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# Phytochemical screening and antidiabetic potentiality of *Pavetta indica* L. (Angiosperms: Rubiaceae) methanol extract on streptozotocin induced diabetic mice

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**Abstract**

In the present study, the methanolic leaves extract of *Pavetta indica* L. (PI) was studied for antidiabetic activity in streptozotocin (STZ) induced diabetic mice. The dried leaves were powdered and extracted with methanol solvent by using Soxhlet method. Preliminary phytochemical investigation was carried out for determination of presence of bioactive constituents. Thereafter, the acute toxicity study was conducted for the selection of the dose and further the activity was studied as per OECD guideline. The antidiabetic activity was performed in STZ induced diabetic rats at the doses of 200 and 400 mg/kg body weight (b.w.) p.o. per day for 28 days. The fasting blood glucose levels (BGL), serum insulin level followed by biochemical parameters, *viz*., glycosylated hemoglobin, total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) were evaluated and all the results were compared with standard glibenclamide (10 mg/kg b.w.). AST (aspartate aminotransferase), ALT (alanine aminotransferase), and ALP (alkaline phosphatase) levels were also estimated. The leaves methanol extract of PI (MEPI) showed the presence of alkaloids, carbohydrate, flavonoids, phenolic and tannins. Further, the results indicated significant increase in the body weight, liver glycogen, serum insulin and HDL levels and decrease in blood glucose, glycosylated hemoglobin, total cholesterol and serum triglycerides when compared with glibenclamide. MEPI at both the doses (200 and 400 mg/kg) showed a significant decrease in glucose, AST, ALT, and ALP levels in diabetic mice and finally concluded that PI has potential antidiabetic activity in STZ induced diabetes.

## 1. Introduction

Diabetes mellitus (DM) is a chronic metabolic and an endocrine disorder which is very common to the people worldwide. This disorder is mainly characterized by insufficiency of insulin action and as a result, disruption in carbohydrates, protein, and fat metabolism (Seshiah, 2016). According to International Diabetes Federation (IDF, 2013), in worldwide, the same is expected to rise to 592 million by 2035. It was estimated that about 65 million diabetic patients in India were affected in 2013 and it is expected to cross 109 million by 2030 (IDF, 2013). The mental tension, change in food pattern and especially diet intake and change in lifestyle in the fast daily life (Manickam and Periyasamy, 2013) are the main responsible for this life threatening disorder. There are many synthetic medicines available in market for the treatment of this disorder but either too costlier or have serious adverse effects like insulin resistance, hypersensitivity and metallic taste, hypoglycemic coma, *etc*. ( Nyunai *et al*., 2009). Therefore, in the recent years, natural plant based treatments gained tremendous success in managing diabetic disorder in both developed and developing countries with the safe or very low adverse effects (Patil *et al*., 2013) with valuable therapeutic agents, both in modern and in traditional medicine. Therefore, all efforts of extensive research have been diverted in the new direction, *i.e*., towards herbal sources (Dubey *et al*., 2020). Ethnobotanical information indicates a vast number of medicinal plants show their hypoglycaemic or antidiabetic potentiality with their bioactive secondary metabolites (Lanjhiyana *et al*., 2011). India with its diverse climatic zones recognized as a hub of medicinal plants. Therefore, the search for safer and effective antidiabetic agents has become the current focus and with this concept, the present activity was selected.

Of late, *Pavetta indica* L. (PI) belongs to the family Rubiaceae, a shrub growing up to 3-5 meters of height. The opposite branches consist of membranous leaves with grey bark, smooth, irregularly scaly when mature greenish cream (Gupta *et al*., 2013). The leaves are simple, glabrous and variable in shape. The inflorescence is corymbose cyme, with white terminal flowers. The fruit is a berry with two pyrenes and seeds, one per pyrene (The Wealth of India, 1991). Traditionally, the leaves are used to treat liver disease, pain from piles, urinary infections and fever (Kritikar and Basu, 1933). The roots are use as purgative, aperient, diuretic and tonic and also show many therapeutic benefits such as visceral obstructions, jaundice, headaches, urinary diseases and dropsical affections (Suresh *et al*., 2015). Thereafter, methanolic leaves extract of PI

