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Effect of *Phyllanthus fraternus* on Fructose Induced Insulin Resistance in Rats

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Abstract: *Phyllanthus fraternus* Webster (syn. *Phyllanthus niruri*) (PF) plant clinically used as a hepatoprotective, has been found to have wide range of biological activities. The purpose of this study is to examine the prophylactic effect of aqueous extract of (PF) on fructose induced-hyperinsulinemia, hypertriglyceridemia, glucose intolerance and hypertension in insulin resistance state in rats. Two groups of Male Sprague-Dawley rats fed either chow or fructose diets were treated with PF aqueous extract dose (250 mg kg⁻¹ p.o.) or vehicle for three weeks in prophylactic treatment. Body weight changes, feed intake, water intake and blood pressure were recorded during experiment. After the completion of treatment period blood samples were collected for estimation of glucose, insulin and lipid profile and then animals were challenged for OGTT. Fructose feeding for three weeks significantly raised triglyceride levels (p<0.01), blood glucose (p<0.01), insulin levels (p<0.01), mean systolic blood pressure (p<0.001) and total area under the curve (AUC) for glucose during OGTT (p<0.01) compared to chow fed rats. Treatment with PF (250 mg kg⁻¹ p.o.) of fructose fed rats was found to significantly prevent hypertriglyceridemia (p<0.01), hyperglycemia (p<0.01), hyperinsulinemia (p<0.01), hypertension (p<0.01) and increase in total AUC (p<0.01) due to fructose feed. Aqueous extract of PF prevented the metabolic changes and hypertension induced by fructose diet in normal rats.

Key words: *Phyllanthus fraternus*, oral glucose tolerance test (OGTT), hypertriglyceridemia, hyperinsulinemia, fructose feed, blood pressure

INTRODUCTION

Insulin resistances (suppressed insulin-stimulated glucose uptake) are characterized by impaired glucose tolerance, hyperinsulinemia and non-insulin dependent diabetes mellitus (NIDDM) (Reaven, 2003). Insulin Resistance (IR) is a central pathophysiological feature of NIDDM, in addition IR is associated with a common metabolic abnormality in obesity, hypertension, dyslipidemia and atherosclerosis recently the term Syndrome X was coined to characterized the syndromes of IR-hyperinsulinemia (DeFronzo and Ferrannini, 1991). IR can be further exacerbated during the progression of the disease because of the deregulation of lipid and carbohydrate metabolism (Reaven, 1988) and enhances free radical generation which is responsible for substantial morbidity and premature mortality (Thirunavukkarasu *et al.*, 2004). In addition glycemic control and management of hyperinsulinemia is also essential for limiting these after effects of NIDDM.

Although currently available pharmacological modalities are not directed to the treatment of impaired insulin action, recent efforts have focused on the development of insulin sensitizing agents, to be found of PPAR γ agonists (glitazones) are in clinical practice, they are meant to target the problem of insulin resistance and enhance insulin action at the cellular level (Saltiel and Olefsky, 1996) however, some of these drugs are linked to liver toxicity (Jaeschke, 2007). There is an increased demand by patients to used natural products with IR and metabolic disorders. Recently reported numerous herbal drugs like *Gymnema sylvestre*, *Zizyphus jujube*, *Ocimum sanctum* and *Trigonella foenum-graceum* to use with IR and metabolic disorders (Tiwari and Madhusudana, 2002).

Phyllanthus fraternus Webster (Syn. *Phyllanthus niruri*), tropical and subtropical species of genus *Phyllanthus* (Euphorbiaceae) commonly known as Bhuiamlki in India. It has been used for as folk medicine in the treatment of liver, kidney and bladder problem, intestinal parasites and diabetes (The Wealth of India,

1995). Particularly *Phyllanthus fraternus* (PF) herb is bitter in taste and reported to possess diuretic, hypotensive, hypoglycemic effect, antihyperlipemic, antihepatotoxic and anti oxidant activity (Calixto *et al.*, 1998).

