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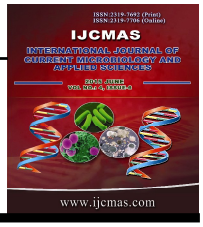

Original Research Article

Efficacy of *Phyllanthus niruri* Linn. Extract in the Management of Type-2 Diabetes Mellitus Associated Hypercholesterolemia in Mice Diabetic Model

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A B S T R A C T

The majority of Type-2 DM patients suffer from visceral obesity and they often have high circulating levels of lipids including cholesterol, triglycerides, low levels of high density cholesterol, which also contribute to the development of vascular complications. I the present study the efficacy of *P. niruri* extract on serum lipid profile of diabetic mice was studied. The results clearly indicated that Diabetic mice treated with *P. niruri* extract (DT_{150}) showed significantly lower values of serum TC (-26.5%; $p<0.001$) and TGs (-55.6%; $p<0.001$), when compared with the DC counterparts. The DT_{250} treatment showed superior lowering effects compared with the DC counterparts as well as DT_{150} group mice by (-34.7%; $p<0.001$) on serum TC levels and (-50%; $p<0.001$) on TGs levels. Contrarily, treatment with rosiglitazone (DT_{RGZ}) showed (-33.2%; $p<0.001$) on TC levels and (-09.6%; $p<0.001$) on TGs levels compared with diabetic control mice.

Keywords

Phyllanthus niruri, Lipid profile, Streptozotocin, Mice

Introduction

Type-2 Diabetes Mellitus (DM) is a heterogeneous disease with both genetic and environmental causative factors. Gerich (1996) has summarized the pathophysiology of Type-2 DM as follows. Initially the pancreatic beta cells are not able to respond with appropriate insulin secretion to glucose challenge. At the same time, an increased demand for insulin due to environmentally induced insulin resistance has also act. At this juncture, a compensatory increase of the insulin secretion is still sufficient to maintain a normal glucose level. By gradual decrease of insulin secretion and increase of insulin resistance, a reduced suppression of hepatic glucose output and impaired glucose tolerance appear. With further increase in insulin resistance, an absolute increase in hepatic glucose output occurs which leads to fasting hyperglycemia. At the same time, pancreas fail to compensate for the increased demand of insulin any further and hyperglycemia sets in. If untreated, hyperglycemia and insulin resistance in Type-2 DM increase the risk of several macro and micro vascular complications such as, hypertension, coronary vascular disease, cardiomyopathy, stroke and

retinopathy, neuropathy, nephropathy (Kannel and McGee, 1979; Garcia, 1974). The majority of Type-2 DM patients suffer from visceral obesity and they often have high circulating levels of lipids including cholesterol, triglycerides, low levels of high density cholesterol, which also contribute to the development of vascular complications. Independent of coronary artery complications, complex changes in the mechanical and electrical properties of the heart may contribute to diabetic cardiopathy. The devastating consequences of these complications include lower-limb amputation, end stage renal failure, loss of vision and myocardial infarction.

It is clear that the contributory abnormalities in Type-2DM are insulin deficiency, insulin resistance and increased hepatic glucose output. The current therapies used to treat patients with these complications are aimed at correcting one or more of these physiological abnormalities. The diabetes control and complications study (DCCT, 1996) and the United Kingdom Prospective Diabetes Study (UKPDS, 1998) demonstrated that good metabolic control through intensive drug therapy and strict lifestyle management could reduce the risk of developing diabetic complications.

Phytochemicals have played an important role in the development of chemotherapeutic agents.

Phytochemicals have multiple beneficial activities including manipulation of carbohydrate metabolism by various mechanisms, preventing and restoring integrity and functioning of β-cells, insulin releasing activity, improving glucose uptake and utilization, and antioxidant properties. Furthermore, natural products are widely viewed as templates for optimization programs with the goal of creating new drugs.

