

Evidence of Anti-hyperglycemic and Anti-oxidant Effect of *Passiflora edulis flavicarpa* (Sims.) in Streptozotocin Induced Diabetic Rats

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Abstract

Diabetes mellitus is a worldwide problem and has no distinct cure. The present study was designed to investigate the antidiabetic, antioxidant and antilipidemic effects of *Passiflora edulis flavicarpa* extract (PefEt) against streptozotocin (STZ) induced diabetic rats. A total of forty wistar rats were randomly divided into four groups (n=10 male rats/group) as control; control+PefEt; diabetic and diabetic+PefEt. Streptozotocin was administered as a single dose (50 mg/kg) to induce diabetes. The effect of *Passiflora edulis flavicarpa* (PefEt-250 mg/kg bodyweight for four weeks) on diabetic rats was investigated by evaluating various biochemical parameters. The levels of blood glucose, C-peptide, insulin; enzymatic antioxidants, total antioxidant status, oxidative markers (Malondialdehyde and Urinary 8-hydroxydeoxyguanosine) and lipid profile were measured. Levels of glucose, MDA and urinary 8-OHdG were significantly decreased, while levels of antioxidants, C-peptide and insulin were significantly increased on administration of PefEt in the STZ-induced rats when compared to control groups. Therefore, it could be concluded that PefEt must be considered as an excellent herb for future studies on diabetes mellitus.

Keywords: antioxidant, blood glucose, lipid profile, *Passiflora edulis*, streptozotocin, urinary 8-OHdG

Introduction

Diabetes is a metabolic disorder due to absolute deficiency or diminished effectiveness of insulin. Due to lack of insulin, hyperglycemia and glycosuria almost invariably occur. The search for a curative agent against this disease resulted in the introduction of several hypoglycemic agents and some of which are used therapeutically. However, various harmful side effects and weak effectiveness of them made their use limited and the search to find more effective agents continues. Investigations in the plant kingdom culminated in the discovery of many herbal hypoglycemic agents. One of them is taken for investigation in this study.

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and hyperlipidemia that predisposes affected individuals go long term micro- and macro vascular complications (Ahmed *et al.*, 2007). Attention has been focused on the relationship between the productions of reactive oxygen species (ROS) in diabetes.

In Diabetes mellitus, hyperglycemia induces non-enzymatic glycation of protein through Maillard's reaction and the resulting products, such as Schiff base and Amadori products can lead to the production of ROS such as hydrogen peroxide and superoxide anion (Brownlee *et al.*, 1984; Sakurai and Tsuchiya, 1988; Njoroge and Monnier, 1989). Experimental evidences suggest that the complication of diabetes is associated with oxidative stress that can induce lipid peroxidation can leads

to the formation of free radicals. All these free radicals might play a role in DNA damage, protein modification reactions and lipid oxidative modification in diabetes (Sies, 1997).

Cells have enzymatic and non-enzymatic scavenger systems against these free radicals. The imbalance between oxidant and antioxidant leads to oxidative damage in cells.

Expression of endogenous antioxidants sometimes not able to prevent oxidative damage, requiring the supply of exogenous antioxidants by consumption of dietary sources (Habib and Ibrahim, 2011). Despite of whole fruits and vegetables intake, certain non-edible parts could be a good alternative to add dietary bioactive compounds (Leite-Legatti *et al.*, 2012).

Passiflora edulis is commonly known as yellow passion fruit. The leaves of *P. edulis* are deeply 3-lobed when matured. The interest in *P. edulis* has been increased because of its antioxidant compounds. Studies with leaves and stems have shown several phenolic compounds like orientin 2"-O-rhamnoside and luteolin 7-O-(2-rhamnosylglucoside) (Coleta *et al.*, 2006), apigenin and luteolin derivatives (Ferrerres *et al.*, 2007). The polyphenols and flavonoids have antioxidant properties as they could neutralize reactive oxidants (Pietta, 2000) and hypoglycemic activity suggests that it will be of more helpful in controlling blood sugar level in diabetes (Devaki *et al.*, 2011).

