory and antioxidative effects of aqueous and ethanolic extracts of *G. latifolium* leaf (Edet et al., 2009; Ugochukwu et al., 2003; Ogundipe et al., 2003; Morebise et al., 2002). Some bioactive phytochemicals found in *G. latifolium* which may contribute to its anti-diabetic property include β-sistosterol, lupenyl esters, pregnane ester, glycosides, essential oils and saponins (Ekundayo, 1980; Morebise et al., 2002).

Selenium is a naturally occurring trace mineral required to maintain good health (Marcason, 2008; Rayman, 2008). Selenium is a key component of a number of selenoproteins involved in essential enzymatic functions such as redox homeostasis, thyroid hormone metabolism, immunity and reproduction (Burk, 1998; Diplock, 1994). The redox homeostatic function helps to maintain membrane integrity, protect prostacyclin production and reduce the likelihood of propagation of further oxidative damage to biomolecules which is usually associated with increased risk of diseases (Neve, 1996).

Alloxan, an oxygenated pyrimidine derivative is a toxic glucose analogue, which selectively destroys insulin-producing beta cells in the pancreas when administered to rodents and many other animal species (Lenzen, 2008). This causes an insulin-dependent diabetes mellitus with characteristics similar to type 1 diabetes in humans. Hence, alloxan-induced diabetes in rats is a good experimental model to study diabetes (Szkudelski, 2001).

The established individual roles of *G. latifolium* and selenium in treatment and management of diabetes informed the objective of this preliminary study which was to evaluate the effect of combined administration of both on some biochemical indices of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection of plant sample and preparation of extracts

Leaves of *G. latifolium* were bought from a local market in Ota, Ogun state, Nigeria and identified by a qualified plant taxonomist. The leaves were picked and left to dry at tropical room temperature. The dried leaves were picked and ground into a coarse powder using a hammermill. Five hundred grams (500 g) of *G. latifolium* was soaked in 2.5 L of 80% ethanol for 48 h. The mixture was sequentially filtered with chess cloth and Whatman's paper (No.1). The final filtrates were concentrated to a fifth of their original volume in a rotary evaporator under reduced pressure at 40°C and later evaporated to a dried residue in a water bath. The extraction gave a yield of 38 g of crude extract.

Animal procurement and housing

A total of forty male wistar rats aged between 4 to 8 weeks and weighing between 110 to 160 g were used in this study. The rats were obtained from the animal house of the University of Agriculture, Abeokuta (UNAAB), Ogun state, Nigeria and were allowed to acclimatize for three weeks prior to the commencement of the experiments in the animal laboratory of the department of Biological sciences, Covenant University Ota, Ogun state. They were kept in well ventilated and clean cages at an average room temperature of 30°C and their beddings changed every two days. The rats were allowed free access to tap water and fed a standard rat chow throughout the period of the experiment. All the processes involved in the handling and experiment were carried out according to standard protocols approved by the animal ethics committee of the Department of Biological sciences, Covenant University, Ota.

Experimental diabetes Induction

The rats were subjected to a 12 h fast and diabetes induced by intra-peritoneal injection of 150 mg/kg body weight alloxan hydrate (Sigma-Aldrich, U.S.A) reconstituted in normal saline. Three days later, diabetes was confirmed in the alloxan-treated rats that had fasting blood glucose level (FBGL) of 200 mg/dl and above. FBGL was estimated using Fine test glucometer, with blood obtained from the tail vein of the rats.

Experimental design

A total of forty rats were randomly divided into eight groups of five rats each. The normal (NC) and diabetic control (DC) groups received normal saline. Four groups namely GLE, GLI, GLS and SEL were made up of diabetic rats. GLE group was treated with 200 mg/kg body weight of *G. latifolium* extract, GLI group received glibenclamide (an anti-diabetic drug), GLS group was given *G. latifolium* extract (200 mg/kg body weight) and selenium supplement, and SEL group was given selenium supplement only. Two groups namely GLN and SLN were non-diabetic and treated with *G. latifolium* extract (200 mg/kg body weight) and selenium, respectively. Treatments were daily administered on a 12 hourly

basis by orogastric intubation for 28 days. At the end of 28 days, the rats were fasted overnight, euthanized under diethyl ether and sacrificed. Organs including pancreas, liver, and kidney were excised, cleaned by blotting with filter paper and fixed in 10% formyl saline for histological examination. Whole blood was collected by cardiac puncture with sterile needles, placed into sterile tubes and allowed to clot for about 3 h. Clotted blood was centrifuged at 3,000 rpm for 10 min and the serum obtained was pooled. The animal grouping and treatment schedule are shown in Table 1.

