

# The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats

N H UGOCHUKWU\*, N E BABADY, M COBOURNE and S R GASSET

Department of Chemistry, 500 Jones Hall, Florida Agricultural and Mechanical University,  
Tallahassee, FL 32307, USA

\*Corresponding author (Fax, 850-561-2434; Email, ngozi.ugochukwu@famu.edu)

Diabetes is known to involve oxidative stress and changes in lipid metabolism. Many secondary plant metabolites have been shown to possess antioxidant activities, improving the effects of oxidative stress on diabetes. This study evaluated the effects of extracts from *Gongronema latifolium* leaves on antioxidant enzymes and lipid profile in a rat model of non insulin dependent diabetes mellitus (NIDDM). The results confirmed that the untreated diabetic rats were subjected to oxidative stress as indicated by significantly abnormal activities of their scavenging enzymes (low superoxide dismutase and glutathione peroxidase activities), compared to treated diabetic rats, and in the extent of lipid peroxidation (high malondialdehyde levels) present in the hepatocytes. The ethanolic extract of *G. latifolium* leaves possessed antioxidant activity as shown by increased superoxide dismutase and glutathione peroxidase activities and decreases in malondialdehyde levels. High levels of triglycerides and total cholesterol, which are typical of the diabetic condition, were also found in our rat models of diabetes. The ethanolic extract also significantly decreased triglyceride levels and normalized total cholesterol concentration.

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## 1. Introduction

Non insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities (Scoppola *et al* 2001). These traits are hypothesized to damage cell membranes which results in elevated production of reactive oxygen species (ROS). This generation of oxygen-free-radicals during cellular metabolism, and by certain environmental factors, including lifestyle, appears to play a critical role in the pathogenesis of NIDDM (Hartnett *et al* 2000). Hyperglycemia, the main symptom of diabetes, not only increases the production of ROS but also affects antioxidant reactions catalyzed by ROS scaveng-

ing enzymes (Uchimura *et al* 1999). NIDDM has also been associated with an increased risk for developing premature atherosclerosis due to increase in triglycerides and low-density lipoprotein levels and decrease in high-density lipoprotein levels (Bierman 1992). All organisms possess antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP)] responsible for scavenging ROS. A defect in ROS scavenging enzyme system has been reported in NIDDM (Kesavulu *et al* 2000).

Many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. *Gongronema latifolium* (Asclepiadaceae), an

**Keywords.** Diabetes; *Gongronema latifolium*; lipid profile; oxidative stress

Abbreviations used: CAT, Catalase; GPx, glutathione peroxidase; MDA, malondialdehyde; NIDDM, non insulin dependent diabetes mellitus; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TG, triglyceride.

edible rainforest plant native to the South Eastern part of Nigeria, has been widely used in folk medicine as a spice and vegetable (Morebise *et al* 2002) for maintaining healthy blood glucose levels (Okafor 1981, 1987, 1989). However, no scientific studies have been done to establish the hypoglycemic, hypolipidemic and antioxidative effects of extracts from *G. latifolium* leaves.

We have evaluated the antioxidant effects of an aqueous and an ethanolic extract from *G. latifolium* leaves on SOD, CAT and GPx activities in hepatocytes of streptozotocin-induced diabetic rats. We also have evaluated their effects on lipid peroxidation in liver by measuring malondialdehyde (MDA) levels, and on triglyceride (TG) and total cholesterol levels in serum.

## 2. Materials and methods

### 2.1 Plant material

*G. latifolium* stem cuts were authenticated and obtained from the Faculty of Pharmacy, University of Nigeria, Nsukka (UNN), Nigeria. The plants were grown in Tallahassee, FL, USA and the mature leaves were harvested bimonthly and stored immediately at  $-80^{\circ}\text{C}$ . Frozen fresh leaves (317 g and 401 g) were homogenized with distilled water and 80% ethanol, respectively, using a PowerGen 1800D homogenizer (Fisher Scientific, Indiana, PA, USA). The mixtures were filtered with Whatman No. 1 filter paper. The filtrates were concentrated to 1/10 of their original volumes at  $38-40^{\circ}\text{C}$  using a rotary evaporator. The aqueous and ethanolic extract concentrates were freeze-dried, yielding 11.05 g of brown powder and 12.6 g of yellowish-green powder, respectively. The powders were resuspended in distilled water before use.

### 2.2 Animals

Male Wistar rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 250–270 g were used. The animals were housed singly in metabolic cages in the animal facility at Florida A&M University under controlled environmental conditions of temperature ( $68-72^{\circ}\text{F}$ ) and relative humidity ( $50 \pm 5\%$ ) and a 12 h light/dark cycle. Upon arrival the animals were acclimatized for at least 7 days and were maintained on a regular commercial rat feed (Harlem Sprague-Dawley, Indianapolis, IN, USA). Deionized distilled water and food were provided *ad libitum*.