Efficacy of *P. niruri* extract has been demonstrated by several workers. Kitisin et al. (1952) have demonstrated the effect of smooth muscle relaxation specific to the urinary and biliary tracts. The anti-hepatotoxic activity of *P. niruri* has been attributed to two novel lignans, phyllanthin and hypophyllanthin (Symasundar et al., 1985). Glycosides (quercitrin and geraniin) found in *P. niruri* demonstrated aldose reductase inhibitory (ARI) activity in studies conducted by a Japanese research group in 1988 and 1989 (Ueno et al., 1988; Shimizu et al., 1989). The ARI effect was also due to the presence of another ellagitannin phytochemical, ellagic acid (Shimizu et al., 1989). The plant also possessed potent analgesic activity against pain models in rats (Santos et al., 1994; Martini et al., 2000).

The diuretic, hypotensive and hypoglycemic effects of *P. niruri* were documented in a human study, which showed a significant diuretic effect (Devi et al., 1986). Similar studies in man revealed that *P. niruri* caused reduction in the systolic blood pressure in non-diabetic hypertensive patients and reduction of blood glucose in diabetic patients (Ramakrishnan et al., 1982; Hukeri et al., 1988). The efficacy of *Phyllanthus niruri* methanol extract on lipid profile of Streptozotocin induced diabetic mice has not been investigated so far and hence the present study was undertaken.

Materials and Methods

Methanol extract of *phyllanthus niruri*. Linn (Euphorbiaceae) was used for assaying the serum lipid profile in Streptozotocin induced mice diabetic models. The whole plant of *Phyllanthus niruri* was washed under running tap water blotted with filter paper and was dried in the shade at room temperature. The dried plant sample (2.6 kg) was then soaked with absolute methanol

under reflux condition for the methanolic extract preparation. The sample was then homogenized with extraction buffer and the supernatant collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40^0C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The obtained methanol extract was stored in deep freezer at -20^0 C until further test.

The mice were allowed to acclimatize for 15 days in an environmentally controlled room under standard environmental conditions (21±2°C, 55±5%Relative humidity, 12 hr Light: Dark cycle) and fed on diet consisted of wheat grains-1Kg, Choker wheat-250gm, Gram grains-250gm, Maize grains-250gm, Soybean grains-250gm, Sundrop oil-50gm, Milk powder-2 table spoon and Jaggery-50gm. This diet provided carbohydrate 48.3%, crude protein 23.5%,crude fat5.9% crude ash5.9% and crude fibre 3.9% (W/W).

The animal model for the present study was based on multiple administration of low dose of freshly prepared streptozotocin (STZ). For induction of diabetes, initially the normal mice were kept 24 hours without food and water. The weight of normal mice was determined.

Diabetes was induced by multiple intra-peritoneal injection of freshly prepared STZ solution in 0.05 M sodium citrate (pH 4.5) at the dose of 35 mg/kg body weight followed by an hour of fasting. The mice were then allowed to access the respective food and water *ad libitum*. Mice with fasting blood glucose level of 200 mg/dl (7.8 mmol/l) or higher were considered to be diabetic and were used in the study. A parallel set of control mice (non-diabetic) were injected with citrate buffer only.

The mice were grouped into five categories viz., Normal control (NC), Diabetic Control (DC), Diabetic Treated (DT_{150}), Diabetic Treated$_{250}$) and Diabetic Treated (DT_{RZG}). NC received only citrate buffer solution. DC group was STZ induced which received citrate buffer only. DT_{150} and DT_{250} received 150mg/Kg and 250mg/Kg body weight of methanol extract respectively. DT_{RGZ} received rosiglitazone at a dose of 2mg/Kg of body weight. All the mice were fed with common pellet diets for 2 weeks after arrival, and then randomly divided into two groups. One group continued to receive common pellet diets and constituted the normal group; the other was fed with diets high in fat and fructose, in order to induce type-2 diabetes. All the mice had free access to food and water.

For the experiment, the mice were divided into five groups having six mice in each group: DC group (diabetic control mice), NC group (non-diabetic control mice) and three DT group (diabetic mice treated with two different doses of extract as well as rosiglitazone/ kg body weight). Body weights were recorded weekly during the experimental period. Treatment with extracts was started after one week of STZ treatment, which was considered as the 1st day of treatment. Blood samples were taken after 8 hrs fasting from the retro-orbital sinus vein prior to the administration of test substances or the buffer and 4 weeks after the treatment under mild ether anesthesia and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out.

