

Full Length Research Paper

# Protective role of *Phyllanthus niruri* extract on serum lipid profiles and oxidative stress in hepatocytes of diabetic rats

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Diabetes mellitus has been associated with lipid abnormalities and oxidative stress. Some phytochemical properties have been shown to possess antioxidant activities, improving the effects of oxidative stress on diabetes. This present investigations confirmed that untreated diabetic rats were associated with lipid abnormalities as indicated by high levels of total- and LDL-cholesterol, triacylglycerols and low levels of HDL-cholesterol and phospholipids, compared to non-diabetic rats (control). The aqueous extract of *Phyllanthus niruri* significantly normalized the serum lipids levels. The study also showed that untreated diabetic rats were subjected to oxidative stress as indicated by significantly abnormal activities of their scavenging enzymes (superoxide dismutase and catalase and significantly low levels of non-enzymic antioxidants (vitamin C, vitamin E and reduced glutathione) in liver and plasma when compared with controls and in the extent of lipid peroxidation (increased malondialdehyde levels) in plasma and liver cells. The aqueous extract of *P. niruri* possessed antioxidant activities as shown by increased activities of enzymic and non-enzymic antioxidants and decrease in malondialdehyde levels.

**Key words:** Oxidative stress, *Phyllanthus niruri*, lipids and hepatocytes.

## INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative insulin deficiency (Aguwa, 1996). It is a multifactorial disease, which is also characterized by 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 life style, appears to play a critical role in the pathogenesis of diabetes mellitus (Hartnelt et al., 2000). Hyperglycemia, the main symptoms of diabetes, not only increases the production of ROS but also affects antioxidant (Uchimura

et al., 1999). Diabetes has also been associated with an increased risk for developing premature arteriosclerosis due to increase in triacylglyceroles and low-density lipoprotein levels and decrease in light density lipoprotein levels (Bierman, 1992).

Recently, there has been increasing interest in the use of traditional medicinal plants in management of various disease conditions especially diabetes mellitus. *Phyllanthus niruri*, commonly called "Enyikwonwa" in Igbo land of the south Eastern part of Nigeria belongs to the family *Euphorbiaceae*. *P. niruri* has been claimed to be hypoglycaemic and hepatoprotective by the traditional healers or herbalist in Eastern part of Nigeria and also by some researchers (Taylor, 2003; Mazumder et al., 2005). It is of medicinal importance for numerous ailments like dysentery, influenza, vaginitis, tumours, diabetes, diuretics, jaundice, kidney stones, dyspepsia, antihepatotoxic, anti-hepatitis-B, antihyperglycaemic and also antiviral and an-

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tibacterial (Chopra et al., 1986).

Although *P. niruri* has been investigated for its various medicinal properties, detailed evaluation of its anti-lipoproteinaemic, antioxidant and lipid peroxidative potentials in diabetic rats are still lacking. In this study, we evaluated the antioxidant potentials of an aqueous extract of *P. niruri* and its effects on enzymic and non-enzymic antioxidants activities in hepapocytes of streptozotocin-induced rats. We also evaluated their effects on lipid peroxidation in liver by measuring malondialdehyde (MDA) Levels and on cholesterols and triacylglycerols levels in serum.

## MATERIALS AND METHODS

### Chemicals

Streptozotocin (STZ), thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), reduced glutathione (GSH), 2,2'-dipyridyl, xynol orange, aspartic acid, alanine,  $\alpha$ -ketoglutarate, 2,4-dinitro phenyl hydrazine (DNPH), 5,5-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Co (St Louis, MO-USA). The rest of the chemical utilized were of analytical grade and were obtained from local firms (Nigeria).

### Plant materials

The plant *P. niruri* was collected in the month of June, 2006 from the Imo State University Campus, Owerri, Nigeria. The plant was taxonomically identified and authenticated by Dr S. E. Okeke, Head, Department of Plant Science and Biotechnology, Imo State University, Owerri. The voucher samples were preserved in the university herbarium for future reference.

### Preparation of the aqueous plant extract

The entire plant of *P. niruri* was sun dried for ten days before the final drying in an oven at 50°C for 24 h. The dried plants were powdered in both manual grinder and electric grinder. 15 g of the *P. niruri* powder was soaked with 350 ml of distilled water in a beaker and the mixture shaken on the laboratory bench for 24 h before filtering. The filtrate was then evaporated by hot-air oven treatment at 40 - 50°C. Appropriate weights of the residue were prepared in distilled water to obtain the various concentration used for the experiments.

