Polyphenolic extract of *Ichnocarpus frutescens* modifies hyperlipidemia status in diabetic rats

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**Abstract**

Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for diabetes mellitus. Decoction prepared from the leaves of *I. frutescens* is used to alleviate the symptoms of diabetes mellitus in folk medicine. The present study was designed to evaluate the anti-diabetic and anti-hyperlipidemic effects of the polyphenolic extract (PPE) of *I. frutescens* leaves in alloxan induced diabetic rats. Diabetes was induced by single intraperitoneal injection of alloxan (150 mg/kg body weight). Polyphenolic extract to alloxan diabetic rats at the doses of 150 mg and 300 mg/kg body weight resulted in a significant reduction of fasting blood glucose (FBG) levels. PPE (300 mg/kg body weight for 21 days) administration showed significant decrease in hepatic HMG-CoA reductase activity of alloxan diabetic rats. No significant effects were found in the normoglycemic rats. Polyphenolic extract exhibited significant hypolipidemic effect as evident from correction of hyperlipidemic indicators (TC, TGs, VLDL, HDL and LDL). Oral administration of polyphenolic extract (100 mg/kg) significantly enhanced the release of lipoprotein lipase enzyme significantly. Polyphenolic extract also inhibited ADP-induced platelet aggregation in vitro. The results indicating the effectiveness of polyphenolic extract against hyperlipidemia and obesity in alloxan-diabetic rats. The histopathological studies of aorta in polyphenolic extract treated alloxan-rats revealed almost recovery to normal appearance. All the results revealed the therapeutic potential of polyphenolic extract against diabetes, hyperlipidemia and atherosclerosis leading diabetic complications and cardiovascular risks.

**Key Words**: *Ichnocarpus frutescens*, hyperlipidemia, diabetes, alloxan, polyphenolic extract

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**Ichnocarpus frutescens** polifenolik ekstraktı diyabetik săçanlarda hiperlipidemik durumda etkiler

**Özet**

Bitkilerden elde edilen doğal ilaçlar diabetes mellitus tedavisi için etkili ve güvenilir bir alternatif tedavi olarak düşünülmektedir. Halk hekimliğinde *I. frutescens* yaprakları kaynatılarak hazırlanan öz, diabetes mellitus semptomlarını azaltmak için kullanılır. Bu çalışma alloxan ile diyabetik olan săçanlarda *I. frutescens* polifenolik ekstraktı (PPE) anti-diyabetik ve anti-hiperlipidemik etkisini değerlendirilmek üzere planlanmıştır. Diyabet alloxan’ın (150mg/kg vücut ağırlığı) bir kerede intraperitoneal enjeksiyonu ile oluşturulmuştur. Alloxan diyabetik farelerde 150-300mg/kg vücut ağırlığı dozlarında kullanılan polifenolik ekstraktı, serum açılık kan şekeri seviyesinde önemli düşüş oluşturmuştur. PPE (21 gün boyunca 300mg/kg vücut ağırlığı) kullanımı hepatik HMG-CoA redüktaz aktivitesinde önemli düşüş oluşturmuştur. Normoglisemik săçanlarda önemli bir etki bulunmamıştır. Hiperlipidemik indikatörlerin (TC, TG, VLDL,
HDL, ve LDL) seviyelerindeki düzelmelerden de anlaşılabilecektir. Polifenolik ekstrakt (100mg/kg) oral kullanımlı lipoprotein lipaz enzim salınımını önemli derecede hızlandırmıştır. Polifenolik ekstrakt ADP’nin neden olduğu trombosit kürlenmesini de önlemiştir. Bu sonuçlar, polifenolik ekstraktin alloksan diyabetik saçlanarda hiperlipidemi ve obeziteye karşı etkinliğini göstermektedir. Polifenolik ekstrakt ile tedavi edilen alloksan saçlanlarının aort damarının histopatolojik incelemesi normal görünümü yakın bir düzeme olduğunu göstermiştir. Bütün sonuçlar, polifenolik ekstraktin diyabet, diyabetik kompleksiyonlara ve kardiyovaskülerler riske neden olan hiperlipidemiye ve aterosklerozu karşı teraparatik potansiyelini göstermiştir.

