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Hypoglycaemic effect of fractions and crude methanolic leaf extract of *Phyllanthus fraternus* in streptozotocin - induced diabetic and normal rats

Nadro, M. S.[1]* and Elkanah, G.[2]

[1]Department of Biochemistry,Gombe State University, Gombe, Nigeria.
[2]Department of Biochemistry, Modibbo Adama University of Technology, PMB 2076, Yola, Nigeria.

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Treatments of diabetes with available agents come with one or more side effects, hence, the need for continual search of alternative treatment agents from medicinal plants. This study was designed to analyse qualitatively and quantitatively some phytochemicals in methanolic extract in *Phyllanthus fraternus* and evaluate their hypoglycaemic activity in both diabetic and normal rats. Sixty-six rats were used of which forty-two were diabetic. Diabetes was induced by intraperitoneal administration of 60 mg/kg body weight of streptozotocin (STZ). Thirty male rats of which twenty-four were diabetic were divided into five (5) groups of six rats each were used for prolonged treatment: Normal, diabetic control, standard control, and two treatments that were orally administered at a dose of 200 and 300 mg/kg body weight of crude methanolic leaf extracts of *P. fraternus* for 28 days. Thirty six (36) rats were used for oral glucose tolerance test (OGTT) which was divided into six groups of three rats each for both normal and diabetic rat. A single dose of 200 mg/kg body weight of crude and fractions (I, II and III) of methanolic leaf extracts of *P. fraternus* were orally administered to diabetic and normal rats before they were loaded with 2 g/kg body weight glucose. The results of phytochemical screening of the crude extract showed the presence of compounds like alkaloids, flavonoids, terpenoids, phenols and saponins. Fraction I contained only flavonoid, fraction II and III contained more than three phytochemicals. Oral administration of 200 and 300 mg/kg body weight of methanolic extracts to diabetic rats significantly reduced ($p < 0.05$) serum glucose levels in all the treatment groups. The results of OGGT showed that fraction I and metformin groups significantly ($p<0.05$) lowered blood glucose level 30 min after glucose load in both diabetic and normal rats when compared with their controls and other treatments groups. These results suggest *P. fraternus* methanolic leaf extract have phytochemicals with glucose lowering ability especially fraction I that competes favorably with metformin.

Key words: Streptozotocin, diabetes, *Phyllanthus fraternus*, phytochemicals, oral glucose tolerance test, *(*OGTT).

INTRODUCTION

Diabetes mellitus is described by world health organization (WHO) as a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (WHO, 2014). This prolonged high blood sugar levels arises either because insulin production is insufficient, or because the body's cell do not respond properly to insulin, or both. Patients with high blood sugar will typically experience frequent urination (polyuria),

increasingly thirsty (polydipsia) and distinctly hungry (polyphagia) (Hayat et al., 2010).

Diabetes can cause many complications if left untreated (WHO, 2013). Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma (Kitabchi et al., 2009). Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes (WHO, 2013). Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with a healthful diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).In spite of these advances and effort made towards treating, managing, and perhaps preventing the health, economic and social effects of diabetes mellitus, the prevalence of the disease globally is on the increase. As of 2014, an estimated 387 million people have diabetes worldwide as contained in the report of International Diabetes Federation (IDF, 2014), with type 2 diabetes making up about 90% of the cases. This is equal to 8.3% of the adult population, with equal rates in both women and men (Vos et al., 2013). In the years 2012 to 2014, diabetes was estimated to have resulted in 1.5 to 4.9 million deaths per year respectively (WHO, 2013; IDF, 2014). The number of people with diabetes is expected to rise to 592 million by 2035 (IDF, 2014). The global economic cost of diabetes in 2014 was estimated to be $612 billion USD (IDF, 2013). Hence, there is an urgent need for new therapeutic drug with high efficacy, low cost, little or no side effects and wider availability if this trend must be reversed. Many plants have been studied in search for antidiabetic activity, some components isolated, but with respect to *P. fraternus,* there has been little scientific record to support its anti diabetic activity and to some extent, its active components.

P. fraternus belongs to the family Phyllanthaceae. It has been used in folk medicine for the treatment of liver, kidney and bladder problem, intestinal parasites and diabetes (The Wealth of India, 1995). Particularly *P. fraternus* 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 lowers blood sugar level in normal and alloxan diabetic rabbits (Ramkrishnan et al., 1982). Different fractions of alcoholic extracts of aerial parts and root of *P. fraternus* were screened for antihepatotoxic activity on carbon tetrachioride (CCL_4) induced liver damage (Ahmed et al., 1998). The aim of this study is to analyse qualitatively and quantitatively some phytochemical components of the *P. fraternus* methanolic leaf extract and its fractions and to evaluate the hypoglycaemic efficacy of fractions of methanolic leaf extract of *P. fraternus* on streptozotocin – induced diabetic rats.

