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Protective role of diets containing *Gongronema latifolium* leaves on Streptozotocin- induced oxidative stress and liver damage

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ABSTRACT

This study was aimed at determining some biomarkers of oxidative stress and liver damage in diabetic rats consuming diets containing *Gongronema latifolium* leaves so as to evaluate the involvement of the diets in the management of oxidative stress and liver damage common among diabetics. Fifty rats were randomly divided into five groups (1 to 5) with10 rats per group. Group 1 (normal control) consumed control diet; Group 2 (diabetic control) consumed control diet, Group 3 and 4 (diabetic treated) consumed *Gongronema latifolium* at 5% and 7.5%, respectively. Group 5 (diabetic treated) consumed control diet and was treated with Insulin. Feed and water were given ad- libitum for 28 days. Results showed that diabetic rats in groups 3 and 4 consuming *Gongronema latifolium* had significant ($P<0.05$) reduction in MDA concentration and in the level of ALT, AST, and ALP in the serum and liver tissue homogenate and a significant increase it the activities of GPx, SOD and Catalase relative to the diabetic control. The results were superior to those on Insulin. It was concluded that consumption of diets containing *Gongronema latifolium* leaves has protective effect on oxidative stress and liver damage associated with diabetes mellitus.

INTRODUCTION

Oxidative stress is a condition of excess formation and insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Cade, 2008; Giacco and Brownlee, 2010) Oxidative stress plays a pivotal role in the development of diabetes mellitus complications, both microvascular and cardiovascular (Cade, 2008).

The metabolic abnormalities of diabetes mellitus cause mitochondrial superoxide overproduction (Cade, 2008). This increased superoxide production is the central and major mediator of diabetes tissue damage, causing the activation of 5 pathways involved in the pathogenesis of complications and direct inactivation of 2 antiatherosclerotic enzymes namely: endothelial nitric oxide synthase and prostacyclin synthase (Jaganjac *et al*., 2013). To prevent oxidative inactivation of these key enzymes, in addition to preventing activation of the pathways involved in the pathogenesis of complications, it is necessary to directly reduce the amount of superoxide production (Brownlee, 2005). Data strongly supports that therapeutic correction of diabetes-induced superoxide overproduction may be a powerful approach for preventing diabetic complications (Brownlee, 2005; Folli *et al*., 2011). Existing methods of treating diabetes do not prevent diabetic complications; therefore new mechanism-based therapeutic strategies are needed (Brownlee, 2005; Folli *et al*., 2011). Therapeutic agents such as transketolase activator, catalytic antioxidants such as the family of SOD/catalase mimetic compounds and dietary antioxidants are potential and current areas of scientific interest (Brownlee, 2005; Folli *et al*., 2011). Diets high in vegetables and fruits are observed to be more beneficial than conventional antioxidants since they contain plethora of antioxidants and other significant dietary factors (Fiorentino *et a*l., 2013). Based on the observations of the beneficial effects of dietary antioxidants in the management of diabetic complications, there have been increased publications in this area (Lobo et al., 2013; Shodehinde and Oboh, 2013).

The effect of consumption of diet containing *Gongronema latifolium* leaves on oxidative stress and liver damage in diabetes mellitus has not been previously reported.

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Congronema latifolium Benth is of the family (Asclepiadaceaae). It is a tropical rainforest plant, a climber with tuberous base limited in distribution to wet and dry forest of tropical Africa (Ugochukwu and Babady, 2002, 2003), Guinea Bissau and western Camerouns.

As a vegetable, it is used in the preparation of many African dishes. In traditional folk medicine, the leaf is used for treatment of diabetes and hypertension (Okafor, 1981, 1987) as well as for treatment of typhoid fever (Okafor, 1981, 1987). It is also used to dispel stomach upset and pains (Okafor, 1975) and to enhance the return of menstrual cycle (Okafor, 1987). Scientific studies have established its chemical (Eleyinmi, 2007) and phytochemical compositions (Antai *et al*., 2009; Atanghwo *et a*l., 2009). Aqueous and methanolic extract of *Gongronema latifolium* were found to exhibit antibacterial activity against a host of bacteria (Eleyinmi, 2007).