The total cholesterol, total triglyceride, Cholesterol-HDL, Cholesterol- LDL and HDL/TC values were assayed and the results

obtained have been presented in table 1. Data were statically analyzed by mean ± S.E and by one-way ANOVA.

Results and Discussion

From the results it is evident that the diabetic mice had higher total cholesterol (TC) (+137%; $p<0.001$) and TGs (+72%; $p<0.001$) values in comparison to normal control (Table-1 and Fig-1).These changes in biochemical parameters are as expected, as when the uncontrolled diabetic status progresses, substantial changes in total cholesterol and triglycerides values are predictable. Diabetic mice treated with lower dose of *P. niruri* extract (DT_{150}) showed significantly lower values of serum TC (-26.5%; $p<0.001$) and TGs (-55.6%; $p<0.001$), when compared with the DC counterparts. The DT_{250} treatment showed superior lowering effects compared with the DC counterparts as well as DT_{150} group mice by (-34.7%; $p<0.001$) on serum TC levels and (-50%; $p<0.001$) on TGs levels. Contrarily, treatment with rosiglitazone (DT_{RGZ}) showed (-33.2%; $p<0.001$) on TC levels and (-09.6%; $p<0.001$) on TGs levels compared with diabetic control mice (Table-1 and Fig-1)

Relative to normal control, the diabetic mice had higher value of low density lipoprotein (LDL) (+256%; $p<0.001$) while diminished value of high density lipoprotein (HDL) (-54%; $p<0.001$).This is because when the unrestrained diabetic condition advances, considerable changes in these biochemical parameters are as expected and predictable. Diabetic mice treated with lower dose of *P. niruri* extract (DT_{150}) showed significantly lower values of serum LDL (-51%; $p<0.001$) and higher value of HDL (-48.4%; $p<0.001$), when compared with the DC counterparts. All over again, the DT_{250} treatment showed even better lowering effects on LDL (-54%; $p<0.001$) compared with the DC counterparts and improved level of HDL (+53%; $p<0.001$). In contrast, treatment with rosiglitazone (DT_{RGZ}) showed a considerable diminished level of LDL (-57%; $p<0.001$) while improved level of HDL (+55%; $p<0.001$) compared with diabetic control mice.

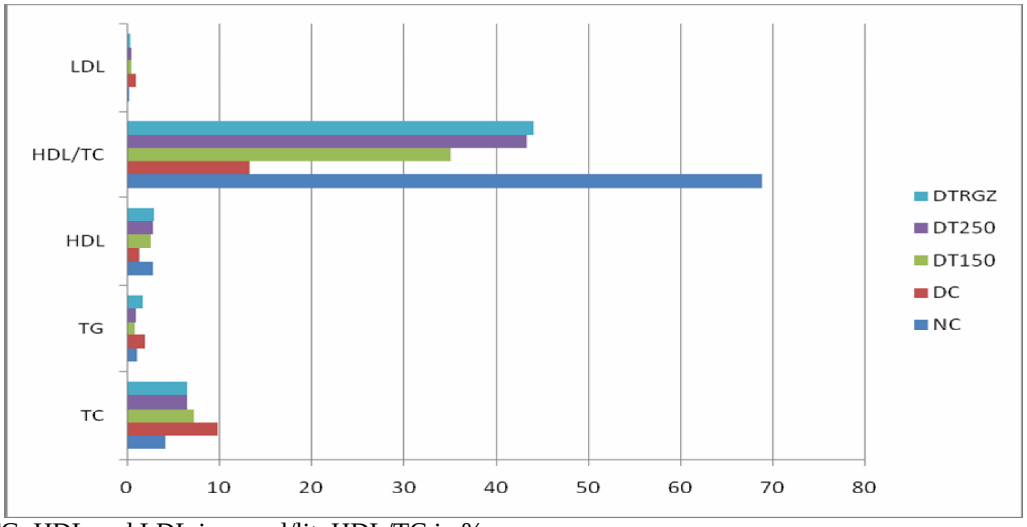
Chronic oral administration of the extract also reduced total cholesterol and triglyceride levels in diabetic and normoglycaemic albino mice consistent with the hypolipidemic effect earlier reported (Khanna et al., 2002). Diabetic dyslipidemia is marked by elevated triglycerides, cholesterol and low density lipoprotein (LDL) particles of altered composition and decreased high density lipoprotein (HDL), and constitutes an important cardiovascular risk factor in diabetics (Agrawal et al., 2006). Reduction in total cholesterol and triglycerides through dietary or drug therapy has been found beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetic patients (Brown et al., 1993; Ahmed et al., 2001). Experimentally, streptozotocin-induced diabetic hyperglycemia is accompanied by increase in serum cholesterol and triglyceride levels (Ahmed et al., 2001) and mimics overt diabetes disease. Thus, in addition to glycaemic control, extract of this plant may further reduce mortality from complications of the disease by ameliorating diabetes induced dyslipidemia. Lipid lowering activity of *P. niruri* alcoholic extracts in triton induced hyperlipidaemia was examined by Chandra et al. (2000). It was observed that administration of triton in mice caused increase in serum cholesterol by 3.5 fold, phospholipids 2 fold and triglyceride 1.2 fold.