Biochemical determinations

Glucose determination

This test was carried out using a glucose enzymatic-colorimetric test kit (GOD-POD), produced by Cypress diagnostics (Belgium). The test principle is based on the oxidation of glucose by glucose oxidase (GOD) to gluconic acid and hydrogen peroxide. The hydrogen peroxide (H_2O_2) forms a red violet color with a chromogenic oxygen acceptor, phenolaminophenazone in the presence of peroxidase (POD). The colour intensity is proportional to glucose concentration in the sample.

Total protein determination

This test was carried out using a total protein test kit produced by Randox laboratories. The test principle involves formation of a coloured complex between cupric ions in alkaline medium with peptide bonds. The intensity of the colour is proportional to the concentration of protein.

Assay for Liver enzymes

Alanine aminotransferase (ALT), Aspartate transferase (AST), and Alkaline phosphatase (ALP) tests were carried out using ultra violet (UV) kinetic test kits produced by Cypress diagnostics. The test is based on photometric determination of rate of nicotinamide adenine dinucleotide (NADH) consumption by pyruvate and oxaloacetate which is directly related to ALT and AST activities, respectively.

Determination of albumin

This test was carried out using an albumin test kit produced by Cypress diagnostics. The measurement of serum albumin is based on its quantitative binding to bromocresol green (BCG). The albumin-BCG-complex absorbs maximally at 578 nm and the absorbance is directly proportional to the concentration of albumin in the sample.

Determination of lipid peroxidation

Lipid peroxidation was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Buege and Aust (1978).

Assay of antioxidant enzymes

Catalase (CAT) activity was determined according to the method described by Sinha (1972). SOD activity was determined according to the method described by Zou et al. (1986). Glutathione-S-transferase (GST) activity was determined according to the method described by Habig et al. (1974).

Statistical analysis

The results are presented as mean ± standard error of mean (SEM) and were analysed for statistical significance by one-way analysis of variance (ANOVA). The values with $p < 0.05$ were considered statistically significant.

RESULTS

Body weight, blood glucose, total protein and albumin concentration

Table 2 shows reductions in body weight in GLE (diabetic rats treated with 200 mg/kg body weight of *G. latifolium* extract), GLS (diabetic rats treated with 200 mg/kg body weight of *G. latifolium* extract and selenium supplement), GLN (Non-diabetic rats and treated with 200 mg/kg body weight of *G. latifolium* extract) and SLN (Non-diabetic rats and treated with selenium) groups. There were also significant reductions in the glucose concentration in GLI, GLS, SEL and SLN against the diabetic control. There were no significant changes in the total protein concentration of the test groups as compared to the diabetic control except GLS. There were significant increases in albumin concentrations of GLE and GLS groups.

Activities of liver enzymes

Table 3 shows that the changes in alanine aminotransferase and alkaline phosphatase activities recorded for the test groups were not significantly different from the controls. However, aspartate aminotransferase in GLE, GLI, GLS and SEL groups were significantly reduced.

Lipid peroxidation level and activities of antioxidant enzymes

The results in Table 4 show that lipid peroxidation levels were not significantly different in the test groups except in GLE, where it was increased. There were significant increases in glutathione-S-tranferase activity in GLE, GLI and SEL as compared to the diabetic control. Superoxide dismutase activity was significantly increased in all the diabetic test groups. The results also showed that catalase activity was significantly increased in the SEL group.

Table 1. Animal grouping and treatment.

Group	Class	Number of rats	Treatment	Dosage
NC	Normal control	5	Normal saline	0.1ml
DC	Diabetic control	5	Normal saline	0.1ml
GLE	Diabetic, *G. latifolium* extract treated	5	*G. latifolium* extract	200 mg/kg body weight
GLI	Diabetic, Glibenclamide treated	5	Glibenclamide	-
GLS	Diabetic, *G. latifolium* extract and selenium treated	5	*G. latifolium* extract and selenium	200 mg/kg body weight and 100 mg/kg body weight
SEL	Diabetic, selenium treated	5	Selenium	100 mg/kg body weight
GLN	Non diabetic, *G. latifolium* extract treated	5	*G. latifolium* extract	200 mg/kg body weight
SLN	Non diabetic, selenium treated	5	Selenium	100 mg/kg body weight

Table 2. Blood glucose and total protein levels in control and test groups.