An aqueous extract of the leaves (PF) lowers blood sugar level in normal and alloxan diabetic rabbits, (Ramkrishnan *et al.*, 1982) hypoglycemic effects due the presence of phytochemical such as flavonoids (Hukkeri *et al.*, 1988) another study documented PF with Aldose Reductase Inhibition (ARI) properties (Shimizu *et al.*, 1989). This activity also supports PF traditional use for diabetes. Different fractions of alcoholic extracts of aerial parts and root of PF were screened for antihepatotoxic activity on carbon tetrachloride (CCL₄) induced liver damage (Ahmed *et al.*, 2002) and also reported allyl alcohol-induced oxidative stress in liver mitochondria (Sailaja and Setty, 2006). PF a clinically used as a hepatoprotective has been found to have wide range of biological activity. However, the effect of PF on IR state is not documented. The purpose of this study is to examine the effect of PF on glucose metabolism and hypertension in IR state.

MATERIALS AND METHODS

Plant material and preparation of aqueous extract:

Authenticated free gift sample of *Phyllanthus fraternus* Webster (syn. *Phyllanthus niruri*) belonging to family Euphorbiaceae was obtained from Himalaya Drug Company Makhali, Bangalore. The whole plant powder (400 g) was macerated with distilled water (2 L) for 7 days with occasional shaking to get the aqueous extract. The extract was then filtered through a cheese cloth then the filtrate transferred to a china dish and evaporated on thermostat controlled water bath at 40°C. The percentage yield of aqueous extract obtained was 18% w/w. In order to determine the presence of different natural compound a preliminary phytochemical study was performed (Harborne, 1998).

Animals: Albino rats (Male Sprague- Dawley) weighing 180-200 g, procured from Sri Venkateswara Enterprises, Bangalore India, were acclimatized for 7 days to the housed individually in an environmentally controlled room temperature (25±1°C) and relative humidity of 45-55% under 12-h light/dark cycle and had free access to water and rodent food pallet *ad libitum* (Lipton, India). All experiment performed at Department of Pharmacology, K.L.E.Ss College of Pharmacy Belgaum India from 2005 to 2006. All experimental procedure were in accordance with

committee for the purpose of control and supervision of experiments on animals (CPCSEA) Chennai, India and this study was revived and approved (Resolution No.-14/24-01-2005) by the institutional animal ethics committee (IAEC).

Experimental design: Male Sprague-Dawley rats initially weighing 200-210 g were used for the experiments. Prior to dietary manipulation, all rats were fed standard pellet rodent diet in addition; rats were acclimatized to the procedure of blood pressure measurement at 16:00 h daily for 1 week. Following the training period, rats were divided into different groups of seven each (n = 7) for the prophylactic testing as shown below.

Daily feed intake, water intake, body weight and weekly blood pressure were measured. At the end of prophylactic treatment period (22nd day) oral glucose tolerance test was carried after 16 h fasting (blood samples were collected by retro-orbital plexus) and initial blood samples (separated plasma) were used for biochemical estimations.

- **Group 1:** C (Received standard chow diet for three weeks)
- **Group 2:** CT (Received standard chow diet plus PF aqueous extract dose 250 mg kg⁻¹ p.o.) once daily for three weeks.
- **Group 3:** F (Received fructose rich diet for three weeks)
- **Group 4:** FT (Received fructose rich diet plus PF aqueous extract dose 250 mg kg⁻¹ p.o.) once daily for three weeks

Fructose feeding: The fructose content provided 60% of total calories in the diet prepared in the laboratory with the following composition (g kg⁻¹) Casein, high protein-207.0; DL-methionine-3.0; fructose-600.0; lard-50.0; cellulose-79.81; mineral mix-50.0; zinc carbonate-0.04; vitamin mix-10. Animals were maintained on these regimens for 3 weeks.

Biochemical measurements: Glucose levels were determined by the glucose oxidase peroxidase method using a kit (Span Diagnostic Ltd Sachin, Surat). The plasma insulin was measured by standard radio immunoassay technique using a standard kit obtained from BRIT, BARC, Mumbai, India. Estimation of total cholesterol and HDL by using a standard kit obtained from Accurex Biomedical Pvt. Limited, as well as triglyceride was estimated by using kit method obtained

from ERBA diagnostics Manheim and serum sodium was estimated by kit method using standard kit obtained from Crest Biosystems.