Table.1 Showing effects of Different doses of *P. niruri* extract and rosiglitazone on serum lipid profile in STZ induced mice diabetic model

Groups	TC (mmol/lit)	TG (mmol/lit)	HDL (mmol/lit)	HDL/TC (%)	LDL (mmol/lit
Normal control (NC)	**4.15±0.86****	**1.14±0.09****	**2.86±0.29****	**68±4.66****	**0.27±0.04****
Diabetic control (DC)	**9.84±1.56***	**1.96±0.29***	**1.31±0.58***	**13.31±1.97***	**.096±0.16***
***P. niruri* extract (150mg/kg) (DT_{150})**	**7.23±0.44****	**0.87±0.08****	**2.54±0.36****	**35.13±3.37****	**0.47±0.06****
***P. niruri* extract (250mg/kg) (DT_{250})**	**6.42±0.64****	**0.98±0.17****	**2.78±0.46****	**43.39±4.26****	**0.44±0.07****
Rosiglitazone 92mg/kg (DT_{RGZ})	**6.58±1.35****	**1.77±0.17****	**2.90±0.55****	**44.07±5.56****	**0.41±0.09****

*P<0.05 as compared with normal control. **p<0.01 as compared with diabetic control.
TC=Total cholesterol; TG= Triglycerides; HDL= High density lipoprotein; LDL= Low density lipoprotein

Fig.1 Effect of *P. niruri* extract on lipid profile in different mice group



TC, TG, HDL and LDL in mmol/lit. HDL/TC in %

Administration of *P. niruri* at the dose of 200mg/kg simultaneously with triton lowered the level of total cholesterol, phospholipids and triglyceride by 27, 25 and 24 percent respectively. In an experiment with cholesterol fed rats, *P. niruri* at a dose of 100 mg/kg lowered the elevated level of low-density lipoprotein lipids in hyperlipidemic and drug fed animals (Chandra et al., 2000).

In the present study, STZ induction to mice resulted in dyslipidemic changes as illustrated by increasing triglycerides (TGs) and total cholesterol (TC), a finding in accordance with that of Woo *et al.*, (2008). Treatment with rosiglitazone or *P. niruri* extract produced significant decreases in serum TGs and TC in diabetic mice. These results are in agreement with those of Okoli et al. (2010) who reported that treatment

with *P. niruri* extract improves dyslipidemia and decreases oxidative stress, with reduction of cardiac parameters. Treatment with *P. niruri* extract to diabetic mice reduces serum hypertriglyceridemia *via* decreased synthesis of triglycerides by the liver or by inhibition of triglyceride release from the liver. Also treatment with *P. niruri* extract induced marked reductions in total serum cholesterol in diabetic mice (Okoli et al., 2010). The active phytochemicals of *P. niruri* extract have hypercholesterolemic activity that might be mediated through increased cholesterol excretion in the feces. In addition anthraquinone glycosides from the *P. niruri* extract have lipid-lowering effects, resulting in depression of lipid accumulation. It consequently has anti-atherosclerotic properties (Okoli et al., 2010).

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