

Studies On The Effect Of Aqueous Extract Of Phyllanthus Niruri Leaf On Plasma Glucose Level And Some Hepatospecific Markers In Diabetic Wistar Rats

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Citation

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Abstract

The effects of various concentrations of aqueous extract of Phyllanthus niruri on plasma glucose level and some hepatospecific markers were investigated in diabetic Wistar strain rats. The classes of chemical components of the aqueous extract of the plant were determined; alkaloids, flavonoids and saponins were found to be present. Acute toxicity test in rats gave an LD₅₀ of 516.2 mg/kg. 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. There were no significant difference (P<0.05) in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities as well as total, conjugated and unconjugated bilirubin levels for the two experimental groups respectively when compared with the control group. The observations show that the aqueous crude extract of Phyllanthus niruri may have hypoglycaemic effect in diabetic rats and that no evidence of hepatotoxicity of the extract was established.

INTRODUCTION

Medicinal plants are being used in traditional system of medicine from hundreds of years in many countries of the world (Oubre et. al, 1970), and which is in line with World Health Organization prescription that medicinal plant researches warrant attention. Most of these medicinal plants with hypoglycaemic properties are being used immensely as alternative forms of treatment and management of diabetes since the use of oral hypoglycemic drugs is restricted by their treatment and management failure and accompanying side effects (Halim and Ali, 2002).

Moreover, the increased dependency rate on this alternative form of treatment for diabetes became possible as a result of many researchers publishing works on a lot of plants with hypoglycemic properties. Phyllanthus niruri also known as "Chanca plectra" belongs to the family Euphorbiaceae. It is known as enyikwonwa in Ibo language of the South Eastern Nigerian. A lot of researchers (Upal et. al., 2005; Ramakrishnan et. al., 1982; and Sivaprakasam et. al., 1995) who worked on Phyllanthus niruri confirmed that it has hypoglycaemic properties. It has also been used for the treatment of other disease conditions in various parts of the world. It is an excellent remedy of jaundice (Kirtikar and

Basu, 1935) and infective hepatitis (Ramanan and Sainani, 1962). It is effective in jaundice in children (Dixit and Achor, 1982). The plant is of medicinal importance for numerous ailments like dysentery, diuretics, kidney stones, influenza, antibacterial, antihyperglycaemic and antiviral (Chopra et. al., 1986).

Thus, as many of these medicinal plants have been used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects. This is always being taken the case with Phyllanthus niruri. However chronic consumption of large amounts of traditional remedies must always be taken with caution as toxicity studies have not been conducted for most of these plants (Shnkar et. al., 1980). A lot of researchers have shown that Phyllanthus niruri has protective action on the different body organs especially the liver and the kidney, and that no form of toxicity has been associated with the usage of this plant extract (Tabasum et. al., 2005; Barros, 2003; and Nishiura et.al 2005). Visveswaran and Santrani (1985) have also shown that 50% alcoholic extract of leaves of Phyllanthus niruri and Ricinus communis have hepatoprotective effect against carbon tetrachloride-induced liver cell damage in rabbits. However, there is a report of

hepatotoxicity in rats (Adedapo et. al., 2005). Based on these varied reports, this research work is designed primarily to examine the actual effect of an aqueous extract of *Phyllanthus niruri* on some biochemical parameters such as blood glucose and hepatospecific markers in diabetic Wistar rats.

MATERIALS AND METHODS

PLANT MATERIALS

The plant *Phyllanthus 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 *Phyllanthus niruri* was sun dried for ten days before the final drying in an oven at 50°C for 24 hours. The dried plants were powdered in both manual grinder and electric grinder. Fifteen gramme of the *P. niruri* powder was soaked with 350ml of distilled water in a beaker and the mixture shaken on the laboratory bench for 24 hours 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.

PHYTOCHEMICAL STUDIES

The chemical classes of constituent in the freshly prepared extract were detected using standard photochemical reagents and procedures as described by Trease and Evans (1983).

In general, test for the presence or absence of phytochemical compound using the above methods involve the addition of an appropriate chemical agent to the extract in a test tube. The mixture is then shaken vigorously or gently as the case may be. The presence or absence of saponins, flavonoids, tannins, alkaloids etc. was observed.