Oral glucose tolerance test and Insulin sensitivity index:

The Oral Glucose Tolerance Test (OGTT) was performed measuring plasma glucose (Cordai *et al.*, 2003) at the end of the study period in each group. The rats were fasted for 16 h and blood samples were collected by retro orbital plexus. A dose of 2 g kg⁻¹ (body weight) glucose solution was given by gastric gavages. Blood samples were obtained from the retro orbital plexus at pre- and 30, 60 and 120 min post- glucose intake. Plasma glucose levels were measured by the glucose oxidase reaction method.

From the blood glucose and insulin levels, the degree of insulin resistance estimated by; using Homeostasis Model Assessment (HOMA) as an index of insulin resistance, as calculated by the following formula (Bergman *et al.*, 2003):

$$\frac{\text{Insulin } (\mu\text{U}) \times \text{Glucose } (\mu\text{mol L}^{-1})}{22.5}$$

Measurement of systolic blood pressure: Systolic blood pressure was measured in conscious rats by the tail cuff method (Hwang *et al.*, 1987). All the rats were pre conditioned to the experimental conditions before actual measurements were conducted, at the time of experiment, the rats were placed in a constant temperature (32°C) chamber for 30 min. Thereafter, the animal were put in a rat holder. The tail cuff and pulse sensor were placed on the tail and were connected to a rat-tail blood pressure monitor (Harvard Apparatus). The pressure in the cuff was displayed on a computer connected with blood pressure monitor. Systolic blood pressure was measured at the point where the reappearance of pulsations is detected by the pulse sensor, for each rat, eight individual readings were obtained. The highest and the lowest measurements were discarded average of remaining was taken as the individual systolic blood pressure.

Statistical analysis: Results are expressed as the Mean±SEM. Statistical analysis was carried out using unpaired student t test and one-way analysis of variance followed by Newman-Keuls test. p<0.05 was considered to be significant

RESULTS

Phytochemicals: The percentage yields of crude aqueous extracts of PF were found 18%w/w and the phytochemical screening crude aqueous extracts revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponin, protein and amino acids.

General characteristics: At the beginning of the study there was no significant difference in general characteristics (mean body weight, food intake and water intake) among the all groups. After three weeks of fructose fed rats was significantly reduced both body weight and feed intake. The weight gain effect on normal chow fed with PF aqueous extract with dose 250 mg kg⁻¹ p.o. has been barred the increase in body weight as compared with control chow fed rats (Table 1).

Fasting plasma glucose, insulin level and Oral glucose tolerance test: Fructose feeding for three weeks in rats showed significantly increased level of plasma glucose and insulin as compared to chow fed rats. Treatment with PF aqueous extract dose (250 mg kg⁻¹ p.o.) in fructose fed rats significantly prevented the increase plasma glucose and insulin level as compared to untreated fructose fed rats, Whereas treatment in chow fed rats with PF dose did not show any change in plasma glucose levels as compared to untreated chow fed rats (Table 1).

The analysis of the oral glucose tolerance test (Fig. 1a) and the comparison between total areas under curve of glycemia evidenced that fructose fed rats developed glucose intolerance assessed by significantly

Table 1: Effects of PF aqueous extract (250 mg kg⁻¹ p.o.) dose on various parameters in four groups of rats after treatment for three weeks with standard chow diet (C), standard chow diet plus PF dose 250 mg kg⁻¹ p.o. (CT), fructose rich diet (F) and fructose rich diet plus PF dose 250 mg kg⁻¹ p.o. (FT) once daily (Values expressed as Mean±SEM, n = 7)