**Anahtar Sözcükler:** Ichnocarpus frutescens, hiperlipidemi, diyabet, alloksan, polifenolik ekstrakt

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**Introduction**

The worldwide epidemic of type 2 diabetes (NIDDM) has been stimulating the search for new concepts and targets for the treatment of this incurable disease. Globally diabetes has shadowed the spread of modern lifestyle and it can be linked to an increase overweight and sedentary population (Vats et al., 2005). Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine based disease. Diabetic patients experience various vascular complications, such as atherosclerosis, diabetic nephropathy and neuropathy (Sheetz, 2002). It is now well established that the hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications (Goldstein et al., 1973; Kaur et al., 2002).

A logical strategy to prevent or treat atherosclerosis and reduce the incidence of cardiovascular disease events is to target the hyperlipidemia by diets and/or lipid lowering drugs (Simons, 2002). Moreover, although glucose levels may be well-controlled, high lipid profiles in serum increase free radical levels, and cause endothelial damage. The American Heart Association has identified the primary risk factor associated with progression of atherosclerosis lesions as elevated levels of cholesterol and triglycerides in serum. Therefore therapies consider the treatment of hyperlipidemia to be one of the major approaches towards decelerating the athrogenesis risk (Rhoads et al., 1986). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease. Many investigations suggested that improvement in defective lipid level, glucose, anti-oxidant levels should be useful in the prevention of diabetes-associated cardiovascular risk (West, 2000).

In India, as in many developing countries, most diabetic patients use medicinal plants as folkmedicine to treat diabetes. Therefore, there is strong interest to search for natural hypoglycemic substances derived from local medicinal plants. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of hyperglycemia and diabetes (Marles and Farnsworth, 1995). Plants are rich sources of hypoglycemic, hypolipidemic and antioxidant agents such as flavonoids, ellagic acids, phenolic acids, phytosterols, gallotannins, and other related polyphenols (Muruganandan et al., 2005; Miyake et al., 2006). The hypoglycemic and hypolipidemic actions of some of these phytochemical constituents have been evaluated and confirmed in animal models (Vinson and Zhang, 2005; Hwang et al., 2005), suggesting that natural products could serve as sources in the search for effective antidiabetic and antihyperlipidemic agents. Therefore, much effort has been focused on the plants for potentially useful products as commercial anti-diabetic and anti-lipidemic agents or as lead compounds.

**Ichnocarpus frutescens**

L.Br. (Apocynaceae) have been used as folkmedicine and as an ingredient in Ayurvedic and Unani preparations against diseases of blood, skin and inflammation. *I. frutescens* is rich in polyphenols and flavonoids (Singh and Singh, 1987). Distribution of various flavonoids and phenolic acids in the leaves of *I. frutescens* have been systematically studied (Daniel and Sabnis, 1978). The pharmacologically active constituents are phenylpropanoids,
phenolic acids, coumarines, iridoid glycosides, flavonoids, sterols and pentacyclic triterpinoids (Lakshmi et al., 1985). The utilization of decoction of leaves of *I. frutescens* in the treatment of diabetes is noteworthy and it is also included in the plant species, which are used by the tribals of Karnataka and Utterpradesh states for treating diabetes (Parinitha et al., 1999). In spite of reported use no systematic clinical experimental studies have been carried out to assess therapeutic uses of this plant. Therefore this study was designed to investigate the effect of polyphenolic extract of *I. frutescens* on hyperglycemia and hyperlipidemia status, which are closely associated with atherosclerosis.

**Materials and methods**

**Drugs and chemicals**

Glibenclamide was purchased from Merck Pharmaceuticals, Mumbai, India. Alloxan, Folin-Ciocalteu reagent, p-anisaldehyde reagent, Fast blue B salt, pyrocatechol, quercetin, serum albumin (BSA), hydroxylamine hydrochloride, anthrone reagent, trichloroacetic acid, ammonium molybdate, sodium periodate, chromotropic acid were purchased from SISCO Research Laboratories Private Limited, Mumbai, India. Serum total cholesterol (TC), triglycerides (TGs), total proteins and HDL cholesterol were estimated by commercially available Diagnostic Kit (Span Diagnostics, Mumbai, India). Fasting blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Accu-chek Active, Roche Diagnostics, USA). All other chemicals and solvents were of analytical grade and purchased locally.