MATERIALS AND METHODS

Plant material

The plant material of *P. fraternus* Webster (Leaves) was collected in the month of May, around 6 am at Hayin gada, in Girei local Government area of Adamawa State which lies on geographical location 9° 21’53.19’’North and 12° 33’28.33’’East Google earth (2014). It was authenticated by a botanist in the Department of Biological Sciences, Modibbo Adama University of Technology, Yola, Adamawa State.

Experimental animals

Male albino rats (5 - 6 weeks) weighing 100 to 130 g numbering 66 were obtained from Veterinary Research Institute VOM, Jos, Plateau State and kept in plastic cages with 12 h dark/light cycle, fed with pelletized grower diet (Vital Feeds, UACN) and given water *ad libitum*. 30 rats were used for prolonged treatment (28 days) while 36 rats were used for OGTT (18 each for diabetic and normal rats).

Equipments

The following equipments were used; electronic balance (Golden mettle-2G2-USA), ACCU-CHEK Glucometer (GC-Roche Diagnostic-Germany) , ACCU-CHEK test strips (Roche Diagnostic-Germany), spectrophotometer (Vis spectrophotometer 721- PEC Medical USA), water bath (HH-2 B-Scientific), column (5cm diameter), silica gel/TLC- cards (Fluka- Germany).

Chemicals

Hexane, dichloromethane and methanol (Sigma-Aldrich Chemie GMbH, Germany), streptozotocin (Tocris Bioscience London), metformin, chloroform, silica gel (Sigma Aldrich-Germany). All other chemicals used were of analytical grade.

Preparation of plant material

The fresh plant material (leaf) was washed with tap water and shade dried for seven days. It was made into powder using mortar and pestle. The powdered plant material was used for the preparation of methanolic extract.

Preparation of methanolic extract

Methanolic extract was prepared by suspending 200 g of thepowdered sample in 2 L of methanol for 24 h with vigorous shaken intermittently, after which it was filtered and then concentrated at 55°C using a water bath (Ugwu et al., 2011).

*Corresponding author. E-mail: msnadro@yahoo.com. Tel: +2348053472090.

Table 1. OGTT following single administration of crude and fractions of methanolic leaf extract of Phyllanthus fraternus in diabetic rats.

Groups	Treatment
Group-A (Diabetic control)	No treatment
Group-B (Standard control)	Metformin 5 mg/kg body weight
Group-C (Fraction I)	Fraction I (200 mg/kg body weight)
Group-D (Fraction II)	Fraction II (200 mg/kg body weight)
Group-E (Fraction III)	Fraction III (200 mg/kg body weight)
Group-F (Crude)	Crude extract 200 mg/kg body weight

Table 2. OGTT following single administration of crude and fractions of methanolic leaf extract of Phyllanthus fraternus in normal rats.

Groups	Treatments
Group -A (Normal control)	No treatment
Group- B (Standard control)	Metformin 5 mg/kg body weight
Group-C (Fraction I)	Fraction I (200 mg/kg body weight)
Group-D (Fraction II)	Fraction II (200 mg/kg body weight)
Group-E (Fraction III)	Fraction III (200 mg/kg body weight)
Group-F (Crude)	Crude Extract 200 mg/kg body weight

Fractionation of the extract by column chromatography

Twenty gram (20 g) of the methanolic leaf extract was subjected to column chromatography for the isolation of the phytoconstituents. Slurry was prepared by dissolving 200 g silica gel in 600 ml hexane (Sarah and Ayesha, 2003).

The fractions were collected and labeled accordingly. Six fractions were obtained, but on subjecting them to thin layer chromatography (TLC), they were pulled together into three broad fractions based on the number of components and retention factor (RF) values of the components in each fraction. The resultant fractions were concentrated by placing them in an oven at a regulated temperature of 40°C. The dried fractions obtained were kept in air tight containers which were later used for OGTT.

Qualitative phytochemical analysis

The qualitative phytochemical screening of the extract was carried out as described by Harborne (1973), Nweze et al. (2004) and Senthilkumar and Reetha (2009). The plant extract was screened for carbohydrates, alkaloids, flavonoids, steroids, phenols, tannins, saponins, terpenoids, glycosides, and proteins.