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have been reported as antipyretic and anti-inflammatory (Mandal *et al*., 2003). The hepatoprotective activity of the ethanolic extract of PI leaves was investigated against acute and chronic liver damage induced by paracetamol in albino rats (Valte *et al*., 2018). Anti-inflammatory activity of methanolic leaves extract of PI was investigated against carrageenan, histamine and dextran as different inflammatory models in male albino Wistar rats (Mandal *et al*., 2003). The Anticancer activities of ethanolic extract of PI showed that the higher concentration had a higher inhibition activity against cancer cells (Suresh *et al*., 2015). The antihelmintic activity of chloroform, petroleum ether, and methanol extracts of PI roots and leaves was investigated against adult Indian earthworms (*Pheretima posthuma*) and roundworms (*Ascaridia gali*) (Prasad and Chaurasiya, 2016). Methanol leaves extract of PI also showed diuretic activity (Ramamoorthy *et al*., 2010). The leaves of PI showed bactericidal activity on Gram-positive bacteria and also inhibited the growth of Gram-negative bacteria (Sujatha and Prakash, 2013). Thereafter, the antidiabetic activity of methanolic extract of PI and its derived $CHCl_3$, -BuOH, and $H_2O$ fractions was revealed using its alpha-glucosidase inhibitory potential against the standard drug acarbose using *in vitro* enzyme assay (Penumala *et al*., 2017). The antidiabetic potential of methanolic extract of PI was revealed in alloxan induced diabetic rats (Natarajan *et al*., 2013). Still the scanty reports on antidiabetic activity of this PI plant using streptozotocin induced diabetic mice. Hence, the present investigation was carried out to re-establish the antidiabetic activity of the PI leaves extract with longer duration, *i.e.*, 28 days of study.

## 2. Materials and Methods

### 2.1 Collection and authentication of plant material

The leaves of *Pavetta indica* L. were collected from the Kolli hills, Namakkal and it was authenticated by Dr. C. Murugan (Scientist and Head) Botanical Survey of India, Coimbatore. The specimen was preserved as herbarium (PI-203/PCOG-2019-20) in the Department of Pharmacognosy, Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalaym, Namakkal.

### 2.2 Drugs and chemicals

Streptozotocin (STZ) (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study. Glibenclamide is an oral antidiabetic preparation with an efficient hypoglycaemic action. Diaonil (Glibenclamide) manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature. All other chemicals and reagents were used of AR (analytical reagent) grade.

### 2.3 Preparation of plant extract

The fresh leaves of PI were washed thoroughly with tap water and then in distilled water. The washed leaves were shade dried for 21 days until complete dryness and then powdered by the electronic mixer grinder to get coarse powder. About 500 g of dry powder was extracted with methanol solvent by using Soxhlet apparatus for 7 h at 40ºC. After completion of solvent extract, it was filtered with Whatman filter paper and further concentrated to a dry viscous mass by using rotary flash evaporator at 35ºC.

### 2.4 Calculation of yield and phytochemical screening

The extract was subjected to calculate the yield followed by phytochemical analysis to test the presence of the group of phytoconstituents present in leave extracts through various specific chemical tests as per the method described by the standard literature (Khandelwal, 2007).

### 2.5 Animals

Swiss albino mice of Sprague - Dawley strain (20-25 g) of either sex (n=36) were procured from animal house of our Institute and used for the present investigation. The animals were fed a standard pellet diet and water *ad libitum*. They were maintained in a controlled environment and temperature (22 ± 5ºC with 12 h of light/dark cycle) as per the standard protocol followed and as per the animal ethical approval given by the Institutional Animal Ethical Committee (12/2009/CPCSEA).

### 2.6 Acute toxicity study

Acute toxicity study of the plant extract of PI was carried out in Swiss albino mice (n = 6) according to OECD (Organization for Economic Cooperation and Development) guidelines No. 423. Extract at different doses up to 2000 mg/kg p.o. was administered and the animals were observed for behavioural changes, toxicity, and mortality up to 48 h (OECD, 2007).