ANIMALS

Male Wistar rats (200-300g) 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-hour 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 hour fast. Diabetes was induced with a single intraperitoneal injection of streptozotocin (STZ) at a dose of 65mg/kg body weight. The STZ was freshly dissolved in citrate buffer (0.01M, 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 w/v, 5 ml/kg). Group 2: Diabetic, received STZ (65 mg/kg body weight) once. Group 3: Diabetic, received STZ (65 mg/kg body weight) once before receiving aqueous extract of *Phyllanthus niruri* (120 mg/kg body weight). Group 4: Diabetic received STZ (65 mg/kg body weight) once before receiving aqueous extract of *Phyllanthus niruri* (240 mg/kg body weight).

After 14 days of treatment, all the rats were decapitated after fasting for 16 hr. Blood was collected into tubes and allowed to clot and the serum collected for various hepatospecific markers estimations. The livers were dissected out and cleared of blood.

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 *Phyllanthus niruri* as described by Ghosh, (1984). After administration of the extract the rats were observed for toxic effects after 48 hours treatment. The toxicological effects were observed in

terms of mortality expressed as LD₅₀. The number of animals dying during a period was noted. The LD₅₀ 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).

HEPATOSPECIFIC MARKERS ANALYSIS

Serum total bilirubin level was estimated based on Van den Berg reaction (Malloy and Evelyn, 1937). Diazotised sulphonilic acid (0.5ml) reacts with bilirubin in diluted serum (0.2ml serum + 1.8ml distilled water) and forms purple coloured azobilirubin, which was measured at 540 nm. Activities of serum aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman and Frankel (1957). 0.2 ml of serum with 1ml of substrate (asparatate and ?-ketoglutarate) for AST, alanine and ?-ketoglutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 minutes for ALT. 1ml of DNPH solution was added to arrest the reaction and kept for 20 minutes in room temperature. After incubation 1ml of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as IU/L.

Based on the method of King and Armstrong (1934) alkaline phosphatase activities was assayed using disodium phenylphosphate as substrate. The colour developed was read at 680 nm after 10 min and activities of ALP expressed as IU/L.

Statistical Analysis: Statistical evaluation of data was performed by using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Duncan, 1957).

RESULT AND ANALYSIS

PHYTOCHEMICAL COMPOSITION OF THE AQUEOUS EXTRACT OF .

The classes of chemical components of the aqueous extract of Phyllanthus niruri are shown in Table I. Alkaloids, flavonoids and saponins were found to be present while tannins, steroids, cyanogenic glycosides and cardiac glycosides were absent.

EFFECT OF EXTRACT OF 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 II. 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 changes in diabetic group was significantly decrease ($p < 0.05$) when compared with 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 EXTRACT OF ON HEPATOSPECIFIC MARKERS ANALYSIS

The changes in the mean value of serum bilirubin and serum hepatospecific enzyme markers in all the groups are shown in table III. There was no significant increase in the levels of total, conjugated and unconjugated bilirubin concentrations and AST, ALT and alkaline phosphatase activities in diabetic rats treated with aqueous extract of P. niruri at a dose of 120 and 240 mg/kg body weight ($P > 0.05$) when compared with both control and diabetic rats. While diabetic rats groups showed significant increase only in serum ALP activities ($p < 0.05$) when compared with the control.