Induction of diabetes mellitus in rats

All the rats were fasted overnight before the administration of Streptozotocin. Diabetes was induced in rats by intra-peritoneal injection of streptozotocin dissolved in distilled water at a dose of 60 mg/kg body weight (Al-Hariri et al., 2011). After the injection, the rats were allowed free access to food and water. To prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were given 5% glucose solution water for next 24 h (Barry et al., 1997). The animals were tested after 72 h of streptozotocin administration. The rats with fasting blood glucose more than 300 mg/dl were considered diabetic and were used for the experiment (Akbarazadeh et al., 2007, Parthasarthy and Ilavarasan, 2009).

Experimental design

Evaluation of hypoglycaemic activity following long term treatment: 30 rats (6 normal and 24 diabetic) were divided into 5 groups of six rats each:

Group 1: Normal control
Group 2: Diabetic control
Group 3: Metformin 5 mg/kgb.w.
Group 4: Diabetic rats treated orally with 200 mg/kg body weight of methanolic leaf extract of *P. fraternus.*
Group 5: Diabetic rats treated orally with 300mg/kg body weight methanolic leaf extract of *P. fraternus*

Estimation of fasting blood glucose level was done on a weekly base for four weeks using a glucometer.

The effect of crude and fractions of methanolic leaf extract of *P. fraternus* on oral glucose tolerance test (OGTT) in diabetic rats is determined as shown in Table 1

The effect of crude and fractions of methanolic leaf extract of *P. fraternus* on oral glucose tolerance test (OGTT) in normal rats is determined as shown in Table 2.

After 30 min of fractions, metformin and crude extract administrations, the rats in all groups were given glucose (2 g/kg body weight). Glucose of blood sample from tail vein was estimated by using glucometer at 0, 30, 60, 90, 120 and 150 min.

Administration of extracts

The extract was administered orally using gastric tube on daily basis for 28 days (long term treatment) while in OGTT, it was a single administration.

Statistical analysis

Values obtained were expressed as mean ± SEM and data were analysed using analysis of variance (ANOVA) with Bonferroni Post

Table 3. Some phytochemicals detected in fractions/crude methanolic leaf extract of *Phyllanthus fraternus.*

Phytochemical	Crude	Fraction I	Fraction II	Fraction III
Saponins	+	-	+	-
Tannins	+	-	+	+
Terpenoids	+	-	+	+
Flavonoids	+	+	-	-
Alkaloids	+	-	+	-
Glycosides	-	-	-	-
Steroids	+	-	-	-
Phenols	+	-	+	+
Protein	+	-	-	+

+ = Present; - = absent.

Table 4. Concentrations of some phytochemicals in methanolic leaf extract of *P. fraternus.*

Phytochemical	Concentrations (mg/g)
Saponins	136.00 ± 0.02
Tannins	37.20 ± 0.02
Terpenoids	6.60 ± 0.04
Flavonoids	159.20 ± 0.05
Alkaloids	96.50 ± 0.01
Phenols	152.00 ± 0.03

Values are mean ± SEM (n=3).

hoc test multiple comparison versus control groups with help of Statistical Package for the Social Sciences (SPSS) software version 21. The values $p< 0.05$ were considered significant (Duncan et al., 1977).

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Results of the qualitative phytochemical screening of the crude/fractions of methanolic leaf extract of *P. fraternus* are presented in Table 3.

Quantitative phytochemical estimation

Results of quantitative estimation of some of the phytochemicals in *P. fraternus* methanolic leaf extract (Table 4).

Effects of *P. fraternus* methanolic leaf extract on blood glucose level in streptozotocin-induced diabetic rats

In prolonged treatments (28 days) (Figure 1), the fasting blood glucose for all the treatments group (except normal control) were all slightly above 350 mg/dl on the initial day (day 0). However, the curve for diabetic untreated group was shown to be at relatively steady level throughout the treatment period. Other treatment groups have shown decrease in fasting blood glucose level from day 7 of treatments with metformin showing the most hypoglycaemic effect. Treatment with Methanolic extract at 300 mg/kg body weight was shown to have the same hypoglycaemic effect with metformin at day 21 of the treatment. On the final day of treatment (day 28), there was no significant difference among all the treatment groups with the fasting blood glucose level of all groups below 150 mg/dl.