### 2.7 Induction of diabetes

Hyperglycemia was induced by streptozotocin at a dose of 60 mg/kg i.p. The animals were kept under observation. After 48 h, the animals were tested for glucosuria using Diastex strips (Eidi *et al*., 2005). 14 days after the STZ injection, mice with fasting blood glucose levels greater than 200 mg/dl were considered diabetic condition, were used for the present investigation.

### 2.8 Treatment protocol

Diabetic animals were randomly assigned into the five groups of six animals each and treated as follows:

**Table 1: Phytochemical analysis of *P. Indica* leaves extract**

| Sl.No. | Groups | Treatments |
|---|---|---|
| I | Normal (Non diabetic) | Normal distilled water |
| II | Diabetic control | STZ induced (60 mg/kg) i.p. |
| III | Standard | STZ induced diabetic mice + glibenclamide (10 mg/kg b.w.) |
| IV | Induced diabetic mice + PI methanol extract | 200 mg/kg |
| V | Induced diabetic mice + PI methanol extract | 400 mg/kg |

### 2.9 Effect on oral glucose tolerance test in STZ-induced diabetic mice

All the above grouped animals except Group-II were given glucose (2 g/kg) 30 min after dosing. Blood samples were collected from the mice tail vein just before glucose administration (0 h) and 30, 60, and 90 min. After the glucose loading, blood glucose levels were estimated by using glucometer (Kalarani *et al*., 2012).

### 2.10 Body weight determination

The body weight of all groups of mice were calculated and documented before treatment (day 0) and after the experimental

period (on days 7, 14, 21 and 28) through electronic weighing balance.

### 2.11 Estimation of blood glucose level

Blood samples were collected at weekly intervals up to the end of the study, *i.e.*, 28 days. Blood glucose was estimated by electronic glucometer using blood glucose strips. On day 28, blood was collected from the mice tail vein (overnight fasted mice) and blood sugar was estimated. Separated serum was analyzed for serum cholesterol and serum triglycerides by enzymatic DHBS colorimetric method, and serum high-density lipoprotein (HDL), serum low-density lipoprotein (LDL), serum creatinine, and serum urea were examined. Furthermore, the activities of alkaline phosphatase (ALP), aspartate, and alanine transaminases (AST and ALT) were also estimated using Randox Assay kits (Singh *et al.*, 2018).

### 2.12 Statistical study

All the values of body weight, fasting blood glucose level, and biochemical parameter estimations were expressed as mean ± SEM and was analyzed for significance by one-way ANOVA followed by Dunnette's multiple comparison test using In Stat v.2.02 software (GraphPad Software Inc.). Differences between groups (p Value) were considered significant at $p<0.05$ level.

## 3. Results

### 3.1 Yield and phytochemical investigation

In the present study, methanol solvent was used for the crude extract preparation for the leaves of PI. The crude extract from the dried leaves of PI showed the yield of 28.27 % w/w. Thereafter, various chemical tests were performed and revealed the presence of alkaloids, carbohydrate, flavonoids, phenolic and tannins as major bioactive constituents.

### 3.2 Acute toxicity study

During acute toxicity study, it was observed that the MEPI was safe to use in animals and showed no mortality at the dose level of 2000 mg/kg b.w. Therefore, 2000 mg/kg dose was considered as a safe dose and further, 1/5th (400 mg/kg b.w.) and 1/10th (200 mg/kg b.w.) dose were selected for carried out the whole experiment and also indicated that $LD_{50}$ for MEPI could be greater than 2 g/kg b.w in mice.