DISCUSSION

The effects of various concentrations of aqueous extract of Phyllanthus niruri on plasma glucose level and some hepatospecific markers were investigated in STZ induced diabetic Wistar strain rats. The classes of chemical components of the aqueous extract of the plant were determined. Alkaloids, flavonoids and saponins were found to be present. Some plants that contain alkaloids have been reported to have hypoglycaemic activity (Bever and Zahad, 1979), so the hypoglycaemic activity associated with extracts of this plant may be attributed to the presence of this alkaloids and flavonoids. These are mainly phenolic compounds, which have been reported to have antidiabetic effects (Farjou et al, 1987). The acute toxicity studies showed that the extract from the aqueous leaf extract of Phyllanthus niruri produced an LD₅₀ of 516.2 mg/kg rat, I.P. 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 administrated 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. This confirms the claims by traditional medical practitioners and herbalists that the *Phyllanthus niruri* extract has blood glucose lowering properties. Food was withheld 12 hr prior to killing in order that the reduction in blood glucose in the test group may not be due to a reduction of food consumed. The precise mechanism by which this leaf extract lowers blood glucose is however not clear. It may be due to increased insulin secretion arising from pancreatic stimulation and probably increased utilization of peripheral glucose (Farjou et al, 1987). It is also believed that some of these hypoglycemic plants perform this function by removing the insulin-inactivating compounds through the SH groups in these inactivating compounds. Nicotinic acid is known to be insulin's inhibitor (Ben-David et al, 1983). Similarly, other hypoglycemic plants containing anthocyanosides appear to act by improving vascularization of the pancreas. Others act by blocking oxidative enzyme of the kreb cycle (succinic dehydrogenase and cytochrome oxidase), thus increasing anaerobic glycolysis and decreasing gluconeogenesis and entailing an increased rate of transfer of glucose from the blood to the tissue (Bever and Zahad, 1979). The results of some liver function parameters were presented in the same table 4.2. The fact that there was no significant change in serum levels of ALT, AST, ALP, total, conjugated and unconjugated bilirubin concentrations even at high dose of administration of the aqueous extract strongly indicates its non-hepatotoxic effect. Thus, since Ojiako and Nwanjo (2006) showed that ALT and AST always increase following hepatotoxicity, and are more reliable markers of liver integrity than ALP, the observed insignificant difference in the activities of ALT and AST of the experimental groups when compared to the control group supports its non-hepatotoxicity. Also, the insignificant difference between the relative liver weight of each of the experimental groups and the control group as presented in table 4.1 seems to support this claim.

In conclusion, these observations show that the aqueous crude extract of *Phyllanthus niruri* may have hypoglycaemic effect in diabetic rats and that no evidence of hepatotoxicity of the extract was established.

Figure 1

Table 1: Phytochemical composition of

Phytochemical substances	Result
Flavonoids	++
Alkaloids	++
Tannins	NP
steroids	NP
Cyanogenic glycosides	NP
Saponins	+
Cardiac glycosides	NP

Keys: ++ = highly present, + = present, NP = not present

Figure 2

Table 2: The mean values of body weight change and mean relative weight of the liver

Groups	Mean initial weight (g)	Mean final weight (g)	Mean weight change (g)	relative liver weight (g)
Control (I)	246.15 ± 5.8	252.53 ± 5.6	6.38 ± 0.45**	4.36 ± 0.52
Group II	250.34 ± 6.1	248.53 ± 6.42	-1.81 ± 0.80	7.23 ± 0.48*
Group III	248.43 ± 5.38	251.47 ± 5.98	3.04 ± 0.52*	5.08 ± 0.39
Group IV	249.24 ± 5.78	252.06 ± 5.00	2.82 ± 0.43*	4.88 ± 0.53

*Significantly different from II (p < 0.05) using Duncan multiple range test

** Significantly different from other groups (p < 0.05) using Duncan multiple range test

*Significantly different from control (p < 0.05) using Duncan multiple range test

Figure 3

Table 3: Mean values of activities of serum AST, ALT, ALP and levels of bilirubin in normal and experimental rats.

	Control (I)	Group II	Group III	Group IV
FBG (mg/dl)	94.46 ± 10.6	226.20 ± 16.3	168.98 ± 15.4	127.67 ± 6.4
AST (IU/L)	75.58 ± 5.24	*102.24 ± 6.0	82.41 ± 6.0	80.28 ± 5.08
ALT (IU/L)	80.82 ± 5.65	80.82 ± 5.65	89.23 ± 7.74	88.09 ± 6.83
ALP (IU/L)	76.43 ± 6.9	*106.43 ± 7.62	80.42 ± 8.32	81.87 ± 7.34
Bilirubin (Umol/L)	4.54 ± 2.8	*6.89 ± 4.43	5.0 ± 4.67	5.08 ± 3.31

*Significantly different from control (p < 0.05) using Duncan multiple range test

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