**Plant materials**

The fresh leaves of *I. frutescens* were collected from delta region of Cauvery River, Thiruchirappalli, India, in February 2005 and authenticated at Botanical Survey of India, Central National Herbarium Howrah, India (Ref No: CNH/I - I/87/2005-TECH/1326). An authentic voucher specimen was deposited in the Herbarium of Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

**Preparation of polyphenolic extract**

The leaves of *I. frutescens* were air-dried for one week at room temperature without exposure to sunlight and were coarsely powdered. The leaf powder (300 g) was macerated for five days at room temperature three times with 800 ml of hydroalcoholic mixture (double distilled water: 99% absolute alcohol; 30:70 % v/v). The three macerates were combined and concentrated using a rotary evaporator (SUPERFIT, India) under reduced pressure and a water bath temperature <35°C. The residue was first dissolved in water and aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The concentrated solution of lower layer was extracted four times with 200 ml of ethyl acetate containing glacial acetic acid (10 ml/l) each time. The four ethyl acetate extracts were combined, evaporated to remove ethylacetate and polyphenolic fraction of *I. frutescens* was obtained as a lyophilized powder and stored at -70°C.

**Fingerprinting and estimation of flavonoids**

Preliminary phytochemical screening revealed the presence of flavonoids, steroids, flavonoids and phenolic acids. Presence of flavonoids in the polyphenolic extract was confirmed by silica gel thin layer chromatography (TLC), according to the procedure described by Wagner and Bladt (1996). TLC profile was established for the polyphenolic extracts by using a solvent mixture of ethyl acetate and chloroform (75:35). HPTLC finger printing analysis was carried out using precoated silica gel 60F254 (20 cm ×20 cm, Merck, Darmstadt). The polyphenolic profile can be visualized with Fast Blue Salt B and anisaldehyde reagent. The total phenolic content and flavonoid content of the polyphenolic fraction was determined by standard methods (Singleton et al., 1999; Chang et al., 2002) using authentic standards.

**Animals and ethical approval**

Male and female Swiss albino rats, weighing about 200-250 g body weight were used in the present study. Animals were collected from breeding colony and acclimatized to the laboratory conditions for two weeks. They were housed in macrolon cages under standard laboratory conditions (light period 7.00 a.m. to 7.00 p.m., 21 ± 2°C, and relative hu-
midity 55-70%). The animals were fed with commercial diet from Hindustan Lever Ltd (Bangalore, India) and had free access to water (ad libitum) during the experiments. Experiments were complied with the rulings of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi, India (Registration No: 0367/01/C/CPCSEA) and the study was permitted by the institutional ethical committee of the Jadavpur University, Kolkata, India.

Acute toxicity study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n = 6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 300 and 2000 mg/kg body weight.

Effect on alloxan induced diabetic rats

Diabetes was induced in rats that had been fasted for 12 h by the intraperitoneal (i.p) administration of alloxan (150 mg/kg body weight), freshly dissolved in sterile normal saline, after anesthesia with ethyl ether. The diabetic state was assessed by measuring non-fasting blood glucose (FBG) concentration 72 h after alloxan treatment. The rats with serum glucose above 350 mg/dl, as well as with polydipsia, polyuria, and polyphagia were selected for the experiment. The animals were segregated into four groups of six rats in each. Group-I served as control, Group-II served as alloxan diabetic control. Group-II and IV received aqueous extract at doses of 150 and 300 mg/kg, once a day, for 21 days. The oral treatments (by gavage) of all groups were carried out the same time (in the morning) and under the same conditions. Blood samples were drawn at weekly intervals till the end of study (i.e. 3 weeks). Fasting blood glucose (FBG) was measured on day 1, 7, 14 and 21 of the study.

Estimation of biochemical parameters

Fasting blood glucose (FBG) levels were estimated by glucose oxidase-peroxidase reactive strips (Acu-chek active, Roche Diagnostics, USA). On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and the serum was separated for the estimation TC, TGs, HDL cholesterol (Henry, 1974) and total proteins were estimated by standard methods using Span Diagnostic Kit (Span Diagnostics, Mumbai, India). LDL cholesterol (Friedwald’s et al., 1972) and VLDL Cholesterol (Friedwald’s et al., 1972) were estimated by Friedwald’s equation. End of the day, all the animals were sacrificed by excess anesthesia, the livers excised out, washed in ice-cold normal saline, patted dry and weighed. Tissue homogenate (10 %) from liver was used for the estimation of HMG-CoA reductase (Rao and Ramakrishnan, 1975). The total protein content was determined from liver homogenate of each animal according to the method of Lowery et al., (Lowery et al., 1951).