**Table 2: Effect on glucose tolerance of MEPI on streptozotocin induced diabetic mice**

| Groups | Treatment | Change in blood glucose levels (mg/dl) | | | |
|---|---|---|---|---|---|
| | | 0th min | At 30 min | At 60 min | At 90 min |
| I | Normal control(Vehicle only) | 62.92 ± 2.10 | 152.10 ± 2.76 | 160.80 ± 2.90 | 154.90 ± 3.10 |
| II | Glibenclamide 10 mg/kg | 70.69 ± 2.08 | 101.17 ± 2.38[a] | 119.60 ± 3.20[a] | 86.18 ± 3.10[a] |
| III | MEPI200 mg/kg | 68.76 ± 1.5 | 130.08 ± 3.87[b] | 146.52 ± 3.26[c] | 98.76 ± 3.18[c] |
| IV | MEPI400 mg/kg | 66.42 ± 2.12[a] | 107.88 ± 4.90[a] | 123.26 ± 2.23[a] | 91.88 ± 2.91[a] |

All values are expressed as mean ± SEM (n=6), statistically significant at [a]*=$p<0.001$ [b]=$p<0.01$; [c]=$p<0.05$. Values are compared with normal by using one-way ANOVA followed by Dunnette's multiple comparison test.

### 3.3 Effect on oral glucose tolerance test

The effects of MEPI on the OGTT in normal mice were estimated. After 30 min of glucose administration, a rapid increase in blood glucose occurred in the fasting animals and then decreased subsequently during the time intervals. The standard glibenclamide administered group (10 mg/kg) had drastically reduced hyperglycemia at 30, 60 and 90 min (101.17, 119.60, and 86.18 mg/dl, respectively) as compared to the normal control group at the same time intervals. Maximum glucose tolerance in MEPI was observed as 98.76 and the minimum was observed as 91.88 in 90 min as compared with Group I (Table 1).

### 3.4 Changes in body weight

At the end of 28 days treatment, the body weight of normal rats, MEPI and standard drug treated group increased significantly; whereas body weight of diabetic control group decreased (Figure 1). The study was showed in 28 days, MEPI at dose levels of 200 and 400 mg/kg resulted significantly increased in body weight from 25 g to 32 g and diabetic control group was resulted decrease in body weight from 25 g to 16 g as compared to normal mice weight (increased body weight from 25 g to 60 g in 28 days). MEPI treated groups were also compared with diabetic control group.

### 3.5 Estimation of lipid profile

The animals treated with MEPI showed significant reductions ($p<0.01$) in lipid profile parameters, *viz.*, 84.41 and 80.11mg/dl cholesterol, 45.33 and 34.24 mg/dl LDL, 16.85 and 18.22 mg/dl VLDL and 86.43 and 74.29 mg/dl triglycerides after treatment with MEPI (250 and 500 mg/kg), respectively when compared with diabetic control mice. Furthermore, a decrease level of HDL observed with the diabetic control group when compared with the normal group but increased with standard drug as well as with MEPI extracts significantly ($p<0.05$) with the dose dependant manner (Table 2).

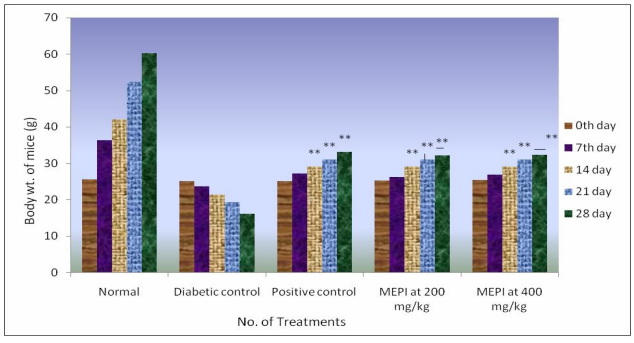


**Figure 1: Effect of MEPI on body weight in STZ-induced diabetic mice.**

*$p<0.05$,**$p<0.01$, values are mean ± SEM, n = 6, when compared with normal and diabetic control by using one-way ANOVA followed by Dunnette's multiple comparison test