Parameters	C	CT	F	FT
Body weight (g)	299.70±9.22	266.70±9.74 [†]	247.50±4.97***	242.00±4.18
Feed intake (g day ⁻¹)	22.20±1.2	20.16±0.72	14.36±0.94***	16.10±1.27
Water intake (mL day ⁻¹)	27.43±2.19	30.67±4.21	26.71±1.08	21.00±0.63
Plasma glucose (mg dL ⁻¹)	96.80±4.03	84.46±6.27	124.30±8.05**	88.32±3.46 ^{##}
Plasma insulin (μU mL ⁻¹)	27.43±4.19	19.57±3.16	44.86±2.75**	24.86±3.82 ^{##}
Insulin sensitivity index	6.55±0.99	3.60±0.61	13.79±1.33	5.48±0.96
Triglyceride	59.56±9.28	62.55±10	100.00±4.76**	75.89±5.2 ^{##}
Total cholesterol	51.34±8.48	58.80±2.06	64.20±2.05	58.87±4.14
HDL	16.95±2	19.53±1.97	14.83±2.95	23.05±1.37 ^{##}

F compared with C using student test ** (p<0.01) and *** (p<0.001), ANOVA followed by Newman-Keuls test [†] (p<0.05) compared with C, ^{##} (p<0.05) and ^{###} (p<0.01) compared with F

increased total area under curve values after fructose feeding for as compared to chow fed rats. Effect of treatment with PF dose (250 mg kg⁻¹ p.o.) significantly prevented the increase in total area under curve values due to fructose feeding (Fig. 1b).

Insulin resistance index: Fructose feeding in rats for three weeks showed increased HOMA-IR levels as compared to chow fed rats. Treatment with PF (250 mg kg⁻¹ p.o.) in fructose fed rats decreased significantly HOMA-IR values as compared to untreated fructose fed rats (Table 1).

Lipid profile: Fructose feeding for three weeks showed significantly increased plasma triglyceride level, but did not affect plasma total cholesterol and HDL levels as

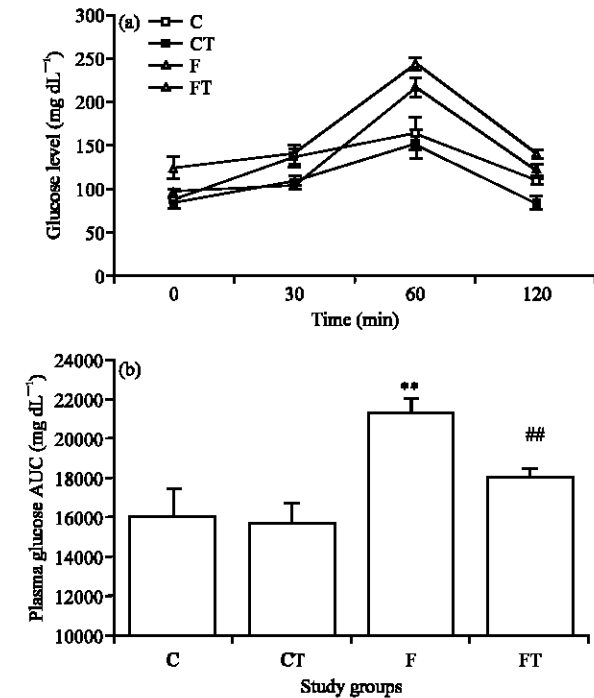


Fig. 1: Effects of PF aqueous extract (250 mg kg⁻¹ p.o.) dose on OGTT (A) and effects on AUC (B) in four groups of rats after treatment for three weeks with standard chow diet (C), standard chow diet plus PF dose 250 mg kg⁻¹ p.o. (CT), fructose rich diet (F) and fructose rich diet plus PF dose 250 mg kg⁻¹ p.o. (FT) once daily. (Values expressed as Mean±SEM, n = 7). (a) OGTT and (b) AUC. FF compared with C using student test ** (p<0.01), ANOVA followed by Newman-Keuls test. ##(p<0.01) compared with F

compared to untreated chow fed rats. The treatment with PF dose (250 mg kg⁻¹ p.o.) in FF rats significantly prevented the increase in triglyceride levels and decrease in HDL- cholesterol level due to fructose feeding but did not affect total cholesterol levels as compared to untreated fructose fed rats (Table 1).