Histopathological studies

Small portions of aorta were fixed in Bouin’s fluid (picric acid: formaldehyde: glacial acetic acid; 75:25:5), dehydrated in graded alcohol and embedded in paraffin wax (Galigher and Kozloff, 1971). They were processed in an automatic tissue processor and embedded in paraffin wax. Sections of 5 \(\mu\)m were cut on a rotary microtome by serial sectioning until the entire thickness of the liver was sectioned. Staining was done by haematoxylin and eosin and later the microscopic slides of the aorta were photographed using light microscope (Biosupreme, India).

Platelet anti-aggregation activity

Platelet rich plasma (PRP) was prepared by centrifugation (1000 rpm for 5 min) of blood collected from normal aspirin free blood bank donors. 1.5 ml of acid citrate dextrose was used as anticoagulant for every 8.5 ml of blood. PRP was taken into siliconized glass cuvettes. Platelet poor plasma (PPP) collected by centrifugation (3000 rpm 5 min) was kept as reference. The cuvettes were incubated at
37 °C for 5 min. The aggregation was initiated by adding 20 µl of ADP (10 µM) to 1ml of PRP. The aggregation was recorded for 5 min at 600 nm. The effect of different concentrations (50–250 µg) of PPE was studied by incubation with PRP at 37 °C for 5 min before the addition of ADP. Commercial heparin (20 µg/ml) was used as reference standard (Subramaniam and Satyanarayana, 1989). The maximal aggregation was recorded. The aggregation is expressed as % inhibition ($X$) calculated by using the following equation:

$$X (%) = \frac{(A-B)}{A} \times 100$$

where A= maximal aggregation of the control, and B = maximal aggregation of drug-treated PRP.

**Lipoprotein lipase releasing activity**

The lipoprotein lipase releasing activity of the drug was determined by the method of Korn (1962). Blood was collected from normal white albino rabbits (1.5-2.0 kg body weight) through the ear vein in EDTA (0.1 M) added tubes. The plasma collected by centrifugation at 3000 rpm for 5 min was used as the enzyme source. The human lipoid serum (TG < 400 mg/dl) was used as the substrate. 0.1 ml of substrate, 0.1–0.4 ml of enzyme, 0.4 ml of 20% albumin (pH 8.5) and 0.1 ml of (NH₄)₂SO₄ were mixed at low temperature and made up to a final volume of 1 ml. The mixture was incubated at 37°C and the aliquots were taken into tubes containing 0.1 ml of 1 N H₂SO₄ at intervals of 0, 1/2, 1, 1 1/2 hrs. The samples treated with 0.1 ml of sodium periodate (0.05 M) and 0.1 ml of sodium arsenate (0.05 M) was kept in boiling water bath for 30 min. After adding 9 ml of chromotropic acid, the volume was adjusted to 10 ml and the optical density was measured after cooling, at 570 nm. The assay was standardized with glycerol solution of known molarity and the glycerol liberated was calculated. The same procedure was repeated after the administration of PPE (50 mg/kg and 100 mg/kg) for a period of 10 min. The glycerol liberated was calculated and compared with normal untreated group.

**Statistical analysis**

The experimental data were expressed as mean ± SEM. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA followed by Dunnett’s multiple comparison test using Gra- phad Instat -3 Software (San Diego, USA). p < 0.05 and p <0.01 were considered as statistically significant.

![Figure 1. Effect of polyphenolic extract of I. frutescens on fasting blood glucose (FBG) levels of alloxan treated diabetic rats. Values are expressed as mean ±SEM (n=6). ** p<0.01 when compared with diabetic control.](image)
Results

Preliminary phytochemical screening of PPE was carried out for the detection of phytoconstituents, using standard chemical tests. Triterpenoids, flavonoids, simple phenolic acids, steroids and tannins were detected in PPE. Chromatography on silica gel 60 with chloroform and methanol mixture as mobile phase in a saturated chamber, allows baseline separation of the target compounds. The PPE profile can be visualized with Fast Blue Salt B reagent.