Ethanolic extract of *Gongronema latifolium* (Owu *et al*., 2012) is antiulcer, analgesic and antipyretic. Aqueous extract of dried leaves of *Gongronema latifolium* were found to exhibit anti-inflammatory activity (Morebise *et al.,* 2002). Its antioxidant (Nwanjo *et al*., 2006; Essien *et al*., 2007; Emeka and Obidoa, 2009) and antitussive properties in the treatment of fowl coughs (Essien *et al.,* 2007) have been reported. Reports are also available on the hematological changes following oral administration of ethanolic root extract of *Gongronema latifolium* (Antai *et al.,* 2009) and the effect on serum protein, haemoglobin, cholesterol, lipid peroxidation, white blood cells, antioxidant enzymes such as glutathione-S-transferase, superoxide dismutase, and liver function enzymes namely alanine transaminase, aspartate transminase and alkaline phosphatase of normal rats on long term consumption of a diet supplemented with leaves of *Gongronema latifolium* (Emeka and Obidoa, 2009).

Histological changes of the liver, intestine and testes following chronic dietary intake by normal rats have also been examined (Emeka and Obidoa, 2009).

Limited information is available on the antidiabetic activity of *Gongronema latifolium*. Ugochukwu and Babady (2003) investigated the effect of aqueous and ethanolic extract of *Gongronema latifolium* leaves on glucose and glycogen metabolism in the liver of normal and Streptozotocin-induced diabetic rats.

The results showed that the ethanolic extract had antihperglycemic potency which was suggested to be mediated through the activation of hexose kinase, phosphofructokinase, glucose-6-phosphate dehydrogenase and inhibition of glucose kinase in the liver (Ugochukwu and Babady, 2003). The effect of the leaf extract of *Gongronema latifolium* in the management of diabetic lipid peroxidation was reported by Nwanjo *et al*, 2006. It was found to exhibit anti-lipid peroxidative activity. Edet *et al*. (2009) investigated its effect on some cardiac enzymes of alloxan-induced diabetic rats and concluded that *Gongronema latifolium* leaf extract was not hepatotoxic and was likely to be of significance in the management of cardiovascular complication in diabetic and non- diabetic users. Studies so far on the effect of this plant on diabetes mellitus have concentrated on the use of extract. There is no report on the use of diets containing this plant leaves. The present study was designed to investigate the effect of consumption of diets containing *Gongronema latifolium* leaves on some biomarkers of oxidative stress and liver damage in Streptozotocin-induced diabetic rats so as to evaluate the potential role of the diets in the management of oxidative stress and liver damage in diabetics. Since *Gongronema latifolium* leaves contain valuable antioxidant, especially antioxidant vitamins including ascorbic acid, α- tocopherol, β-carotene and phenolics (Chu et al., 2002; Oboh, 2007), it is suspected that consumption of these leaves might reduce oxidative stress and prevent liver damage.

This study is justified because it will provide information on the possible dietary role of *Gongronema latifolium* in the management of diabetic oxidative stress and liver damage and may be more applicable than work on extract since users could easily prepare such diets than extraction which require sophisticated equipment. This may present a household, more available and accessible prophylactic and therapeutic options for diabetics in African countries where this vegetable is popularly used in the preparation of many dishes. In Africa the prevalence of diabetes is rising and comparatively high in young to middle-aged people unlike the West where the older are most affected. Conventional treatments are scarce, expensive and have some undesirable side effects. Therefore, cheap, immediate, and available remedies are needed.