Diabetes was induced by intraperitoneal injection of 65 mg/kg body wt. of streptozotocin (STZ) (Sigma, St Louis, MO, USA) dissolved in citrate buffer (0.01 M, pH 4.5). Control rats received only the buffer. A week after

injection of STZ, diabetes was confirmed in STZ-treated rats showing fasting blood glucose levels above 250 mg/dL. The experimental animals were then divided into six groups (table 1). Treatment was administered twice daily by gavage at a dosage of 100 mg/kg body wt. of both extracts (as previously determined using oral glucose tolerance test) in a 12 h cycle (8:00 am and 8:00 pm) every day for 14 days. After 14-day treatment, the rats were euthanized by anesthesia using the inhalant, halothane. Blood was collected from heart and transferred into EDTA tubes immediately. Blood was then centrifuged at 4,000 g for 10 min to remove red blood cells and recover plasma. Livers were surgically removed, immediately washed with ice-cold saline and stored at  $-80^{\circ}\text{C}$ .

### 2.3 Biochemical assays

Weighed amounts of liver tissues were homogenized with ten times their weight in volume of the appropriate buffers using Sonic Dismembrator (Fisher Scientific, Indiana, PA, USA). The homogenized liver tissues were used for measurement of scavenging enzymes SOD (Ukeda *et al* 1997), CAT (Aebi 1984), GPx (Paglia and Valentine 1967); and lipid peroxidation using MDA levels (Sunderman *et al* 1985). Triglycerides and total cholesterol concentration as well as protein content were evaluated using assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA).

### 2.4 Statistical analysis

The results were analysed by one-way ANOVA test using GraphPad Prism software package version 3.0 (San Diego, CA, USA). All data are expressed as mean  $\pm$  SEM. Differences between groups were considered significant at  $P < 0.001$ , 0.05 and 0.01.

## 3. Results

To evaluate the effects of aqueous and ethanolic extracts from *G. latifolium* leaves on serum lipid profile and oxidative stress in diabetic rats, these extracts were adminis-

**Table 1.** Experimental design.

Group	Number of rats	Treatment
Diabetic control (DC)	6	Saline solution
Diabetic (DA)	6	Aqueous extract
Diabetic (DE)	6	Ethanolic extract
Normal control (NC)	6	Saline solution
Normal (NA)	6	Aqueous extract
Normal (NE)	6	Ethanolic extract

tered twice daily for 14 days to normal and diabetic rats. Their livers and blood plasma as well as those of the controls were used to measure the activities of ROS scavenging enzymes (SOD, GPx, CAT) and the levels of MDA, TG and total cholesterol.

### 3.1 Effect of *G. latifolium* leaf extracts on SOD activity

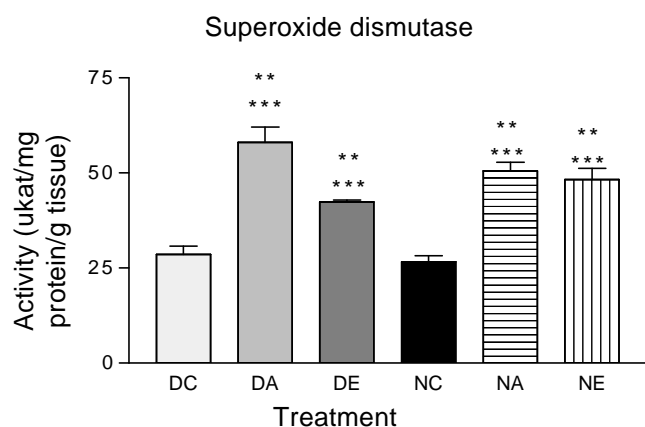
The activities of SOD (figure 1) were significantly higher in diabetic rats treated with ethanolic and aqueous extracts compared to control diabetic rats ( $P < 0.05$ ). There was no significant difference among the treated diabetic and normal groups, and between diabetic and normal controls.

### 3.2 Effect of *G. latifolium* leaf extracts on GPx

GPx activity (figure 2) was significantly higher in diabetic rats treated with the ethanolic extract ( $P < 0.01$ ) compared to control diabetic rats. There was no significant difference between control diabetic rats and diabetic rats treated with aqueous extract ( $P > 0.05$ ). There was no significant difference among rats in the normal groups.

### 3.3 Effect of *G. latifolium* leaf extracts on CAT activity

There were non-significant differences in CAT activity (figure 3) in all the normal and diabetic groups. However, the activity of CAT in diabetic rats treated with the ethanolic extract was lower than in both the diabetic control rats and the diabetic rats treated with the aqueous extract, though the differences were not significant.