These findings demonstrated that the *P. edulis* leaf extract have potent in-vitro and ex-vivo antioxidant properties and might be considered as possible new sources of natural antioxidants (Rudnicki *et al.*, 2007). Several studies have

reported that the fruit of *P. edulis* has been used in the treatment of diabetes, mainly due to the presence of soluble fibers such as pectin (Krahn *et al.*, 2008; Weickert and Pfeiffer, 2008; Salgado *et al.*, 2010; Sandra *et al.*, 2011; 2012; Correa *et al.*, 2014; Sousa *et al.*, 2015) but literature contains only few studies that used leaves and stem of the species *P. edulis* for their antioxidant effect (Rudnicki *et al.*, 2007; da Silva *et al.*, 2013) and hypoglycemic effect in diabetes rats (Devaki *et al.*, 2011).

The literature survey has shown that many works has been carried out to verify the claims on the antioxidant (Farhana *et al.*, 2009) and hypolipidemic (Maricelma *et al.*, 2012) effect of *P. edulis* fruit extract in rats and rat models. The purpose of the study was to determine the effect of *P. edulis* leaf extract on oxidative stress, hyperglycemia and hyperlipidemia in STZ induced diabetic rats. So the present study clearly reveals that the leaves and stem extract of *P. edulis* have shown to reduce blood glucose after administration of STZ which confirms the hypoglycemic nature of the plant.

In the light of the above, the use of *P. edulis* leaf extract represents a therapeutic prospect for diabetes mellitus and also contributes to reduce free radicals and lipid peroxidation, which is also a highly positive factor for lowering the risk of cardio vascular diseases. In view of these facts, the present work aimed to evaluate the effect of *P. edulis* leave extract on biochemical profile of Wistar rats.

Materials and Methods

Animals

Male Wistar albino rats (5-8 weeks old), weighing 200-250 g were purchased from the Tamilnadu Veterinary Animal Science, Chennai, India were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of $22 \pm 2^\circ\text{C}$, fed with a standard pellet diet (VRK Nutritional Solution, Maharashtra, India) and water *ad libitum*.

The rats were handled according to the University and Institutional legislation, regulated by the ethical committee on animal care of Mahatma Gandhi Medical College and Research Institute, Puducherry (India). All the procedures performed on animals were approved and conducted in accordance with the National Institute of Health Guide (Reg. No. 686/02/2012/CPCSEA).

Source of chemicals

Streptozotocin was purchased from Sigma chemical company, St. Louis, Mo, USA. 8-OHdG ELISA kit was purchased from Cell Biolabs, Inc., San Diego, CA 92126, USA. All other chemicals used for the experiments were of analytical grade obtained from local firms.

Plant material

P. edulis flavicarpa leaves and stems were collected from the Calicut, Kerala, India. The plant materials were authenticated by experts from the Botanical Department, Annamalainagar, Chidambaram where a voucher specimen has been deposited.

Preparation of ethanolic extract of *Passiflora edulis flavicarpa* (PefEt)

The fresh leaves and stems were collected and sun dried for seven days. The 50 g of this powder was mixed with 300 ml

ethanol for 24 hours. The extract was filtered using a muslin cloth and concentrated under the room temperature. The solution was evaporated giving a fine residue. The yield of the extract was 20% and the preliminary phytochemical screening was done by using standard procedure and the extract was tested for alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds. The residual extract was dissolved in sterile water and used in the investigation. The previous study by Ana Beatriz *et al.* (2007) confirmed that a dose of 250 mg/kg PefEt was found to be effective. Hence, a dose 250 mg/kg PefEt was orally administered daily for 4 weeks.

Induction of diabetes

Wistar rats were made diabetic by oral injection of STZ (50 mg/Kg bodyweight) dissolved in 0.05 M citrate buffer, pH 4.5 according to Ketan *et al.* (2006). Three days after the injection, blood glucose level was estimated. The rats were considered diabetics when fasting blood glucose level was more than 140 mg%.