Total phenolic contents of PPE were expressed as mg of pyrocatechol equivalent per gram of dry weight of PPE extract. 1000 µg of PPE was used to determine the amount of total polyphenolic contents. The level of total polyphenolic compounds was 184.52 mg of pyrocatechol equivalent per gram of PPE. The present study showed the flavonoid content determined by two independent colorimetric methods, one for the determination of flavones and flavonols and other for determination of flavanones, as reported by earlier. The contents of total flavonoids in the PPE of *I. frutescens* were expressed as the sum of two complementary methods for the determination of flavones, flavonols and flavonones and the results were found to be 36.42 mg of quercetin and naringenin equivalent per gram of PPE. Major types of phenolic constituents identified in the leaves of *I. frutescens* are simple phenolic acids, flavonol, flavones, flavonones and flavonoid glycosides.

Acute toxicity studies revealed the non-toxic nature of the polyphenolic extract of *I. frutescens*. After the administration of polyphenolic extract, rats were immediately observed for 2 h for behavioral, neurological and autonomic profiles for any changes or lethality for the next 48 h. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. However, at the above mentioned doses, PPE did not show any untoward effects on behavioral response, normal reflexes and so on. According to OECD guidelines for acute oral toxicity, an LD_{50} dose of 2000 mg/kg and above is characterized as unclassified and hence the drug is found to be safe.

Figure 1 shows the effect of polyphenolic extract on FBG levels of alloxan diabetic and non diabetic animals after the daily treatment (150 mg/kg and 300 mg/kg) for 21 days. Significant decrease in the FBG levels were observed in the

![Graph](image-url)
groups treated with both the doses of PPE, as compared to the same group before treatment. No significant changes were seen in diabetic controls after distilled water treatment. In the PPE treated groups, although a significant anti-hyperglycemic effect was evident from the first day onwards, the decrease in FBG levels was maximum by the end of the second week, in the group received 300 mg/kg/day of PPE.

Figures 2 and 3 represent the effect of PPE on general biochemical parameters such as serum cholesterol, TGs, HDL cholesterol, LDL cholesterol and VLDL cholesterol of diabetic rats treated with PPE (150 mg/kg and 300 mg/kg) once days for 21 days. The serum cholesterol, triglyceride, lipoprotein levels and HMG CoA reductase activity were significantly increased, while serum HDL-Cholesterol and total protein levels were significantly decreased in diabetic group as compared to normal rats. However, following treatment with PPE (300 mg/kg) for 21 days, the serum TGs, TC, LDL-c, VLDL-c and were reduced significantly, while HDL-c levels has been increased significantly in PPE treated group when compared to diabetic control group.

Histopathological examination of aortic roots of control animal demonstrated that the rat had no aortic abnormalities (Figure 4). In contrast the aortic roots of alloxan treated diabetic rats did not show significant atheromator lesions and an accompanying slight macrophages infiltration (Figure 5). Histological examination of the aortic rats of polyphenolic extract showed good preservation of aortic wall morphology (Figure 6).

The PPE treated animals for a period of 10 min showed increased production of glycerol as an index of the greater release. The glycerol liberated in the PPE treated animals was found to be increased significantly (Table 1). In vitro inhibitory activity of PPE against ADP-induced platelet aggregation were measured (Table 2). PPE inhibited platelet aggregation in vitro potently compared to heparin reference drug widely used as anti-platelet agent in clinical practice.

**Discussion**

Diabetes mellitus belongs to a heterogeneous group of disorders that have hyperglycemia as common feature (Reaven, 1988). It is well known

![Figure 3](image-url)  
**Figure 3.** Effect of polyphenolic extract of *I. frutescens* on liver HMG CoA reductase activity alloxan-treated diabetic rats. Values are expressed as mean ±SEM (n=6). #HMG CoA reductase activity is expressed as a ratio of HMG CoA and mevalonate levels.
that the incidence of diabetes mellitus is high all over the world, especially in Asia. The chronic hyperglycemia of diabetes is associated with long term dysfunction and damage to various organs, especially the tissues requiring insulin for glucose uptake. It is multi-faceted and dynamic expressions of practice many plants are used to treat diabetes mellitus in south India. Most of these plants are not scientifically validated for their therapeutic efficacy and safety. Scientific studies on these plants are like to provide invaluable antidiabetic drugs. Further, most of the oral hypoglycemic agents used in allopathic therapy are reported to have side effects in the long run (Khan and Schchter, 1991). Therefore, there is a need to search for effective and safe drugs for diabetes. Survey of literature revealed that, the utilization of decoction of leaves of *I. frutescens* in the treatment of diabetes and jaundice are noteworthy and it is included in the plant species, which are used by the tribal of Karnataka, India for treating diabetes (Bhandary, et al., 1995). Many investigations of oral anti-hyperglycemic agents of plant origin used in traditional medicine have been conducted and many of the plants show positive activity (Bailey and Day, 1989; Saravanan et al., 2005).