Blood pressure: It was observed that the blood pressure was similar in all the experimental groups before the dietary intervention no change in blood pressure was observed in chow fed control rats after three weeks. Fructose feeding in rats resulted in a progressive increase in mean systolic blood pressures, which reached an average of 138.9 mmHg after three weeks as compared to untreated chow fed rats. Treatment with PF dose (250 mg kg⁻¹ p.o.) in fructose fed rats completely prevented the rise in mean systolic blood pressure as compared to untreated fructose fed rats (Fig. 2).

Serum sodium level: Fructose feeding in rats for three weeks showed increased the levels of serum as compared to chow fed rats, prophylactic treatment with PF dose (250 mg kg⁻¹ p.o.) in fructose fed rats significantly reduced serum sodium level as compared to untreated fructose fed rats (Fig. 3).

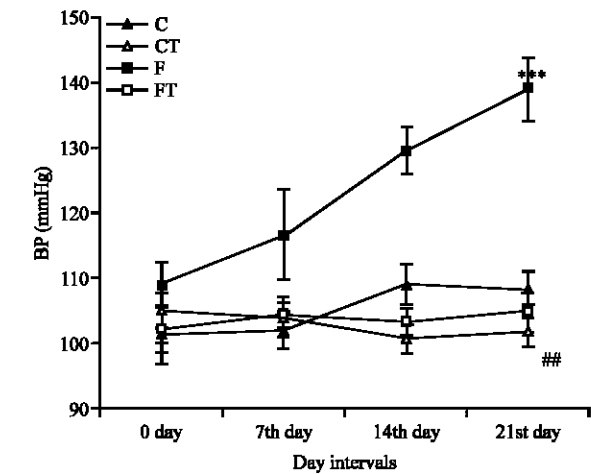


Fig. 2: Effects of PF aqueous extract (250 mg kg⁻¹ p.o.) dose on blood pressure in four groups of rats after treatment for three weeks with standard chow diet (C), standard chow diet plus PF dose 250 mg kg⁻¹ p.o. (CT), fructose rich diet (F) and fructose rich diet plus PF dose 250 mg kg⁻¹ p.o. (FT) once daily (Values expressed as Mean±SEM, n = 7). F compared with C using student test ***(p<0.001), ANOVA followed by Newman-Keuls test ##(p<0.01) compared with F

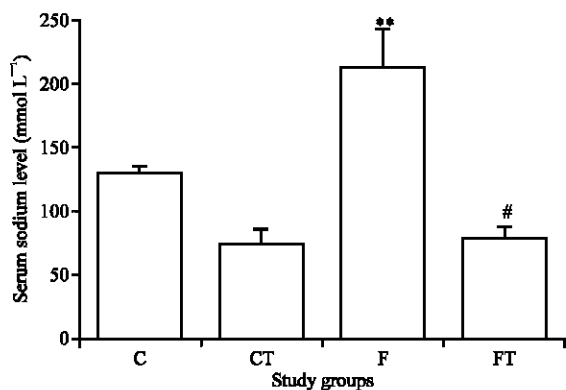


Fig. 3: Effects of PF aqueous extract (250 mg kg⁻¹ p.o.) dose on serum sodium level in four groups of rats after treatment for three weeks with standard chow diet (C), standard chow diet plus PF dose 250 mg kg⁻¹ p.o. (CT), fructose rich diet (F) and fructose rich diet plus PF dose 250 mg kg⁻¹ p.o. (FT) once daily. (Values expressed as Mean±SEM, n = 7). FF compared with C using student test ** (p<0.01), ANOVA followed by Newman-Keuls test # (p<0.05) compared with F

DISCUSSION

Hyperinsulinemia affects the development and clinical course of at least three major related diseases namely: non- insulin dependent diabetes mellitus, essential hypertension and coronary heart diseases, (Reaven, 1988) in addition, it is a common metabolic abnormality in obesity and dyslipidemia (DeFronzo and Ferrannini, 1991). Numerous studies in rats have demonstrated that chronic fructose feeding induces a state of hyperinsulinemia, hyperglycemia and hypertriglyceridemia with loss of *in vivo* insulin sensitivity (Hwang *et al.*, 1987; Elliott *et al.*, 2002). Fructose does not stimulate insulin secretion from pancreatic β -cells (Elliott *et al.*, 2002) the subsequent hyperglycemia, along with increase in glucose-dependent insulin tropic polypeptide and glucose like peptide-1 secreted from the gut stimulate pancreatic insulin secretion, causing an acute rise in plasma insulin concentrations (Cordai *et al.*, 2003).