Blood collection

One ml of blood drawn from lateral tail vein was collected in a herparinized tube and then centrifuged at 3000 rpm for 10 min to obtain plasma for the estimation of TAS, TBARS, lipid profile and FBS. Enzymatic antioxidants were estimated with hemolysate.

Urine collection

One hundred and fifty μL of urine was collected in a sterile container for the analysis of urinary 8-hydroxydeoxyguanosine (8-OHdG). The rats were housed individually in metabolic cages for 24 hours to collect urine then centrifuged at 2000 rpm for 10 min and supernatants were collected and stored at -20°C until assayed for the urinary 8-OHdG (Perline, 1971).

Experimental design

Animals were randomly divided into four groups of ten rats each:

Group I: Control rats were orally injected with the same volume of citrate buffer (pH 4.5). Group II: Control rats treated with PefEt alcoholic extract (250 mg/kg/bodyweight) daily by oral administration for 4 weeks.

Group III: Rats were made diabetic by oral injection of streptozotocin (50 mg/kg bodyweight) dissolved in 0.05 citrate buffer (pH 4.5).

Group IV: Diabetic rats treated with PefEt alcoholic extract (250 mg/kg/bodyweight) daily by oral administration for 4 weeks.

Biochemical assay

Blood glucose was estimated by portable glucometer (One Touch Ultra, Jhonson and Johnson). C-Peptide as well as insulin was estimated in sera by ELISA kit by the method of Kao *et al.* (1994); Ashby and Frier (1981) respectively.

Measurement of oxidative markers

Lipid peroxidation product, thiobarbituric acid reactive species (TBARS) was determined by Ohkawa *et al.* (1979).

Urinary 8-OHdG: Urine specimens were used for the measurement of the 8-OHdG levels using a competitive in-vitro enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs,

Inc., San Diego, CA 92126, USA). The detection range of the ELISA assay was 0 to 20 ng/mL. The urinary 8-OHdG was expressed as total amounts excreted in 24 h.

Measurement of antioxidant status

Antioxidants such as Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assayed by the method of Kakkar *et al.* (1984), Sinha (1972) and Rotruck *et al.* (1973) respectively. Total antioxidant status of serum was determined by ferric reducing antioxidant power assay (Benzie and Strain, 1996; Cevahir, 2002), whereby at low pH, reduction of a ferric tripyridyl triazine (Fe^{3+} -TPPTZ) complex (Sigma Aldrich, St. Louis, MO, USA) to a ferrous form, which has an intense blue color, that can be monitored by measuring the absorbance at 593 nm using spectrophotometer. It is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. The results were expressed as micro mole per liter.

Measurement of lipid profile

Plasma triglycerides, total cholesterol, LDL and HDL and fasting glucose concentration were measured with an auto analyzer (Randox Daytona, clinical biochemistry analyzer) by using IFCC approved commercial kits. VLDL was calculated by Friedwald equation.

Statistical analysis

All data measurements were expressed as mean \pm SD for control and experimental animals. Statistical comparisons were performed using one way analysis of variance (ANOVA) test which was applied for multigroup comparison. Comparison of different variables in various groups was carried out using Post Hoc LSD multiple comparisons. Pearson's correlation test was used for correlating oxidant and TAS variables. For all tests a probability ($p < 0.05$) was considered significant. The data analysis was carried out by using statistical package of social science (SPSS) version 12 for windows.

Results

The present study revealed that the induction of diabetes using streptozotocin (STZ) caused a significant increase in blood glucose levels and inversely significant decrease in serum insulin and C-peptide as compared to the control group. Oral administration of PefEt significantly reduced blood glucose level reaching about 50%.

The changes in the lipid profile of normal and STZ-induced rats are illustrated in Table 1. Untreated diabetic rats were

characterized by a significant elevation in the levels of total cholesterol, triacylglycerols, LDL cholesterol and VLDL cholesterol compared with the control rats. Diabetic rats treated with PefEt showed near normal levels of lipid profile. Significantly ($p < 0.05$) decreased levels of serum HDL cholesterol were observed in diabetic rats when compared with the non-diabetic rats. Administration of PefEt to diabetic rats significantly increased serum HDL cholesterol, when compared with STZ-induced rats.