In our normoglycemic study, we couldn’t find any significant effect of PPE on blood glucose levels of normal rats for 15 days. The reason for the low hypoglycemic activity in normoglycemic conditions may be due to their in ability to disturb the carbohydrate homeostasis maintenance. In the OGTT both
Polyphenolic extract modifies hyperlipidemia status

The doses increases the tolerance for glucose suggesting peripheral utilization of glucose in glucose loaded rats. This indicates the efficacy of PPE to control elevated blood sugar level.

The diabetogenic agent alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which is toxic to pancreatic beta cells because it can generate toxic free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine (Szkudelski, 2001; Wilson et al., 1984). In the present study, the diabetogenic effect of alloxan is in accord with previous studies (Huang et al., 2000; Whitton et al., 1975). Alloxan caused rapid release of insulin initially and then sharp decline due to liberation of stored insulin. Presence research work reveals that the polyphenolic extract of I. frutescens leaves for 21 days at the doses of 150 mg/kg and 300 mg/kg in alloxan diabetic rats. The significant decrease in the levels of FBG in alloxan diabetic rats treated with the PPE (Figure 1) may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose as postulated by Erah et al. (1996).

Diabetes is also associated with hyperlipidemia and hypertriglyceridemia (De seredday et al., 2004). The rise in blood sugar is accompanied with the increase in TC, TGs, VLDL and fall of HDL. Administration of PPE normalized serum lipids, secondary to the diabetic state. Diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to underutilization of glucose (Krishnakumar et al., 2000). The regression of diabetic state on PPE administration increases the utilization of glucose, thereby depressing the mobilization effect. However, polyphenolic extract of leaves of I. frutescens at a dose of 300 mg/kg exhibited anti-hyperlipidemic effects while at the same time increasing HDL-c. HMG-CoA reductase is the enzyme which catalyses the conversion of HMG-CoA to mevalonate using NADPH as reducing equivalent and is the major rate limiting step in cholesterol biosynthesis (Kedar and Chakrabarti, 1982). The activity of HMG-CoA reductase activity in liver for cholesterol biosynthesis was also found to be decreased after the PPE treatment.

These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin (Sharma et al., 2003). Significant modification/attenuation of the lipoprotein levels (VLDL and HDL) in the serum towards the control level which strengthens the hypolipidemic effect of PPE.

Table 1. Modulatory effect of polyphenolic extract of I. frutescens on lipoprotein releasing activity in normal white albino rabbits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycerol releasing activity (mg/dl/hr)</th>
</tr>
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<tbody>
<tr>
<td>Normal Rabbits</td>
<td>4.13 ± 0.42</td>
</tr>
<tr>
<td>PPE (100 mg/kg)</td>
<td>9.00 ± 0.92</td>
</tr>
<tr>
<td>PPE (200 mg/kg)</td>
<td>9.56 ± 0.69</td>
</tr>
<tr>
<td>Heparin (1 mg/kg)</td>
<td>11.53 ± 0.70</td>
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Table 2. Modulatory effect of different concentrations of polyphenolic extract of I. frutescens on platelet aggregation activity. Values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inhibition of platelet aggregation (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>---</td>
</tr>
<tr>
<td>PPE</td>
<td></td>
</tr>
<tr>
<td>100 (µg/ml)</td>
<td>25.8 ± 2.78</td>
</tr>
<tr>
<td>200 (µg/ml)</td>
<td>41.66 ± 1.68</td>
</tr>
<tr>
<td>300 (µg/ml)</td>
<td>70.6 ± 2.96</td>
</tr>
<tr>
<td>Heparin (20 µg/ml)</td>
<td>82.67 ± 2.40</td>
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</table>
in alloxan diabetic rats. Moreover, TC, TGs, VLDL, HDL and atherogenic index are biomarkers of hyperlipidemia and atherosclerosis and human studies indicate that a higher proportion of HDL-c is anti-atherogenic (Elisaf et al., 1995). These levels are increased in alloxan diabetic rats, and after the PPE supplementation/administration these levels resettled towards the control level.