**Table 3: Effect of MEPI on serum lipid profile after 28 days**

| Group | Cholesterol (mg/dl) | LDL (mg/dl) | HDL (mg/dl) | VLDL (mg/dl) | Triglycerides |
|---|---|---|---|---|---|
| I | 68.12 ± 0.05 | 20.64 ± 0.12 | 12.03 ± 0.04 | 16.20 ± 0.04 | 60.21 ± 0.21 |
| II | 87.33 ± 0.11 | 96.43 ± 0.33 | 9.10 ± 0.24 | 20.34 ± 0.03 | 117.03 ± 0.10 |
| III | 73.22 ± 0.23** | 38.56 ± 0.20** | 14.43 ± 0.21** | 17.98 ± 0.43** | 73.21 ± 0.14** |
| IV | 84.41 ± 0.31** | 45.33 ± 0.21** | 16.21 ± 0.42** | 16.85 ± 0.03** | 86.43 ± 0.44** |
| V | 80.11 ± 0.11** | 34.24 ± 0.10** | 20.76 ± 0.12** | 18.22 ± 0.10** | 74.29 ± 0.20** |

*$p$<0.05, **$p$<0.01, values are mean ± SEM, n=6, when compared with diabetic control by using one-way ANOVA followed by Dunnette's multiple comparison test.

### 3.6 Blood glucose level estimation

Blood glucose was estimated at 0, 7, 14, 21, and 28 days for all the treated groups of mice. The glibenclamide and MEPI treated groups (200, 400 mg/kg b.w), showed a significant reduction ($p$<0.05) from 7th to 28th day. STZ induced MEPI at dose level of 400 mg/kg b.w showed a significant reduction in blood glucose level ($p$<0.05) (Figure 2). Animals with STZ induced diabetes 60 mg/kg b.w. (i.p) had elevated blood glucose on first day and after 28 days, it was reduced a little but was higher than that of normal group.

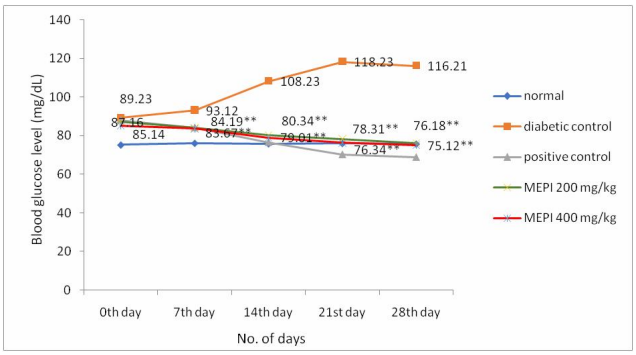


**Figure 2: Effect of MEPI on blood glucose level in STZ-induced diabetic mice.**

*$p$<0.05, **$p$<0.01, values are mean ± SEM, n=6, when compared with diabetic control by using one-way ANOVA followed by Dunnette's multiple comparison test

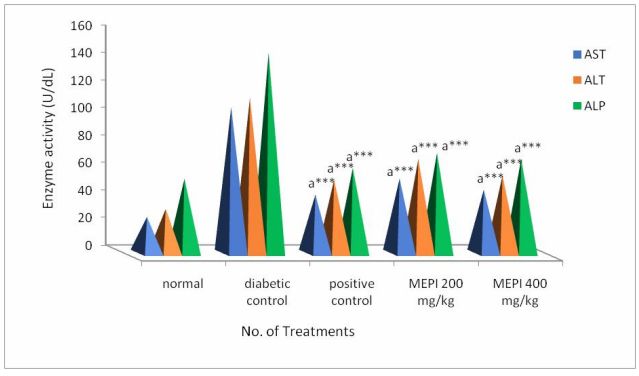


**Figure 3: Effect of MEPI on enzyme activity in STZ-induced diabetic mice.**

Values are mean ± SEM, n=6; $^a p$ < 0.05, when compared with normal control ***$p$<0.001, when compared with diabetic control by using one-way ANOVA followed by Dunnette's multiple comparison test.