### Animals

Male Wistar rats (200 – 300 g) bred in the Animal House of College of Medicine and Health Sciences, Imo State University, Owerri were used in this study. They were housed in stainless steel cages and kept in a room where a 12 h light/dark cycle was maintained. They were allowed free access to water and feed diet (product of Pfizer Nigeria Ltd) *ad libitum* throughout the period of the experiment.

### Induction of diabetes in rats

After one week of acclimatization, the rats were subjected to a 16 h fast. Diabetes was induced with a single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight. The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) (Ozsoy-

Sacan, 2000). The injection volume was prepared to contain 1.0 ml/kg (Murali et al., 2002). After 5 days, blood glucose levels were measured and the animals with a concentration of more than 230 mg/dl were classified as diabetic (Cetto et al., 2000).

### Experimental design

Twenty four male Wistar rats were used in this study. The rats were randomized and divided into four groups of six animals each.

**Group 1:** Normal, received normal saline solution (0.9% NaCl, 5 ml/kg).

**Group 2:** Diabetic, received STZ (65 mg/kg body weight) once

**Group 3:** Diabetic, receive STZ (65 mg/kg body weight) once before receiving aqueous extract of *P. niruri* (120 mg/kg body weight)

**Group 4:** Diabetic received STZ (65 mg/kg body weight) once before receiving aqueous extract of *P. niruri* (240 mg/kg body weight).

After 14 days of treatment, all the rats were decapitated after fasting for 16 h. Blood was collected in two different tubes i.e. one with fluoride oxalate anticoagulant for plasma separation and another without anticoagulant to separate serum for various biochemical estimations. The livers were dissected out and cleared off blood. They were immediately transferred to ice-cold containers containing 0.9% NaCl and homogenized in 0.1 N Tris-HCl buffer (pH 7.4), and used for the estimation of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity.

### Measurement of blood glucose levels

The body weight and blood glucose levels were measured at the beginning and end of the experiment. Blood samples were obtained by tail vein puncture of both the normal and STZ-induced diabetic rats. Blood glucose levels were determined using a glucometer (Lifescan Johnson and Johnson Company, Milipitas, CA).

### Acute toxicity tests

The acute toxicity of the extract was tested using 30 Wistar rats divided into 5 groups of 6 rats each, with each group receiving graded dose (250 - 1500 mg/kg body weight, intraperitoneally) of the aqueous extract of *P. niruri* as described by Ghosh (1984). After administration of the extract the rats were observed for toxic effects after 48 h treatment. The toxicological effects were observed in terms of mortality expressed as LD<sub>50</sub>. The number of animals dying during a period was noted. The LD<sub>50</sub> of the extract was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract, probit 5 being 50% (Litch field et al., 1959).

### Estimation of serum lipids

Extraction of lipids from serum carried out according to the procedure of Folch et al. (1957) by using chloroform-methanol (2:1, v/v) mixture. From this total cholesterol LDL- and HDL-cholesterol triacylglycerols and phospholipids were estimated as earlier described (Nwanjo, 2005; Nwanjo and Ojiako, 2005).

### Estimation of lipid peroxidation

Lipid peroxidation in plasma and liver was estimated colorimetrically

**Table 1.** Mean values of body weight and blood glucose in experimental and control rats.

Treatment	Body weight (g)			Fasting blood glucose (mg/dl)	
	Initial	Final	change	Initial	Final
Control (Group 1)	180.8 ± 6.2	198.82 ± 8.4	18.2 ± 4.0**	96.34 ± 12.8	82.8 ± 10.5
Diabetic Group II	182.2 ± 4.4	158.71 ± 6.9	-23.49 ± 3.2	230.56 ± 20.0	262.4 ± 32.0
Diabetic + 120 mg/kg <i>P. niruri</i> (Group III)	183.4 ± 6.8	192.26 ± 5.2	8.86 ± 2.6*	231.23 ± 11.6	138.92 ± 13.2 <sup>#</sup>
Diabetic + 240 mg/kg <i>P. niruri</i> (Group IV)	180.9 ± 10.8	196.98 ± 112	16.08 ± 4.4**	231.89 ± 13.2	97.9 ± 6.8 <sup>#</sup>

\*Significantly different from diabetic rats (group II) ( $P < 0.05$ ).