The status of enzymatic antioxidants (SOD, GPX, CAT,) and total antioxidant status were measured in erythrocyte lysate and plasma respectively in all experiment groups are depicted in Table 2. Activities of enzymatic antioxidants were markedly reduced in diabetic rats compared with control and control treated groups. The induction of diabetes using streptozotocin developed a state of oxidative stress as denoted by a significant increase in TBARS and DNA damage represented by 8-OHdG. The treatment of diabetic rats with PefEt led to a significant decrease in serum levels of lipid hydroperoxide. However, the antioxidant enzyme activity and TAS was significantly higher in the PefEt treated diabetic group when compared to the untreated diabetic rats. PefEt administration to normal rats did not alter the activity of enzymatic antioxidants (SOD, CAT, GPX).

The correlation analysis between total antioxidant status and urinary 8-OHdG was depicted in the Fig. 1 which revealed a significant negative correlation in all the four groups (control: $r = -0.9346$; PefEt: $r = -0.9491$; diabetic: $r = -0.8311$; DM+PefEt: $r = -0.9057$). The treatment of diabetic rats with PefEt led to a significant decrease in urinary 8-OHdG levels with significant increase in total antioxidant levels compared to untreated diabetic rats.

Discussion

Diabetes is characterized by hyperglycemia with diminished secretion of insulin and its function. Over a long period, diabetes generates a larger number of reactive oxygen species (ROS) which induces oxidative stress. There were many reasons for increased oxidative damage. In case of diabetes mellitus, hyperglycemia itself contributes to increased generation of ROS by producing reactive ketoimine and ketoamine adducts during oxidative glycosylation and glycoxidation, which further produce ROS (Park *et al.*, 2001). Enhanced oxidative stress may be closely related to pathogenesis of diabetic macro and micro angiopathy by exerting direct oxidative stress (Nishikawa *et al.*, 2000).

In the diabetic state, increased ROS production and decreased antioxidant capacity has been observed in many studies

Table 1. Fasting blood sugar, insulin, C-peptide and lipid profile in the different studied groups

Groups	Control	PefEt	Diabetic	Diabetic \pm PefEt
Glucose (mg/dL)	94.1 \pm 10.8	86.1 \pm 9.8	271.9 \pm 49.4 ^a	122.9 \pm 32.4 ^b
C-Peptide (ng/mL)	0.051 \pm 0.012	0.077 \pm 0.028	0.025 \pm 0.009 ^a	0.038 \pm 0.006 ^b
Insulin (μ IU/mL)	1.86 \pm 0.22	2.81 \pm 0.42	0.93 \pm 0.21 ^a	1.42 \pm 0.25 ^b
Lipid Profile				
Total Cholesterol (mg/dL)	68.04 \pm 0.11	60.12 \pm 10.8	87.30 \pm 0.26 ^a	70.1 \pm 0.31 ^b
TAG (mg/dL)	83.96 \pm 0.27	70.24 \pm 0.41	175.61 \pm 1.72 ^a	85.88 \pm 0.29 ^b
LDL Cholesterol (mg/dL)	21.45 \pm 0.21	16.01 \pm 0.08	31.21 \pm 0.13 ^a	23.01 \pm 0.16 ^b
VLDL Cholesterol (mg/dL)	16.79 \pm 0.05	14.04 \pm 0.08	35.12 \pm 0.34 ^a	17.17 \pm 0.05 ^b
HDL Cholesterol (mg/dL)	28.79 \pm 0.18	34.48 \pm 0.19	17.58 \pm 0.11 ^a	29.78 \pm 0.12 ^b

Values are given as mean \pm SD in each group.

a=Statistical significant difference between diabetic and control rats at ($p < 0.05$).

b=Statistical significant difference between diabetic rats treated with PefEt and the diabetic at ($p < 0.05$).