MATERIALS AND METHODS

Collection and processing of plant materials

Fresh but matured leaves of *Gongronema latifolium* were collected from the Endocrine Research Farm, University of Calabar, and from University of Calabar Staff Village, Calabar in March, 2011. These leaves were authenticated by a Taxonomist and Voucher Specimens were deposited in the herbarium in the Department of Botany, University of Calabar. The leaves were selected to remove extraneous materials, washed and rinsed with distilled water and dried under shade until the leaves were dried. Dried leaves were milled using commercial feed mill machine (Artec model 40) to powder and sieved with 1mm mesh to obtain fine leaf powder.

Fine leaves powder were packaged in a well labeled amber container and stored in the refrigerator at temperature 2-8 °C until used for the preparation of rat chow.

Formulation of experimental diets

Feed ingredients include: leaf powder, soybean meal, maize meal, garri, mineral/ vitamin premix, L-lysine L-methionine and corn oil. Standard rat chows (grower) were formulated according to NRC, 1992 (Table 1).

Three (3) different diets were formulated namely: Control, GL5%; and GL-7.5%. All diets were isocaloric and isonitrogenous. The percentage composition and nutrient analysis of the experimental diets are shown in Table 1.

Table 1: percentage composition and nutrient analysis of experimental diets

Feed ingredient	Diets:		
	Control	**Va-5%**	**Va-7.55**
Soybean Meal (%)	33.78	31.03	30.53
Garri (%)	26	25	25
Maize meal (%)	38	37	35
L-Lysine(%)	0.18	0.18	0.18
L-Methionine (%)	0.17	0.17	0.17
Min/ vitamin (%)	0.25	0.25	0.25
DCP(%)	2.00	2.00	2.00
Bone meal (%)	1.00	1.00	1.00
Corn oil (%)	0.25	0.25	0.25
G. latifolium(%)			
Nutrient Analysis:			
CP(%)	18.40	18.31	18.47
CFAT(%)	4.30	4.01	3.97
CFIBRE(%)	3.71	4.27	
ME (kcal/kg)	3219	3214	3213

Composition of premix: (nutrient in amount in 2.5kg) vit A(I.U) 12000000,vit $D3_{3(LU)}$ 2500000, vit E(mg) 20000,vit $K_{3(mg)}$ 2000,vit B1(mg) 2000,vit B1(mg) 5000,vit B6(mg) 4000,vit B12(mg) 15,niacin (mg0 30000, pantotheic acid (mg) 11000 folic acid (mg) 1500, biotion 60, choline chloride (mg) 220000, antioxidant (mg) 1250, manganese (mg) 50000, zinc (mg) 40000, iron(mg)20000, copper, (mg) 3000,lodine(mg) 1000,selenium (mg)200,cobalt(mg)200.

Animals

Seventy (70) albino rats of Wistar strain (female only) weighing between 83-121g were purchased from the animal house of the Faculty of Basic Medical Science, University of Uyo, Uyo, and transported in well ventilated cages to the animal house of the Department of Biochemistry, University of Calabar, Calabar, where they were kept throughout the duration of the experiment. The animals were allowed to acclimatize for two weeks. They were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25 $\pm 5^{o}$C), relative humidity (50 ± 5%) and twelve hour light/dark cycle.

Approval was granted by the ethic committee of the College of Basic Medical Science, University of Calabar and the animals were kept under the care of a trained animal technician and cared for according to Canadian council on animal care: guide to care and use of experimental animals (1998). Animals were allowed free access to water and chow over a two weeks adaptation period and closely monitored.

Experimental design and induction of experimental diabetes mellitus

The design consisted of fifty (50) female rats divided into 4 groups of diabetic and 1 groups of normal rats with 10 animals in each group.

Diabetes mellitus were induced in the diabetic groups after an overnight fast by intraperitoneal injection of 55mg/kg body weight of Streptozotocin, (STZ) (Sigma St. Louis, MO. USA) reconstituted in 0.1% M sodium citrate buffer. Rats whose fasting blood glucose concentration were higher or equal to 200 mg/dl three days after the induction were confirmed diabetic and recruited in the study. Blood glucose concentration was determined using one touch Glucometer (Lifescan, Inc. 1995, Milpas, Galifornia, U.S.A) with blood obtained from the tail vein of the rats.