\*\*Significantly different from group II and group III ( $P < 0.05$ ).

<sup>#</sup>Significantly different from initial fasting blood glucose ( $P < 0.05$ ).

by measuring malondialdehyde (MDA) by the method of Nichans and Samuelson (1968). In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCL reagent (TBA 0.37%: 0.25 N HCL: 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank.

#### Estimation of enzymic antioxidants

Superoxide dismutase (SOD) activity was determined by the modified method of NADH-phenazinemetosulphate-nitroblue-tetrazolium formazon inhibition reaction spectrophotometrically at 560 nm (Kakkar et al., 1984). A single unit of enzyme was expressed as 50% inhibition of NBT (nitroblue tetrazolium) reduction /min/mg protein.

Catalase (CAT) was assayed calorimetrically as described by Sinha (1972) using dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1.3 ratio). The intensity was measured at 620 nm and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity.

#### Estimation of non-enzymic antioxidants

Reduced glutathione (GSH) was determined by the method of Ellman (1959). 1 ml of supernatant (0.5 ml plasma/0.5 ml liver homogenate precipitated by 2 ml of 5% TCA) was taken and 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) were added. The colour developed was read at 412 nm.

Vitamin C (ascorbic acid) concentration was measured by Omaye et al. (1979) method. To 0.5 ml of plasma/0.5 ml liver homogenate, 1.5 ml of supernatant, 0.5 ml of DNPH reagent (2% DNPH and 4% thiourea in 9 N sulphuric acid) was added and incubated for 3 h at room temperature. After incubation 2.5 ml of 8.5% sulphuric acid was added and colour developed was read at 530 nm after 30 min.

Vitamin E ( $\alpha$ -tocopherol) was estimated by the method of Desai (1984). Vitamin E was extracted from plasma/liver homogenate by addition of 1.6 ml ethanol and 2.0 ml petroleum ether to 5.0 ml plasma and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2 ml of 0.2% 2,2-dipyridyl, 0.2 ml of 0.5% ferric chloride was added and kept in dark for 5 min, an intense red colour layer obtained on addition of 4 ml butanol was read at 520 nm.

#### Statistics

Statistical evaluation of data was performed by using one-way

analysis of variance ANOVA followed by Duncan's multiple range test (DMRT) (Duncan, 1957).

## RESULTS

### Effect of extract of *P. niruri* on blood glucose levels and body weight changes

The results of blood glucose levels and body weight changes in normal, STZ-induced diabetic rats and *P. niruri* treated diabetic rats were shown in Table 1. There was a significant ( $p < 0.01$ ) increase in blood glucose levels in STZ-induced diabetic rats (Group II) when compared with normal rats. Administration of aqueous extract of *P. niruri* at a dose of 120 and 240 mg/kg body weight significantly ( $p < 0.05$ ) decreased blood glucose in STZ induced rats (Group III and IV). The results were found to be in a dose dependent manner.

The body weight in the diabetic group was significantly less ( $p < 0.05$ ) than the normal control which then returned to near normal in diabetic rats treated with aqueous extract of *P. niruri* at a dose of 120 and 240 mg/kg body weight

### Effect of aqueous extract of *P. niruri* on serum lipids

The changes in the levels of serum lipids in control and experimental rats are illustrated in Table 2. The total cholesterol, LDL-Cholesterol and triacylglycerol significantly increased and HDL-Cholesterol and phospholipids significantly decreased in STZ-induced diabetic rats (group II) ( $P < 0.05$ ) when compared with the normal (group I) rats. The aqueous extract of *P. niruri* (120 mg/kg and 240 mg/kg body weight) offered a significant protection against alteration in the serum lipids of diabetic rats. The results were also dose dependent.