Hypoglycaemic effects of fractions of methanolic leaf extract of *P. fraternus* in Streptozotocin induced diabetic rats

The hypoglycaemic effects of the three fractions (F I, F II and F III) obtained were carried out in diabetic rats following a glucose load and from the result obtained (Figure 2), the blood glucose level increases rapidly in all the groups 30 min after commencement of the glucose tolerance test, but fraction one (FI) and the standard

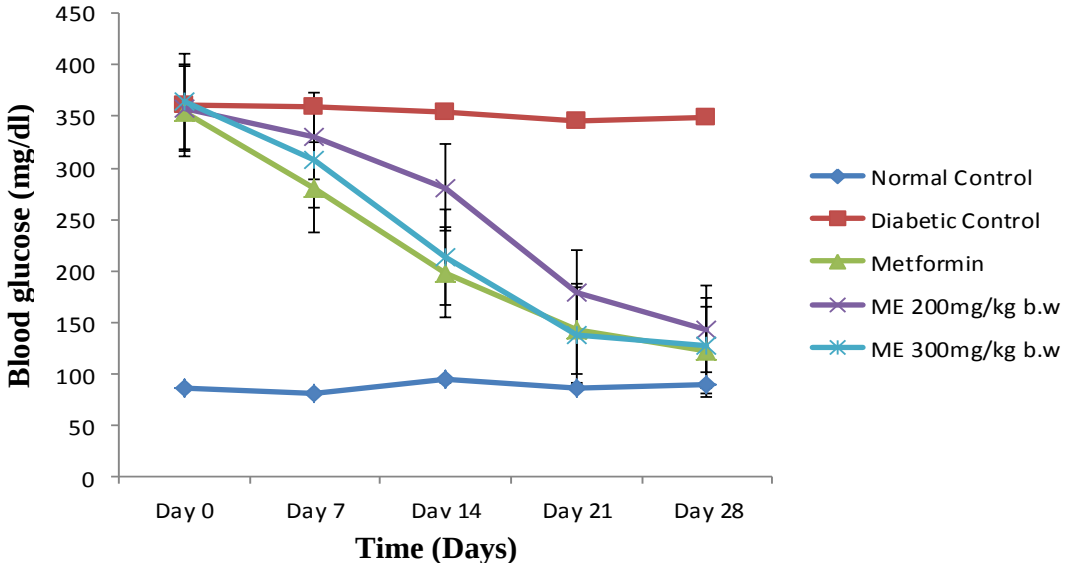

Figure 1. Effects of methanolic leaf extract of *P. fraternus* on fasting blood glucose level (mg/dl) in Streptozotocin - induced diabetic rats.