Table 2. Antioxidants, MDA and urinary 8-OHdG in the different studied groups

Groups	Control	PefEt	Diabetic	Diabetic + PefEt
SOD (U ^A /mg Hb)	8.10±0.48	8.16±9.2	4.13±0.31 ^a	8.15±0.48 ^b
CAT (U ^B /mg Hb)	142.01±11.15	140.41±11.18	71.23±10.11 ^a	93.82±12.98 ^b
GPX (U ^C /mg Hb)	26.31±1.26	24.01±1.22	9.39±1.71 ^a	21.42±2.15 ^b
TAS (μM/L)	1129±148.8	1131±174.9	779±127.4 ^a	1090±128.4 ^b
TBARS (nM/mL)	1.31±0.24	1.30±0.19	2.64±0.22 ^a	1.38±0.41 ^b
Urinary 8-OHdG (ng/day)	5.41±0.91	4.98±0.89	27.29±5.60 ^a	11.25±3.35 ^b

Values are given as mean±SD in each group.

a=Statistical significant difference between diabetic and control rats at (p<0.01).

b=Statistical significant difference between diabetic rats treated with PefEt and the diabetic at (p<0.01).

U^A=enzyme concentration required to inhibit to 50% in one minute

U^B=μM of H₂O₂ consumed/minute

U^C=μg of GSH utilized/minute

TAS=Total Antioxidant Status;

TBARS=Thiobarbituric acid reactive substances

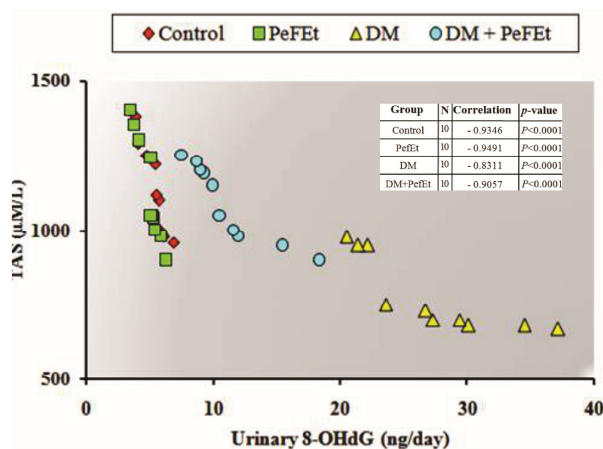


Fig. 1. The correlation of urinary 8-OHdG with TAS

The urinary 8-OHdG was independently correlated with TAS in all the groups.

The negative correlation was highly significant (p<0.0001) in all the four groups.

(Oberley, 1988; Baynes, 1991; Giugliano *et al.*, 1996; Durdi and Timur, 2007). ROS generated during metabolism can enter into reactions that when uncontrolled, can affect certain processes leading to clinical manifestations (Winkler and Moser, 1992; Halliwell, 1993; Kesavulu *et al.*, 2000).

In common, oxidative stress can affect various bio-molecules, mainly nucleic acid results in the generation of modified bases in DNA. 8-OHdG often plays a crucial role in mutagenesis (Kuchino *et al.*, 1987). Elevated levels of 8-OHdG have been reported in urine (Leinonen *et al.*, 1997; Hinokio *et al.*, 1999), mononuclear cells (Dandona *et al.*, 1996), and skeletal muscles (Suzuki *et al.*, 1999) of diabetic patients. In previous studies, the levels of 8-OHdG increased in kidney tissues of STZ-induced diabetic rats (Fraga *et al.*, 1990; Ha *et al.*, 1994).

Several studies demonstrated that a plasma 8-OHdG level was increased in diabetic patients (Kasai *et al.*, 1986; Richter *et al.*, 1988; Shigenaga *et al.*, 1989; Shin *et al.*, 2001). In agreement with previous studies, STZ induced rats had significantly higher levels of 8-OHdG compared with control rats in the present study. This finding suggests that urinary 8-OHdG is a more sensitive marker for oxidative DNA damage. The lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic scavenger system (Halliwell and Gutteridge, 1994).