### Effects of *P. niruri* aqueous extract on MDA

The levels of MDA in plasma and liver were significantly ( $p < 0.05$ ) increased in STZ-induced rats as compared to normal rats. Treatment with aqueous extract of *P. niruri*

**Table 2.** Mean values of serum lipids in experimental and control rats.

Treatment	Total-C (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)	Phospholipids (mg/dl)
Control (Group I)	92.1 ±8.62**	46.2 ± 3.58*	31.99 ±2.0**	69.55 ±3.4**	92.3 ±6.5*
Diabetic (Group II)	172.5 ±9.5	30.2 ±4.2	100.68 ± 4.2	158.1 ± 8.4	65.1 ± 3.2
Diabetic + 120 mg/kg <i>P. niruri</i> (Group III)	130.66 ±10.6*	42.8 ± 3.6*	66.22 ±3.5*	108.2 ±5.8*	80.2 ± 6.1*
Diabetic + 240 mg/kg <i>P. niruri</i> (Group IV)	98.9 ± 9.22**	37.82 ±3.9*	44.66 ±3.8**	82.1 ± 6.1**	88.3 ± 5.6*

\*Significantly different from diabetic rats (Group II) (P < 0.05).

\*\*Significantly different from group II and group III (P < 0.05).

**Table 3.** Mean values of plasma and liver lipid peroxidation in experimental and control rats.

Treatment	Plasma MDA (nmol/ml)	Liver MDA (nmol/ml)
Control (Group I)	2.92 ± 0.48*	10.42 ±2.9*
Diabetic (Group II)	6.89 ± 1.04	25.26 ± 4.8
Diabetic + 120 mg/kg <i>P. niruri</i> (Group III)	4.26 ± 0.98*	14.48 ± 3.6*
Diabetic + 240 mg/kg <i>P. niruri</i> (Group IV)	3.68 ± 1.12*	12.87 ±4.1*

\*Significantly different from the diabetic (p < 0.05)

**Table 4.** Mean activities of liver of enzymic antioxidant in both experimental and control rats.

Treatment	Liver SOD (units/mg protein)	Liver CAT (units/mg protein)
Control (Group I)	5.89 ± 0.56*	70.56 ± 5.3*
Diabetic (Group II)	3.01 ± 0.58	46.2 ± 4.6
Diabetic + 120 mg/kg <i>P. niruri</i> (Group III)	4.99 ± 0.42*	62.34 ± 3.8*
Diabetic + 240 mg/kg <i>P. niruri</i> (Group IV)	5.04 ± 1.21*	65.22 ± 4.02*

\*Significantly different from the diabetic (p < 0.05).

**Table 5.** Mean values of plasma and liver non-enzymic antioxidant in experimental and control rats.

Treatment	GSH (mg/dl)		Vitamin C (mg/dl)		Vitamin E (mg/dl)	
	Plasma	Liver	Plasma	Liver	Plasma	Liver
Control (Group I)	19.82 ± 0.8*	38.2 ± 3.5*	1.39 ± 0.3*	1.28 ± 0.16*	1.71 ± 0.2*	0.91 ± 0.04*
Diabetic (Group II)	10.45 ± 1.7	19.67 ± 2.8	0.69 ± 0.2	0.68 ± 0.08	0.83 ± 0.15	0.52 ± 0.04
Diabetic + 120 mg/kg <i>P. niruri</i> (Group III)	16.12 ± 0.9*	32.2 ± 3.4*	1.21 ± 0.32*	1.25 ± 0.08*	1.42 ± 0.13*	0.87 ± 0.04*
Diabetic + 240 mg/kg <i>P. niruri</i> (Group IV)	18.0 ± 1.33*	35.8 ± 2.99*	1.22 ± 0.29*	1.30 ± 0.11*	1.61 ± 0.15*	0.88 ± 0.3*

\*Significantly different from the diabetic (p < 0.05).

resulted in a significantly decrease in the levels of lipid peroxidation products (MDA) in diabetic rats (Table 3).

#### Effects of *P. niruri* aqueous extract on enzymic antioxidant status

A significant decrease (P < 0.05) in the activities of enzymic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) of the liver was noted in STZ-induced diabetic rats when compared with the normal rats. Upon administration of 120 and 240 mg/kg body weight of

aqueous extract of *P. niruri*, the activities of both SOD and CAT were significantly reversed to near normal (Table 4).

#### Effects of *P. niruri* aqueous extract on non-enzymic antioxidant status

The levels of plasma and liver vitamins C, E and reduced glutathione (GSH) were significantly depleted in STZ-induced diabetic rats. Treatment with aqueous extract of *P. niruri* significantly increased the levels of these non-enzymic antioxidants in diabetic rats (Table 5).