The decrease of serum TGs level is an important finding of this experiment. Recent studies also show that triglycerides are independently related to coronary heart diseases (Bainton et al., 1992) and most of the anti-hyperlipidemic drugs don’t decrease TGs levels, but PPE lowered it significantly and this effect might be related to increase the endothelium bound lipoprotein lipase which hydrolysis the TGs into fatty acids. Lipoprotein lipase playing a major role in the transport and metabolism of TGs of exogenous origin (Taskimen, 1987). It is the key enzyme, which regulates the disposal of lipids fuels in the body. PPE showed an enhancing role of releasing and activating the lipoprotein lipase resulting in the metabolic degradation of lipids. This is further supported by the in vitro releasing effect of lipoprotein lipase enzyme by polyphenolic extract of I. frutescens.

Platelets play a pivotal role in health and diseases, given their central involvement in homeostasis and thrombosis. The interaction between platelets and blood vessel walls are important in the development of thrombosis and cardiovascular diseases such as myocardial infarction, stroke and atherosclerosis (Mustard et al., 1990). Among the family of platelet activating factors (PAF), arachidonic acid, and ADP are three important platelet stimulants which induce platelet aggregation via different mechanism. Recently several natural natriplatelet agents from natural products including polyphenols (Luceri et al., 2007) and flavonoids (Pignatelli et al., 2002) have been reported. In this study polyphenolic extract selectively inhibited ADP induced platelet aggregation in vitro. Plant preparations containing polyphenols/flavonoids have been used for centuries as herbal remedies for a variety of diseases and found to have an impact on diabetes and obesity related disorders (Mary et al., 2003).

Polyphenols may inhibit platelet aggregation through a number of different mechanisms, including inhibition of cyclooxygenase, lipoxygenase and phosphodiesterase activities (Hong et al., 2001). Leaves of I. frutescens containing a variety of polyphenolic compounds. It appears that something, possibly by flavonoids in the PPE inhibits in vitro platelet activity. Antioxidants that prevent oxidative stress, such as vitamin E or polyphenolic flavonoids, as well as polyphenol-rich foods, protect (Aviram and Fuhrman, 2002; Kaplan et al., 2001) LDL from oxidation and, in parallel, reduce the development of atherosclerotic lesions. Thus, frequent consumption of polyphenol-rich foods may confer a health benefit against cholesterol accumulation and may inhibit enhanced development of atherosclerosis. This is consistent with in vitro studies document a decrease in platelet aggregation with flavonoids (Ruf, 1999).

In Indian traditional medicine, the plant has been on popular remedy for the treatment of diabetes mellitus (Patel and Srinivasan, 1997). Nowadays herbal drugs are gaining popularity in the treatment of diabetes and its complications. We found that the polyphenolic extract is rich in flavonoids, coumarins and phenolic compounds. These polyphenols are certainly one of the active components responsible for the antidiabetic and anti-hyperlipidemic effect. Polyphenols have been reported to have a major role in reducing oxidative stress-associated diabetes which in turn helps the regulation of plasma glucose concentration and hepatic glucose metabolism (Du Thie and Crozier, 2000). Flavonoids isolated from different sources have been documented to show antidiabetic and anti-hyperlipidemic activities (Vessal et al., 2003; Fuhrman and Aviram, 2001). Thus, the significant antidiabetic and antihyperlipidemic activity of PPE in our study may be attributed to the presence of various flavonoids and phenolic acids in the leaves of I. frutescens including apigenin, luteolin, kaemferol-3-glycoside, quercetin, ferulic acid caffeic acid. Another interesting feature is that the histopathological abnormalities seen in the pancreas and aorta of diabetic animals are also reversed showing almost normal appearance.

All these data suggest that oral administration of PPE to alloxan diabetic rats causes a considerable reduction of hyperglycemia and hyperlipidemia, mainly through a restoration of serum lipid levels and decrease in cholesterol synthesizing enzyme. However, the observed effects of PPE of I. frutescens could contribute very well to its antidiabetic potential, by minimizing the diabetes associated oxidative stress linked complications such as hyperlipidemia and atherosclerosis. However, more
studies are required not only to identify and characterize the polyphenols present in leaves of *I. frutescens*, but also to study the *in vivo* efficacy of this fraction in the prevention of atherosclerotic lesions induced in experimental animal models. Based upon the data reported here we propose that polyphenolic fraction from *I. frutescens* may have anti-diabetic and anti-hyperlipidemic effect.

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References