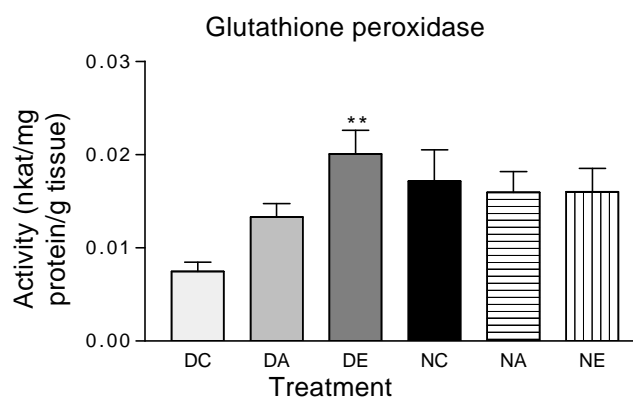
**Figure 1.** SOD activities in normal and diabetic rats treated with the aqueous and ethanolic extracts from *G. latifolium* leaves. \*\* $P < 0.05$  compared with NC; \*\*\* $P < 0.05$  compared with DC.

### 3.4 Effect of *G. latifolium* leaf extracts on MDA levels

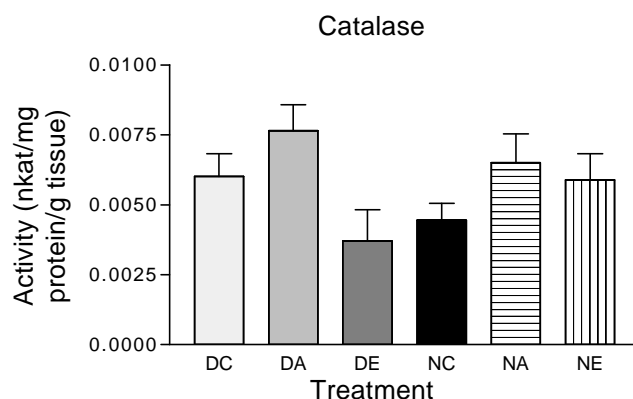
A significantly elevated level of MDA (figure 4) was observed in diabetic control rats and those treated with aqueous extract when compared with diabetic rats treated with the ethanolic extract and the normal groups ( $P < 0.001$ ). However, the MDA levels in diabetic rats treated with the ethanolic extract were higher than in normal groups. There was no difference in the levels of MDA among normal rats.

### 3.5 Effect of *G. latifolium* leaf extracts on TG levels

Serum TG concentration (figure 5) was significantly higher in diabetic control rats compared to the treated diabetic and normal rats ( $P < 0.001$ ). TG level in those treated with ethanolic extract significantly ( $P < 0.05$ )



**Figure 2.** GPx activities in normal and diabetic rats treated with the aqueous and ethanolic extracts from *G. latifolium* leaves. \*\* $P < 0.01$  compared with DC.

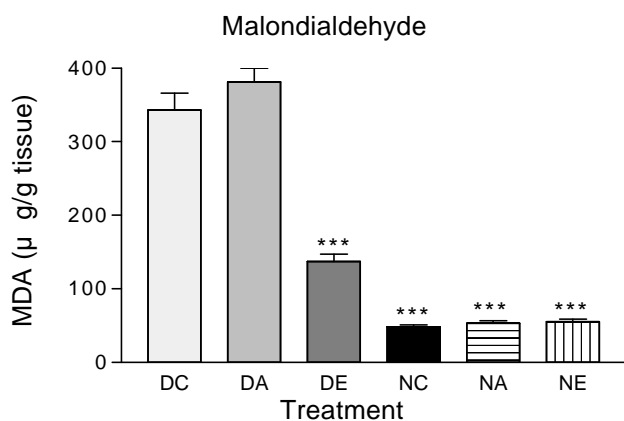


**Figure 3.** CAT activities in normal and diabetic rats treated with the aqueous and ethanolic extracts from *G. latifolium* leaves.

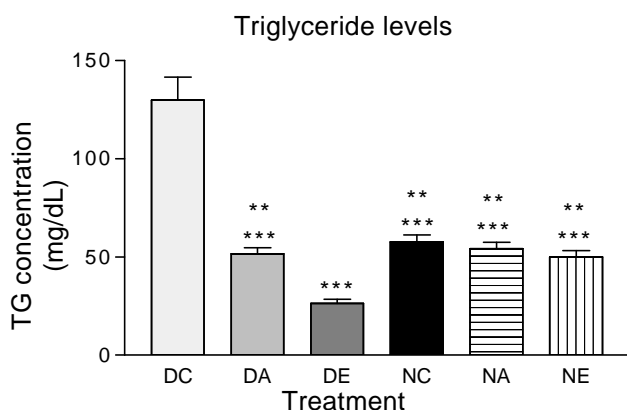
decreased in comparison to the diabetic rats treated with aqueous extract and the normal control rats.