## DISCUSSION

This study demonstrated the hypoglycemic, hypolipidaemic and antioxidant effect of aqueous extract of *P. niruri* in STZ-induced diabetic rats. Streptozotocin induction causes a significant elevation in the level of blood glucose and loss of body weight in rats. In this study we observed that the administration of aqueous extract of *P. niruri* at the doses of 120 and 240 mg/kg body weight to diabetic rats not only caused a significant decrease in blood glucose but also has a significant effect in controlling the loss of body weight, which is caused during diabetes. High level of blood glucose, the primary clinical manifestation in diabetes, is associated with certain complications of diabetes. Brownlee et al. (1981) reported that in diabetic there is increase in cholesterol/phospholipids ratio, thereby disrupting membranes fluidity and leading to membrane alteration of function. In agreement with this, we also observed that the levels of serum lipids significantly changed in diabetic rats, suggesting that hyperglycemia has devastating effect on membrane stability and functional state. Similar results were observed in our previous study (Nwanjo, 2005). Administration of aqueous extract of *P. niruri* at doses of 120 and 240 mg/kg body weight showed protection against serum lipid changes in STZ-induced diabetic rats. The activities were found to be in a dose dependent manner.

Membrane lipids succumb easily to deleterious actions of reactive oxygen species (ROS) (Reiter, 1995). ROS induce oxidative tissue damages plays an important role in many clinical disorders such as heart disease, diabetes, gout and cancer associated with the peroxidation of cellular lipids, which is determined by measurement of thiobarbituric acid (TBA)-reactive substance. The concentration of lipid peroxidation products may reflect the degree of oxidative stress in diabetes. It has been reported previously (Baynes, 1991; Kakkar et al., 1995) that in tissues and blood of rats with STZ-induced diabetes, malondialdehyde, one of the by-products of lipid peroxidation is increased. Further, increased level of TBA-reactive substances results in increased levels of oxygen free radicals, which attack the polyunsaturated fatty acid in cell membranes and cause lipid peroxidation. STZ is toxic to pancreatic  $\beta$ -cells and is thus widely used for induction of experimental diabetes in animals. It can give rise to oxygen free radicals because of the increased blood glucose level in diabetes (Rakieten et al., 1963). The measurement of lipid peroxidation is a convenient method to monitor oxidative damage (Viani et al., 1991). In this study, the increased level of TBA-reactive substances in plasma and liver of STZ-induced diabetic rats reflected the lipid peroxidation as the consequence of oxidative stress caused by increased blood glucose levels in diabetes. Treatment with aqueous extract of *P. niruri* protects the cells through attenuation of lipid peroxidation and decreased the production of free radical derivatives as evident from the decreased levels of plasma

and liver TBA-reactive substances. Thus aqueous extract *P. niruri* offered protection against oxidative stress by scavenging of free radicals.

The enzymic antioxidants defense systems are the nature protector against lipid peroxidation. SOD scavenges the superoxide ions produced as cellular by products. SOD is a major defense for aerobic cells in combating the toxic effects of superoxide radicals (McCord et al., 1976). CAT reduces hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisomes. The reduced activities of SOD and CAT in STZ-induced diabetic rats resulted in the accumulation of superoxide radical and  $H_2O_2$  respectively, which produce deleterious effects (Mazunder et al., 2005). Administration of aqueous extract of *P. niruri* showed normalization of activities of SOD and CAT in liver of STZ-induced diabetic rats.

Non-enzymic antioxidants such as reduced glutathione (GSH), Vitamin C and Vitamin E play an excellent role in protecting the cells from oxidative damage (Anuradha and Selvam, 1993). GSH is a major endogenous non-enzymic antioxidant which counter balance free radicals mediated damage. It is well established that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals by participation in detoxification reaction and keeps up the cellular levels of the active forms of Vitamin C and vitamin E by neutralizing the free radicals. When there is a reduction in GSH the cellular levels of vitamin C and vitamin E are closely inter linked to each other (Pari and Amadi, 2005). In agreement with this report, the decreased levels of GSH, vitamin C and Vitamin E on STZ-induced diabetic rats were observed in this study. Administration of aqueous extract of *P. niruri* to these diabetic rats, maintained the level of non-enzymic antioxidants to near normal by the possible role of this extract in improving the GSH status.

In conclusion, *P. niruri* exhibits strong hypoglycemic and hypolipidamic activities. Our study also showed that aqueous extract of *P. niruri* have antioxidant activity. Therefore aqueous extract of *P. niruri* is suggested to be effective for reducing oxidative stress and free radical-related diseases including diabetes.

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