### 3.7 Serum enzyme level

Activities of serum enzymes such as ALP, AST and ALT were determined. The activity of enzymes is increased much higher with STZ induced mice than normal, which gave significant ($p$<0.05) results in 28 days. Furthermore, diabetic animals treated with the standard drug showed a significant decrease in enzyme activity. MEPI at 200 and 400 mg/kg b.w resulted significant decrease in enzyme activity at dose dependent manner but the values are close with the standard group mice (Figure 3). Data revealed that MEPI at dose of 400 mg/kg b.w showed the values of AST- 46.12 U/dl; ALT-56.34 U/dl; ALP-68.43 U/dl which were close to the STZ induced glibenclamide group of animals (AST-42.43 U/dl; ALT-53.23 U/dl and ALP-61.80 U/dl) but the values were significant when compared with positive control group ($p$<0.001) and normal group of animals ($p$<0.05).

## 5. Discussion

DM is a chronic condition and being considered a public health problem in terms of population growth and ageing, greater urbanization, the increasing prevalence of obesity (Whiting *et al.*, 2011). In the present study, phytochemical evaluation with respect to chemical tests is required to identify preliminary phyto-constituents present in herbal extracts. Plant active constituents are essential for therapeutic efficacy. Hence, the chemical test for MEPI was carried out for the identification of the presence of group of constituents. Mechanisms of action of phytoconstituents involve regulating glycemic metabolism or decreasing cholesterol levels or increasing secretion of insulin or by improving microcirculation (Das *et al.*, 2020). The present investigation was carried out for qualitative identification of the phytoconstituents present in the MEPI. Methanol extract was selected because most of the important phytoconstituents related to antidiabetic activity are soluble. Further, the methanolic leaves extract of PI (200 and 400 mg/kg body weight) was investigated for antidiabetic activity on STZ diabetic mice and resulted significant declines of body weight gain in STZ-diabetic mice were noted after 28 days. Similar observations were noted in many experimental diabetes researches (Jayaprasad *et al.*, 2015; Jayaprasad *et al.*, 2016; Das *et al.*, 2020).

Increased food consumption and decreased body weight observed in diabetic mice in comparison to normal mice, indicates a polyphagic condition and weight loss due to excessive breakdown of tissue proteins (Chatterjea and Shinde, 2002). In the present study, the levels of blood glucose, total protein, cholesterol, LDL and VLDL were significantly higher, while the level of HDL was statistically decreased in diabetic mice. All the observations were similar with the previous scientific investigations (Achi *et al.*,

2016; Hu *et al*., 2016; Zhang *et al*., 2016). This may be due to the presence of polyphenolic compounds, especially flavonoids in the leaves, which are incorporated into lipoprotein within the liver or intestine and transported within the lipoprotein particles which are inversely associated with mortality from coronary heart disease. Therefore, flavonoids and phenolics may be located for protection of LDL from oxidation. The same result was revealed earlier (Safdar *et al*., 2003). Furthermore, flavonoids are reported to suppress glucose level and also found to be a strong inhibitor of α-glucosidase (Kim *et al*., 2000). MEPI leaf extract also showed the presence of phenolics in higher content and that is the reason for the decrease in blood glucose level.

In the further investigation, there was a significant rise in the serum enzyme levels such as AST, ALT, and ALP. Results revealed the increased level of all the enzymes in the diabetic control group compared to the normal control group, and thereafter the standard drug decreased the values, the activity was may be due to the cell membrane damage of hepatocytes or due to increased cell membrane permeability (Nwufo *et al*., 2017). The activities of ALT, AST, and ALP in serum are increased due to the leakage of these enzymes (in the liver cytosol) and as a result diabetes may induce hepatic dysfunction.

## 5. Conclusion

In the present study, the leaves methanolic extract of *P. indica* was investigated for antidiabetic activity by STZ induced diabetic mice for the duration of 28 days. The study showed the presence of various plant constituents and among the all, flavonoids are the one that are possibly responsible for the antidiabetic activities may be by triggers insulin secretion, and demonstrated significant lowering of blood glucose level, and biochemical parameters. Further, statistical improvement in the body weight of animals showed in a dose-dependent manner by enhanced peripheral glucose utilization by direct stimulation of glucose uptake and reduced blood glucose level.

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## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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