Group 1 (normal control, **NC**) was fed with control diet; Group 2 (diabetic control, **DC**) was fed with control diet Group 3 (diabetic treated, **5%GL)** was fed with 5% *Gongronema latifolium* diet. Group 4 (diabetic treated, **7.5%GL**) was fed with 7.5% *Gongronema latifolium* diet. Group 5 (diabetic treated, **INSULIN**) was fed with control diet and treated with insulin, a standard therapeutic agent, which was introduced for comparison. Insulin dose used was 5U/kg body weight (b.w), given subcutaneously (s.c) according to Sonia and Scrinivasan, (1999). It was given once per day by 4.00pm. Feed and water (Tap water) was given ad-libitum. Treatment lasted for 28 days

Collection of sample for analysis

At the end of the 28 days, food and water were withdrawn. The rats fasted overnight. They were then euthanized under chloroform vapor and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles into sterile tubes. Serum was separated for biochemical assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).The liver was surgically removed. It was immediately washed with physiological saline and then weighed with an analytical balance.

It was collected into paper bag and stored frozen until needed for tissue homogenate preparation. Tissue homogenate was prepared in 0.1M Tris-HCl buffer (pH 7.4) and used for the determination of MDA, and activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), as well as assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).

Biochemical assays

Serum was used for biochemical assay of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using Biosystem diagnostic kits (Barcelona,Spain).

Tissue homogenate was used for the quantitation of lipid peroxidation by the method of Ohkawa *et al.* 1979, Glutathione peroxidase (GPx): by the method of Paglia 1967, Catalase (CAT**)** by Takahara *et al.* 1960 and Superoxide dismutase (SOD as described by Misra 1972 as well as assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using Biosystem diagnostic kits (Barcelona,Spain).

Statistical analysis

The results were analyzed for statistical significance by one-way ANOVA using the SPSS statistical program and least square test (LSD) between group using MS excel programme. All data were expressed as mean + SEM. P value <0.05 was considered significant.

Table 2: Effect of consumption of *Gongronema latifolium* leaf diets on oxidative stress indices of diabetic rats.

Treatment	MDA (nmole/mg protein)	SOD(nmole/min/mg protein)	GPx(nmole/min/mg protein)	CAT(nmole/min/mg protein)
NC	$26.62\pm0.35_a$	$140.44\pm1.42_a$	$180.00\pm2.55_a$	$11.24\pm0.09_a$
DC	$39.05\pm0.49_b$	$46.16\pm1.66_b$	$125.00\pm1.25_b$	$7.68\pm0.12_b$
5%GL	$17.58\pm0.52_c$	$45.54\pm5.31_a$	$240.34\pm0.24_c$	$12.04\pm0.12_a$
7.5%GL	$14.59\pm0.36_c$	$158.14\pm3.62_a$	$186.21\pm2.59_a$	$11.97\pm1.02_a$
INSULIN	$27.20\pm0.39_c$	$140.40\pm4.31_a$	$142.00\pm186_c$	$11.47\pm0.11_c$

Means within the same column with different superscript are significantly different (P<0.05).n=10

Table 3: Effect of consumption of diets containing *Gongronema latifolium* on markers of liver damage in serum of STZ induced diabetic rats.

Treatment	AST(U/L)	ALT(U/L)	AST/ALT	ALP(U/L)
NC	$1.68\pm0.21_a$	$4.73\pm0.22_a$	$0.35\pm0.95_a$	$33.68\pm0.39_a$
DC	$13.17\pm2.17_b$	$8.70\pm2.28_b$	$1.51\pm0.95_b$	$63.01\pm1.69_b$
5%GL	$3.97\pm0.15_c$	$4.65\pm13.35_c$	$0.85\pm0.01_c$	$35.34\pm1.03_a$
7.5%GL	$1.68\pm0.18_c$	$5.09\pm1.85_c$	$0.33\pm0.09_c$	$33.04\pm1.02_a$
INSULIN	$4.10\pm10.29_c$	$8.98\pm15.69_a$	$0.45\pm0.66_a$	$44.90\pm1.29_a$

Means within the same column with different superscript are significantly fifferent (P<0.05).n=10

Table 4: Effect of consumption of diets containing *Gongronema latifolium* on markers of liver damage in liver homogenate of STZ induced diabetic rats.