Results of our study confirms these observations as feeding of fructose rich diet for 21 days raised triglyceride levels (+68%), blood glucose (+28%) and insulin levels (+64%) these are cardinal features of syndrome X. Development of hypertension is secondary to hyperinsulinemia in fructose fed rats (Hwang *et al.*, 1987) and it is evident in our study. Fructose feeding in rats resulted in a progressive increase in mean systolic blood

pressures, which reached an average of 138.9 mm Hg⁻¹ (+28%) after 21 days as compared to chow fed rats. Possible reasons for development of hypertension in fructose fed rats include activation of central sympathetic nervous system, (Sechi *et al.*, 1997) and renin angiotensin system (Kamide *et al.*, 2003) and increased sodium retention (Iyes and Katovich, 1996). The importance of this model can be gauged from the fact that it has been used for assessing the therapeutic efficacy of presently available insulin sensitizers; therefore this animal model was selected for the study.

Whereas treatment of PF dose (250 mg kg⁻¹ p.o.) on fructose fed rats was significantly prevent hypertriglyceridemia (-24%), hyperglycemia (-29%), hyperinsulinemia (-45%) and hypertension (-29%) as compared to untreated FF rats. This dose of PF significantly prevented all biochemical changes and hypertension suggesting preventive effect of PF in this model of hypertension with IR.

On the other hand, traditional medicinal plants with various active principles and properties have been used since ancient times to treat a great variety of human diseases. The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of β -cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics. Recently reported numerous herbal drugs like *Gymnema sylvestre*, *Zizyphus jujube*, *Ocimum sanctum* and *Trigonella foenum-graceum* to use with IR and metabolic disorders (Tiwari and Madhusudana, 2002).

Geranin, a compound isolated from this plant is reported to have ACE inhibitory property (Shimizu *et al.*, 1989). ACE inhibitors have been reported to reduce hypertension and increase insulin sensitivity in FF rats (Navarro-Cid *et al.*, 1995). So we have state that the antihypertensive property of the PF may be attributed to the presence of this compound. Sodium retention is one of the main mechanisms by which hypertension are developed in fructose fed rats (Iyes *et al.*, 1996). In the present study, PF significantly reduced sodium retention (-63%) in fructose fed rats, this could be another possible explanation for reduction in mean systolic blood pressures in PF treated FF rats.

Oxidative stress plays an essential role in the development of hyperinsulinemia and insulin resistance in fructose fed rats (Thirunavukkarasu *et al.*, 2004) and chronic feeding of rats with a diet high in fructose causes high blood pressure, IR and oxidative stress as revealed by increasing lipid per oxidation (DeFronzo and

Ferrannini, 1991; Thirunavukkarasu *et al.*, 2004). It is also reported that oxidative stress in turn leads to mitochondrial damage (Evans *et al.*, 2003) PF is known to possess antioxidant property and protection against mitochondrial dysfunction (Sailaja and Setty, 2006). More recently studies have found that anti oxidants, especially α -lipoic acid improve insulin sensitivity in FF rats (Thirunavukkarasu *et al.*, 2004). Several clinical trials, albeit small and short duration, have also demonstrated that treatment with vitamin E, vitamin C or glutathione improves insulin sensitivity in IR state (Evans *et al.*, 2003). Thus antioxidant property of PF may be effective against fructose feed induced insulin resistance.

In this study we have found that treatment of fructose fed rats with PF dose of 250 mg kg⁻¹ p.o. was found to be effective in prophylactic treatment they significantly prevented all biochemical changes and hypertension. This study gives an experimental evidence for the use of PF in the treatment of insulin resistance.

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