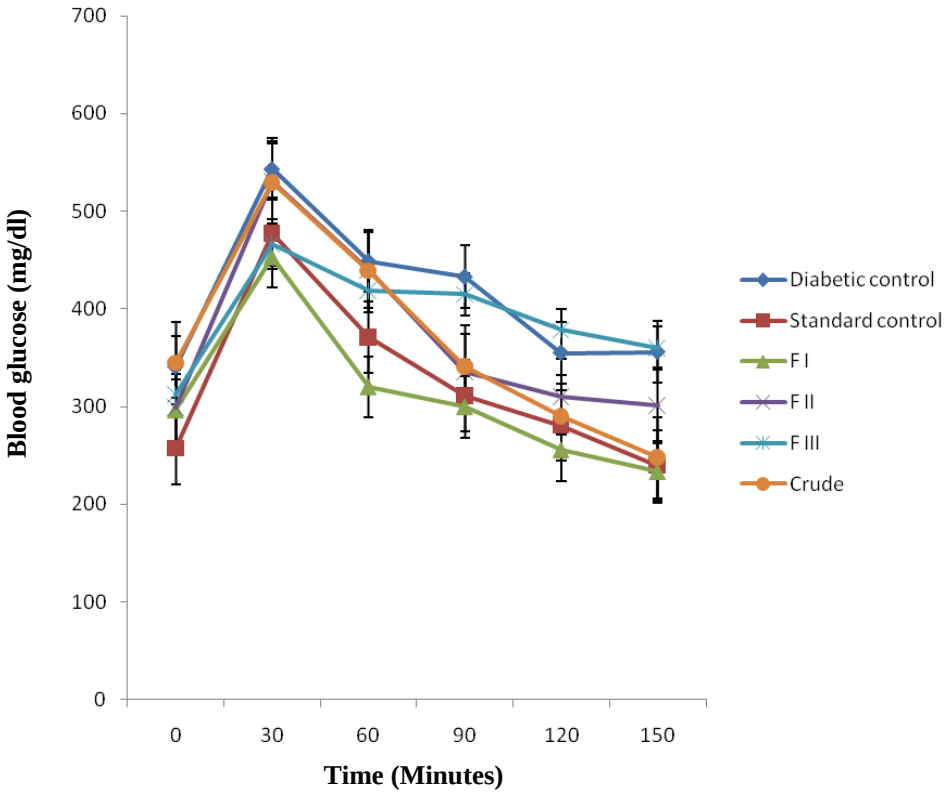

Figure 2. Effect of pre-treatment with 200 mg/kgbw of methanolic leaf extract of *P. fraternus* on oral glucose tolerance test in STZ-induced diabetic rats.

control (metformin) were significantly lower at $p<0.05$ when compared against the diabetic control. There was sharp fall in the glucose level in both metformin and FI treated groups with FI treated group giving the most

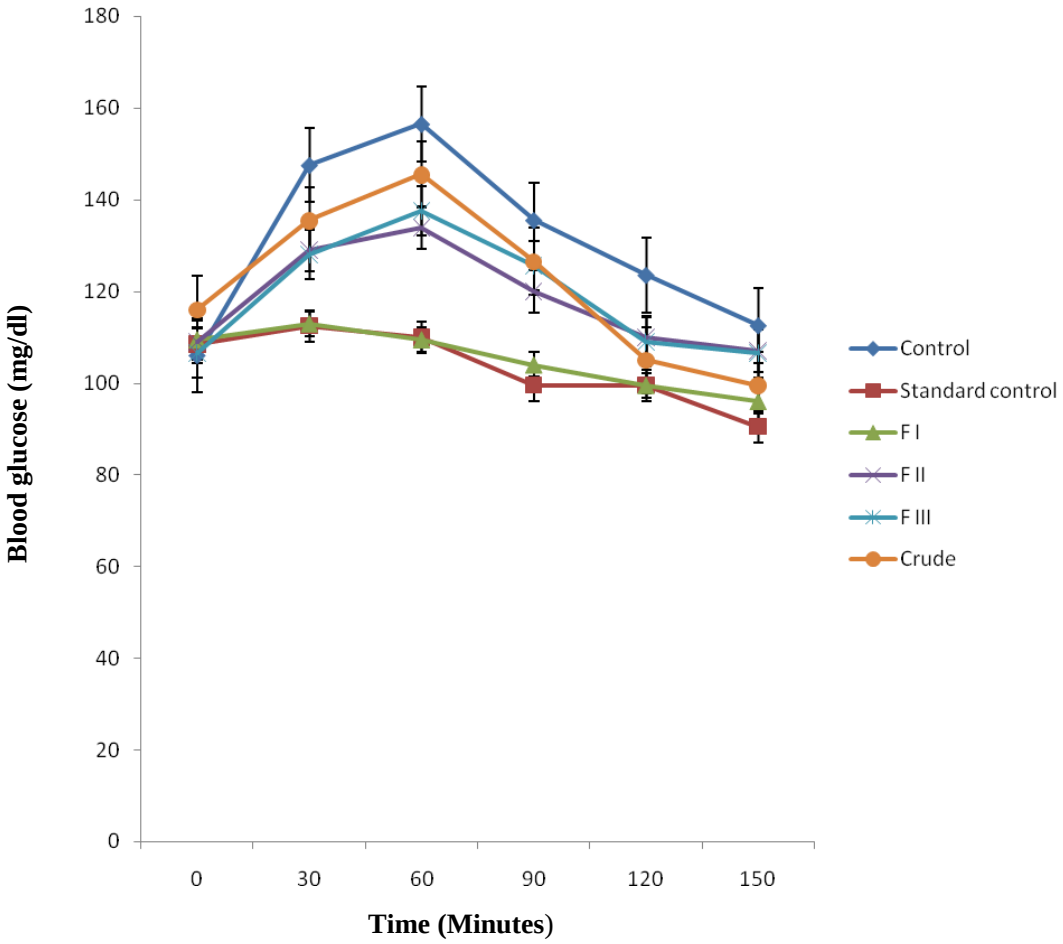

Figure 3. Effect of pre-treatment with 200 mg/Kgbw of methanolic leaf extract of *P. fraternus* on oral glucose tolerance test in normal rats.

glucose tolerance all through the test period followed by metformin. Fraction three (FIII) showed the least hypoglycaemic effect all through the period of the test, at 150 min, FIII and diabetic control showed no difference. On the other hand, crude extract and FII had similar effect at 90 min, but FII maintained a relatively steady level through the remaining period while the crude extract further decreases the glucose level. The crude extracts showed maximum hypoglycaemic effects at 150 min where it exerts similar hypoglycaemic effect with metformin and FI.