Treatment	AST(U/L)	ALT(U/L)	AST/ALT	ALP(U/L)
NC	$9.08\pm4.04_a$	$48.33\pm15.35_a$	0.18±0.26	$209.12\pm45.23_a$
DC	$49.81\pm4.87_b$	$97.41\pm5.39_b$	0.51±0.90	$387.32\pm12.09_b$
5%GL	$44.95\pm10.62_b$	$69.74\pm17.69_c$	0.64±0.60	$206.80\pm0.56_c$
7.5%GL	$16.29\pm10.36_a$	$22.20\pm77.24_d$	0.73±0.13	$180.06\pm21.90_c$
INSULIN	$21.68\pm47.01_c$	$60.48\pm14.81_c$	0.36±3.17	$270.71\pm045_d$

Means within the same column with different superscript are significantly different (P<0.05)n=10

RESULTS

The effect of consumption of diet containing *Gongronema latifolium* leaves on the activities of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) and on the concentration of malondialdehyde (MDA) is shown in table 2.

Rats in the diabetic control group had GPx, SOD and catalase activities that were significantly lower (P<0.05) (125±1.52, 45.16±1.66, and 7.68±0.12 nmole/min/mg protein respectively) compared to those of the normal control (180±2.55, 140.44±1.42, 11.24±0.09 nmole/mim/mg protein respectively). MDA concentration was significantly higher (P<0.05) for the diabetic control (39.05±0.49 nmole/g protein) compared to the normal control (26.62±0.35 nmole/mg protein).

Treatment with diets of the *Gongronema latifolium* significantly increased (P<0.05) the GPx, SOD and the catalase activities but significantly reduced (P<0.05) the MDA concentration in the diet treated groups compared to the diabetic control. The diets appeared to have more impacts on the concentration of MDA and activity of GPx than Insulin. The activities of SOD and CAT of the diet groups compared to the group on Insulin were not significantly different (P< 0.05). Table 3 shows that the AST, ALT, AST/ALP and ALP level were significantly increased (P<0.05) in the diabetic control group compared to the normal control. Treatment with the diets and Insulin significantly reduced (P<0.05) their levels compared to the diabetic control. Results for diets were generally superior to Insulin. The results for serum markers of liver damage followed the same trend as those of liver tissue homogenate (Table 4).

DISCUSSION

The diabetic control rats showed profound alteration in the concentration of lipid peroxidation end product (MDA) and antioxidant enzyme status in the liver (Table 2). MDA level was significantly (P<0.05) elevated whereas the enzymes level were significantly reduced (P<0.05. Elevated lipid peroxidation and the drastic alterations in the antioxidant enzyme status is a manifestation of oxidative stress in the diabetic control rats. The Increased MDA level (Table 2), in the liver of diabetic control rats was an indication of hyperglycemia caused lipid peroxidation. MDA is more cytotoxic and stable than reactive oxygen species (Esterbauner et al., 1991). The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and nonenzymatic antioxidants. Oxidized lipids are able to produce MDA as a decomposition product and the mechanism is thought to involve formation of prostaglandins, like endoperoxides, from polyunsaturated fatty acid (PUFA) with two or more double bonds (Esterbauner et al., 1991). Increased lipid peroxidation in the membrane of liver (Sanjay *et al*., 2001) is reported in diabetic cases. Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications and is associated with vascular complications (Sanjay *et al*., 2001). Increase in lipid peroxidation results in tissue damage (Sanjay *et al*., 2001). Liver damage is followed by leakage of liver enzymes ASP, ALT. ALP. Significant high levels of these enzymes were seen in the diabetic control rats. Reduction in the level of these enzymes in the diabetic rats consuming diet showed that the diet was able to counteract lipid peroxidation and perhaps heal the damaged cells. The