Lipid peroxidation leads to oxidation of polyunsaturated fatty acids and forms root cause for variety of diseases including

cancer, diabetes and cardiovascular disorders (Amina and Hamza, 2006). The present results showed a significant increase in lipid peroxidation in plasma and RBC of rats treated with STZ. This confirms the previous report on the ability of this diabetogenic compound to induce oxidative damage through generation of free radicals (Sakudelski, 2001).

Enzymatic antioxidants such as SOD, CAT and GPx and TAS provide protection against ROS. Decrease in the antioxidant activity results in the accumulation of lipid peroxides and increase oxidative stress in diabetic rats (Hicks *et al.*, 1988; Johansen *et al.*, 2005). The enzymatic antioxidants were significantly reinstated to near normal in PefEt treated rats. This signifies the antioxidant effect of PefEt in scavenging the free radicals.

A large no of plant derived products as a cure for hyperglycemia in ever growing. The present study confirms the antihyperglycemic, antihyperlipidemia and antioxidant effect of *Passiflora edulis flavicarpa* extract in STZ induced rats. Administration of PefEt to diabetic rats significantly reduced the blood glucose levels to near normal.

The administration of PefEt to diabetic rats significantly reduced MDA, a marker of lipid peroxidation suggesting the antioxidant property of the extract. Previous studies have shown that various plant extract can also stimulate the synthesis of cellular antioxidants (Ahmed and Sharma, 1997; Essa and Subramanian, 2006). The phytochemical screening of PefEt revealed the presence of flavonoids and polyphenols, which plays an important role as natural antioxidants for the prevention of oxidative damage. It is believed that phenolic antioxidants can scavenge deleterious free radicals and prevent their oxidative reactions with vital biological molecules (Rice-Evens *et al.*, 1996).

The administration of *Passiflora edulis flavicarpa* extract (PefEt) reduced MDA, a marker of lipid peroxidation in the diabetic rats suggesting that the extract possesses potent antioxidative properties. The levels of lipid peroxidation in cells are controlled by free radical scavenging system (Gutteridge, 1995; Tho, 1988). SOD, CAT, GPx and TAS constitute a mutually supportive of defense against ROS. Decreased activity of these antioxidant enzymes results in the accumulation of lipid peroxides and increase oxidative stress in diabetic rats. The level of these antioxidants was significantly restored to near normal after treatment of PefEt.

In the present study PefEt is a source of many potentially active antioxidants and active constituents, such as flavonoid, glycosides, hibiscetin cyanidine, cyanine glycoside. This would be the reason for the enhanced activities of antioxidant levels in extract treat groups.

During diabetic conditions lipogenesis is decreased while lipolysis is increased in the hepatic tissue. The increased level of cholesterol is due to the decreased level of high density lipoprotein (HDL) cholesterol. The antihyperlipidemic effect shown by the PefEt extract was also in accordance with earlier findings. Significantly lowering of total cholesterol and rise in HDL cholesterol in biochemical state for prevention of atherosclerosis (Schwab, 2000).

Several research work suggest that *Passiflora edulis* species is well known to possess anxiolytic (Deng et al., 2010), cytotoxic, antioxidant and anti-inflammatory effect (Ana Beatriz et al., 2007), but very few work done in diabetes mellitus and lipid profile and no work was done in *Passiflora edulis* as a preventing tool for DNA damage. In the present study, we have shown a correlation between urinary 8-OHdG and TAS. This suggests that urinary 8-OHdG may be a useful biomarker for the assessment of oxidative DNA damage in diabetic patients. Our data suggests that PefEt would be helpful for the reduction of blood glucose and oxidative DNA damage in diabetes.

Conclusions

The present study reinforces that, *Passiflora edulis flavicarpa* extract revealed antioxidant, antihyperglycemic and antihyperlipidemic activities without showing any toxic effects and serves as natural remedy against hyperglycemia that can be used for prevention of diabetic vascular complications. Further studies on the characterization and the active principles responsible for antidiabetic activity of *Passiflora edulis flavicarpa* are highly warranted.

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