Groups	Class	Body weight change (g)	Blood glucose (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
NC	Normal control	4.00±3.41	66.51±8.79	11.38±0.69	2.06±0.28
DC	Diabetic control	10.00±0.00	176.32±0.00	10.70±0.00	1.79±0.00
GLE	Diabetic, *G. latifolium* extract treated	-8.00±18.76[a]	105.23±10.87[a]	10.59±0.64	2.50±0.07[b]
GLI	Diabetic, glibenclamide treated	40.00±0.00[b]	67.86±0.00[a]	9.25±0.00[a]	2.21±0.00
GLS	Diabetic, *G. latifolium* extract and selenium treated	-54.00±0.00[a]	49.89±0.00[a]	12.67±0.00[b]	2.32±0.00[b]
SEL	Diabetic, selenium treated	0.00±10.00	59.73±10.04[a]	11.93±0.02	1.78±0.05
GLN	Non diabetic, *G. latifolium* extract treated	-10.50±23.75[c]	62.53±5.88[c]	11.32±0.58	3.80±0.23[d]
SLN	Non diabetic, selenium treated	-26±8.51[c]	46.58±8.30[c]	11.60±1.20	1.51±0.13[c]

[a]significant reduction with respect to diabetic control; [b]significant increase with respect to diabetic control; [c]significant reduction with respect to normal control; [d]significant increase with respect to normal control.

DISCUSSION

Diabetes mellitus is a disease condition characterised by alterations in carbohydrate, lipid and protein metabolism (Das et al., 1996). The management of diabetes mellitus is considered a global problem because a successful and effective treatment is yet to be discovered. Most of the modern anti-diabetic drugs, including insulin and oral hypoglycaemic agents only control blood sugar levels as long as they are regularly administered and are associated with a number of undesirable effects (Upadhyay et al., 1996; Cheng and Caughey, 2007). This generates the need for better, convenient and less toxic treatment options. The treatment of diabetes mellitus has been attempted with different indigenous plants and polyherbal formulations (Chandel et al., 2011).

The results from this study revealed significant loss of weight of untreated diabetic rats compared to non- diabetic animals. This is attributed to the

Table 3. Activities of liver enzymes in control and test groups.

Groups	Class	ALT (mg/dl)	AST (mg/dl)	ALP (mg/dl)
NC	Normal control	3.68±0.93	15.93±5.61	2.64±0.40
DC	Diabetic control	7.86±0.00	59.5±0.00	1.65±0.00
GLE	Diabetic, *G. latifolium* extract treated	9.84±3.94	12.03±5.48[a]	2.2±0.55
GLI	Diabetic, Glibenclamide treated	7.86±0.00	11.38±0.00[a]	3.3±0.00
GLS	Diabetic, *G. latifolium* extract and selenium treated	13.13±0.00	18.38±0.00[a]	3.3±0.00
SEL	Diabetic, selenium treated	7.44±4.81	87.5±32.38[b]	4.13±2.48
GLN	Non diabetic, *G. latifolium* extract treated	12.47±4.26	71.97±28.59[d]	3.3±0.95
SLN	Non diabetic, selenium treated	10.79±7.77	7.00±1.75[c]	2.48±0.83

[a]significant reduction with respect to diabetic control; [b]significant increase with respect to diabetic control; [c]significant reduction with respect to normal control; [d]significant increase with respect to normal control.

Table 4. Lipid peroxidation levels and activities of antioxidant enzymes in control and test groups.

Groups	Class	Lipid peroxidation (Mol/L)	CAT (Units/L)	SOD (Units/L)	GST (Units/L)
NC	Normal control	5.31±0.67	155.63±3.52	0.14±0.08	1.76±0.90
DC	Diabetic control	3.27±0.00	159.99±0.00	0.01±0.00	0.07±0.03
GLE	Diabetic, *G. latifolium* extract treated	7.45±0.84[b]	181.15±11.49	0.12±0.06[b]	0.80±0.21[b]
GLI	Diabetic, Glibenclamide treated	3.85±0.00	154.47±0.00[a]	0.11±0.00[b]	0.48±0.00[b]
GLS	Diabetic, *G. latifolium* extract and selenium treated	6.41±0.00	148.73±0.00[a]	0.09±0.04[b]	0.24±0.00[a]
SEL	Diabetic, selenium treated	4.13±0.99	202.52±45.34[b]	0.09±0.04[b]	2.16±0.72[b]
GLN	Non diabetic, *G. latifolium* extract treated	6.17±0.54	163.21±45.34[d]	0.07±0.05	1.62±0.40
SLN	Non diabetic, selenium treated	7.06±0.23	162.92±4.82[d]	0.11±0.02	1.32±0.12

[a]significant reduction with respect to diabetic control; [b]significant increase with respect to diabetic control; [c]significant reduction with respect to normal control; [d]significant increase with respect to normal control.

loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids (Granner, 1996). Consumption of *G. latifolium* is not readily associated with increase in weight (Iweala and Obidoa, 2009). Oral administration of extracts of *G. latifolium* and selenium supplementation and their co administration had a hypoglycaemic effect in the diabetic and non-diabetic rats. The group that was administered the anti diabetic drug, glibenclamide, showed a reduction in blood glucose level due to its insulin- stimulating actions on the beta cells of the pancreas (Srinivasan et al., 2008). The enhanced reduction in blood glucose by combined action of *G. latifolium* extract and selenium supplementation may be attributed to the action of selenium which is an insulin mimetic and possibly the ability of the plant extract to alter the inhibitory activity of alloxan on glucokinase which is the glucose sensor of the beta cells (Stapleton, 2000; Steinbrenner et al., 2011). Generally selenium has been found to potentially promote an overall improvement in islet function (Campbell et al., 2008)

Assay for liver enzymes namely ALT, AST and ALP is important in assessing optimal liver function during diabetes. Increase in the level of liver enzymes in the plasma is an indication of liver dysfunction (Dame, 1981). The increase in ALT and ALP were not statistically different from the control group, indicating possible hepato-protective effect of the plant extracts. The group that was administered *G. latifolium* and selenium supplement showed a significant reduction in AST levels as compared to the diabetic control. AST is not a good indicator of liver dysfunction and this further substantiates the possible hepato protective effects of co-administration of the plant extracts and selenium in diabetes.

Total protein and albumin levels were not statistically different between the test groups and the control groups. This could be attributed to low protein content of *G. latifolium* (Atanghwo et al., 2009). However, the increase in total protein and albumin in the group treated with *G. latifolium* and selenium supplement further supports a possible preservation of liver function.

Lipid peroxidation which is one of the characteristic features of chronic diabetes was not significantly changed possibly due to its stabilization by the antioxidant

components of *G. latifolium* and selenium (Lyons, 1991). Catalase which is known to scavenge and detoxify hydrogen peroxide showed a reduced activity in the diabetic control due to increased oxidative stress associated with diabetes. The increase in the activity of catalase in the rats treated with the *G. latifolium* extract and selenium supplement agrees with the study carried out by Atanghwo et al. (2009). *G. latifolium* contains flavonoids and polyphenols which are antioxidants that could bring about reduction in oxidative stress (Iwueke et al., 2010). The results suggest that selenium plays important roles in quenching reactive oxygen species and reduces the oxidative stress associated with diabetes (Diplock, 1994; Mukherjee et al., 1998).

The treatment with glibenclamide elicited a negligible reduction in catalase activity as compared with the co-administration of the plant extract and selenium. The increased activity of SOD and glutathione-S-transferase in the groups administered plant extracts and selenium further confirms their high antioxidant capacities (Ogundipe et al.*,* 2003). Increased activity in SOD by *G. latifolium* has been reported by other studies (Ugochukwu and Babady, 2002; Iweala and Obidoa, 2009). SOD is the major enzyme involved in scavenging of reactive oxygen species (Mahdi, 2002). The increase in the activity of SOD suggests this process as one of the mechanisms by which *G. latifolium* and selenium produces their synergistic anti-diabetic actions.

The increase in GST can be attributed to phyto-chemicals, especially flavonoids and anthocyanidins in *G. latifolium* which induce detoxification enzymes through up regulation of their genes by interacting with antioxidant response elements (ARES) (Birt et al., 2001; Ren et al., 2003; Ross and Kasum, 2002; Ferguson, 2001). The ability of substances to induce GST is due to possession of electrophilic centers that are able to react with sulfhydryl groups through oxido-reduction or alkylation (Prestera et al., 1993). An increase in GST activity translates to increased capacity to conjugate and excrete toxic intermediates that can cause diseases such as diabetes (Pantuck et al., 1984).

The results obtained from this study indicate that the combined administration of *G. latifolium* and selenium supplementation have superior blood glucose level lowering effects comparable to standard anti-diabetic drugs such as glibenclamide (Ugochukwu et al., 2003). This superior effect is possible due to synergism usually associated with bioactive compounds from medicinal plants and other agents (Tiwari and Rao, 2002; Asuquo et al., 2010). The results indicate the beneficial effect of micronutrients combination in controlling diabetic hyper-glycemia (Aly and Mantawy, 2012). The combined administration also showed promises in effective amelioration of complications associated with diabetes which are linked to oxidative stress, liver dysfunction and

lipid peroxidation (Atangwho et al., 2010). This will influence the pattern of use of herbal medicine by diabetic patients (Egede et al., 2002).

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