diabetic rats treated with diets exhibited considerable protection against the oxidative stress at both dose levels though; the higher dose level was numerically superior. The GPx, SOD and CAT level were significantly increased (P<0.05) while MDA was significantly reduced (P<0.05) in the diabetic treated groups compared to the diabetic control. This shows the protective effect of the diets on the antioxidant enzyme status of diabetic rats. The ASP, ALT and ALP level (Table 3) support the fact that the diet played a protective role since their levels were significantly reduced (P<0.05) in the groups of diabetic rats consuming the diets compared to the diabetic control.

Hepatocellular injury, cell death and liver fibrosis occur when ROS and RNS are generated in excess (Feher *et al*., 1987). Glutathione peroxidase, Supper oxide dismutase and Catalase are important antioxidants enzymes involve in cellular defense against wide variety of free radicals (Medina and Moreno- otero, 2005). There has been conflicting reports on the effects of diabetes on the activities of these enzymes in the liver. Maritime, 2003 reported elevation of their activities in the liver while Rajasekaran and Kalaichavan, 2014 reported a decrease in their activities in the liver. In our study, we obtained a decrease in its activity and our results agree with Rajasekaran and Kalaichavan, 2014. The discrepancy may be due to the severity and duration of diabetes and the time the samples were collected. Some animals might have been able to recover from the diabetogen before the samples were collected. Reduction in the activity of these enzymes compromises their function and might account in part for the increased oxidative stress in the diabetic control rats in this study. Diabetes-induced alterations in glutathione peroxidase activity were reversed by treatment with probucol, combined vitamins C/ E, carotene, quercetin (in liver and brain, though not in kidney or heart), coenzyme Q10 and isoeugenol (only in liver), piperine (in kidney) (Maritime, 2003). The diets in this study were effective in restoring the activity of glutathione peroxidase. Alterations of SOD activity in well established diabetes was possible with coenzyme Q10 and piperine, but not with vitamin C, vitamin E, and carotene (Rajasekaran and Kalaichavan, 2014.) The diets in this study were also effective in restoring the activity of SOD. Treatment of established diabetes of 4 weeks or more did not alter Catalase level. For example, no reversals were seen after treatment with melatonin, quercetin, coenzyme Q10, piperine, isoeugenol, gemfibrozil, combined vitamin C/ vitamin E, and beta-carotene (Maritime, 2003). It was interesting to observe that *Gongronema latifolium* leaf diets were able to elevate the activity of Catalase enzyme to normal.

Reports show that conventional antioxidants do not prevent diabetic complications effectively (especially in sustained hyperglyceamia) because conventional antioxidants neutralize reactive oxygen molecules on a one-for-one basis, whereas hyperglycemia-induced overproduction of superoxide is a continuous process (Rajasekaran and Kalaichavan, 2014). Rather diets high in vegetables and fruits are more beneficial than conventional antioxidants since they contain plethora of antioxidants and other significant dietary factors. We believe that the *Gongronema latifolium* leaf diets was able to protect diabetic oxidative stress and liver damage because it is rich in diverse antioxidants vitamins, minerals, cofactors and phytochemicals (Essien, 2007; Atangwho, 2009) that work in synergy with each other and against different types of free radicals (Maritime, 2003). Some of these factors act as Insulin as well as antioxidants enzymes mimetics.

CONCLUSION

The presents study demonstrates the protective role of *Gongronema latifolium* leaf diets against oxidative stress and liver damage in diabetic wistar rats. The effect of the diets may be due to the plethora of antioxidants in the leaves including vitamins, minerals, cofactors, and phytochemicals acting in synergy against all oxidants. The diets may be useful as therapy against oxidative stress and liver damage in diabetes mellitus and is therefore recommended for further studies.

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