### 3.6 Effect of *G. latifolium* leaf extract on total cholesterol levels

Total cholesterol levels (figure 6) of diabetic rats treated with the ethanolic extract were nonsignificantly lower than those of the normal control rats. However, there was a significant decrease in the values for the normal groups and those treated with the ethanolic extract compared to diabetic controls and rats treated with the aqueous extract.



**Figure 4.** MDA levels in normal and diabetic rats treated with the aqueous and ethanolic extracts from *G. latifolium* leaves. \*\*\* $P < 0.001$  compared with DC and DA.

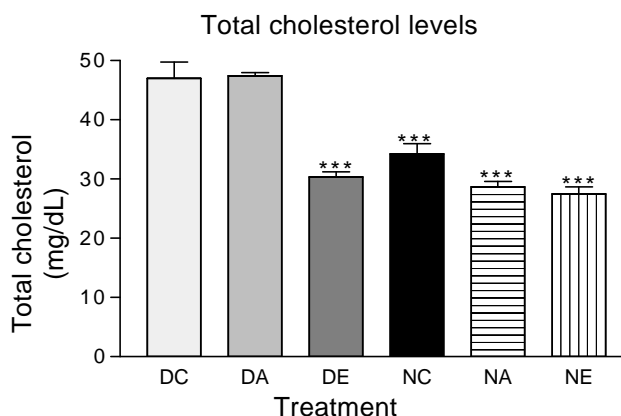


**Figure 5.** TG levels in normal and diabetic rats treated with the aqueous and ethanolic extracts from *G. latifolium* leaves. \*\* $P < 0.05$  compared with DE; \*\*\* $P < 0.001$  compared with DC.

## 4. Discussion

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial/heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes (Akhtar and Ali 1984), but only a few have been scientifically evaluated. Therefore, we investigated the effect of ethanolic and aqueous extracts from *G. latifolium* leaves on lipid profile, in serum, and biomarkers of oxidative stress, in hepatocytes, of diabetic rats.

Impaired glucose metabolism leads to oxidative stress (Ceriello *et al* 1992), and protein glycation produces free radicals (Wolff *et al* 1991). Therefore, the decreases in SOD and GPx activities, in control diabetic rats when compared to rats treated with the aqueous and the ethanolic extract from *G. latifolium* leaves, could at least in part result from inactivation of the enzymes by  $H_2O_2$  or by glycation, which are known to occur during diabetes (Sozmen *et al* 2001; Hodgson and Fridovich 1975; Searle and Willson 1980). In our previous studies, the ethanolic extract significantly reduced blood glucose levels, which in turn could have reduced the potential glycation of the enzymes and the ensuing decreases in their activities. We did not detect any differences in catalase activity between the diabetic and normal rats or between diabetic control and treated diabetic rats. The activities of GPx and CAT increased and decreased, respectively, in the diabetic rats treated with the ethanolic extract. Perhaps GPx shares  $H_2O_2$  with CAT but its action is geared more towards low level of oxidant stress rather than severe oxidant stress (Izawa *et al* 1996).



**Figure 6.** Total cholesterol levels in normal and diabetic rats treated with the aqueous and ethanolic extracts from *G. latifolium* leaves. \*\*\* $P < 0.05$  compared with DC.

We also evaluated lipid peroxidation by measuring hepatic levels of MDA. In agreement with other studies (Cho *et al* 2002; Aydin *et al* 2001), we observed a significant increase in MDA levels in hepatocytes of diabetic rats when compared to normal control rats. The ethanolic extract caused a significant decrease in MDA levels in diabetic rats when compared to diabetic control groups and diabetic rats treated with the aqueous extract. This decrease could be attributed to the increase in GPx activity in rats treated with the ethanolic extract since GPx has been known to inactivate lipid peroxidation reactions (Levy *et al* 1999).

Lipid profile, which is altered in the serum of diabetic patients (Orchard 1990; Betteridge 1994), appears to be a significant factor in the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol levels. In this study, both extracts significantly reduced the triglyceride levels in treated diabetic rats when compared to untreated diabetic rats. The ethanolic extract was also able to significantly decrease the total cholesterol concentration in treated diabetic rats when compared to both untreated diabetic rats and diabetic rats treated with the aqueous extract. These reductions could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics (Cho *et al* 2002).

Further, studies will be needed to purify the bioactive compound(s) in the ethanolic extract, and use the purified compound(s) for bioassay-directed experiments.

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