Hypoglycaemic effects of fractions of *P. fraternus* extract in normal rats

Treatments with different fractions of *P. fraternus* extract prior to glucose load in normal rats have shown positive hypoglycaemic effects after glucose load Figure 3. The control (untreated group) has shown a rapid increase in blood glucose in the first 60 min after glucose load before showing gradual decrease at 90 min and finally going back almost to the initial value at 150 min.

Treatment with metformin (Standard control) and Fraction I prevented glucose induced hyperglycaemia 30 min after commencement of the glucose tolerance test. Treatment with metformin have significantly ($p<0.05$) lowered the blood glucose level at 30, 60 and 90 min of the tolerance test when compared with other treatments except for fraction I which in all cases showed similar hypoglycaemic effect with the standard control (metformin). More so, fraction I had significantly decreased blood glucose level ahead of the crude extract at 30, 60 and 90 min. All the treatment groups showed similar hypoglycaemic effect at 120 min differing significantly ($p<0.05$) against the normal control.

DISCUSSION

Phytochemical screening of *P. fraternus* methanolic extract revealed the presence of alkaloids, flavonoids,

tannins, saponins, steroids, phenols, carbohydrates, terpenoids and proteins. This finding is similar to the research findings of Matur et al. (2009) and Okokon et al. (2005). Plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects (Garg et al., 2010). Earlier reported studies have already confirmed that flavonoids and tannins are the class of compounds which are responsible for several therapeutic activities (Garg et al., 2010; Iwu, 1983). Several Authors also reported flavonoids, sterols, alkaloids and phenolics as bioactive antidiabetic principles (Nadro and Onoagbe, 2012). Fortunately the leaf of *P. fraternus* contains all these bioactive antidiabetic principles in reasonable quantities.

Streptozotocin induced diabetes has been described as a useful experimental model to study the activity of hypoglycaemic agents (Paul et al., 2006). Blood glucose level was increased consistently and significantly in the diabetic untreated groups with relative stability after seven days. This rapid increment may be due to decreased glucose clearance as a consequence of a defect in glucose transport (Wi et al., 1998).

Prolonged treatment (28 days) with methanolic extract of *P. fraternus (*MEP) (200 and 300 mg/kg body weight) and metformin (5 mg/kg body weight) showed continual decrease of blood glucose, suggesting long term maintenance of blood glucose level in diabetic rats. Several medicinal plants have been reported to restore activity of key enzymes of glucose and glycogen metabolism which are strongly disturbed in streptozotocin diabetic rats (Eddouks et al., 2003, Sharma et al., 2010). Hypoglycaemic effect of MEP may arise from the inhibition of hepatic glucose production, or insulin signalling (Qin et al., 2003). Prasad et al. (2009) reported also that the hypoglycaemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimictic action or by other mechanism such as stimulation of glucose uptake by peripheral tissues, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles.

Fractions obtained from the fractionation of the methanolic extract of *P. fraternus* showed hypoglycaemic effects following a single dose administration at 200 mg/kg body weight in both normal and diabetic rats. Fraction one (F I) had significantly lowered blood glucose level 30 min after glucose load in oral glucose tolerance test in diabetic rats and had favourably competed against metformin all through the 150 min the test lasted. Similarly, in normal rats, FI was able to significantly prevent glucose induced hyperglycaemia better than the other fractions and much better than the crude extract. This suggests that the fractionation has helped to free the hypoglycaemic agent in fraction one (FI) which had consequently exhibited faster hypoglycaemic effect at 30 min even better than metformin (standard drug). Whereas the slow performance of the crude extract suggests that the hypoglycaemic agent is bound with other components that required time to be freed up, hence the maximum effect coming after 2 h, probably, after digestion had taken place to free up the hypoglycaemic agent.

Conclusion

From this study, it can be deduced that flavonoids from Fraction 1 of methanolic leaf extract of *P. fraternus* is a potent hypoglycaemic agent. Similarly, repeated oral administration of methanolic leaf extract of *P. fraternus* was shown to evoke hypoglycaemic effect on the fasting blood glucose profile of streptozotocin induced diabetic rats. These results support the traditional usage of *P. fraternus* in the treatment of diabetes mellitus.

Conflicts of interests

The authors have not declared any conflict of interests.

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