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Original Article

FIBRINOLYSIS ENHANCEMENT BY *PUERARIA TUBEROSA* (INDIAN KUDZU) IN PATIENTS WITH CORONARY ARTERY DISEASE: A PLACEBO CONTROLLED STUDY

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Abstract: The Indian Kudzu (Pueraria tuberosa DC.) is an important medicinal plant widely used in Indian (Ayurveda) and Chinese systems of medicine. The present study is an attempt to evaluate its effect on fibrinolysis in patients with coronary artery disease. Acute study shows a dose dependent effect of P. tuberosa tuber powder on fibrinolytic activity; where a maximum of 150 percent rise was observed after administration of 5 g tuber powder and the minimum significant ($p<0.05$) rise was observed with 0.75 gram dose. One month administration of P. tuberosa in two doses of 3 g and 1.5 g, to the patients with coronary artery disease significantly increased fibrinolytic activity ($p<0.01$ for 3 g and $p<0.05$ for 1.5 g daily doses). The plasma fibrinogen level was also reduced; but the statistical significance was achieved only with 3 g dose ($p<0.01$). It was tolerated well without any untoward side effects. It is probably the first time that fibrinolysis enhancement of P. tuberosa has been demonstrated in human volunteers with coronary artery disease.

Key words: *Pueraria tuberosa*, Coronary artery disease

INTRODUCTION

Herbal preparations are used since ancient times to prevent diseases and regain a healthy state of mind and body. Advances in phytochemistry and identification of plant compounds which are effective in curing certain diseases have renewed the interest in herbal medicine. A number of studies have been conducted to find out the herbs and natural food sources and their supplements having anti-thrombotic effect and there is evidence that consuming such foods leads to prevention of coronary defects and stroke [1,2].

Pueraria tuberosa is an important medicinal plant widely used in various formulations in the Indian and Chinese traditional systems of medicine. It is a perennial climber and member of the family Fabaceae. It is distributed throughout India up to the height of 1200 meters. It is commonly known as Bilaikanda, Vidarikanda, Ghora-bel (Hindi), Bhumikushmanda (Sanskrit), Indian Kudzu (English) [3].

In Ayurveda, the tubers of *P. tuberosa* are described as sweet, refrigerant, emollient, laxative, aphrodisiac, galactogogue, diuretic, emetic, cardiotonic, expectorant, febrifuge and used for the treatment of hepatosplenomegaly, leprosy, dyspepsia, spermatorrhoea, tuberculosis and cough [4]. In ethnomedicine, tubers are used as edible and to treat diarrhea, fever, chest pain, rheumatism, abdominal pain etc [5].

These tubers are rich in isoflavanoids such as puerarin, daidzein, genistein and genistin; pterocarpenes and coumestans such as hydroxytuberosone, 3-O-methylanhydrotuberosin, anhydrotuberosin, tuberostan, tuberosin, puerarone, puerarostan, beta sitosterol, stigmasterol [6,7].

Recent researches worldwide have shown its antioxidant [8], hypoglycemic [9], hypolipidaemic [10,11], vasorelaxant [12] and cardioprotective [13] activities. In view of its traditional use and important pharmacological properties, the present study was envisaged to observe the effect of phytoestrogen rich tubers of *P. tuberosa* on plasma fibrinogen and fibrinolysis in human volunteers.

MATERIALS AND METHODS

Collection of plant material: Tubers of *P. tuberosa* were collected from Phulwari Wildlife Sanctuary, in Udaipur district. A voucher specimen of the plant (EA-264) was deposited at the Herbarium in Laboratory of Ethnobotany and Agrostology, Department of Botany, M.L. Sukhadia University, Udaipur for future reference.

Preparation of plant drug: Tubers were cut in small pieces and air-dried in shade at ambient temperature. After complete drying these were ground in an electrical grinder to make fine homogenous powder and filled in airtight containers. The dried powder was filled in gelatin capsules. Each capsule contained 0.75 g of the drug. Matched Placebo was also prepared by filling the capsules with lactose powder.

Study protocol and methodology: The study was approved by Institutional ethical committee. It was a single blinded, placebo controlled study. The study was in accordance with the guidelines of the Declarations of Helsinki and Tokyo (2004). After informed consent, study subjects were selected from the out patient department of Maharana Bhopal General Hospital attached to RNT Medical College, Udaipur, Rajasthan, India.

Subject distribution and drug administration:

Group I: Ten, middle aged (50-70 years), non obese (BMI<25), healthy individuals who were not suffering from any disease and not receiving any form of medication were selected for the study of 24 h profile of fibrinolytic activity. Out of these ten, five individuals were given a single dose of 3 g *P. tuberosa* and their fibrinolytic activity were assessed at 4, 8, 12 and 24 hours. Similarly, another five individuals were given a single dose of placebo and the same procedure was repeated.

Group II: Twenty five individuals between the ages of 50-70 years and having coronary artery disease (healed myocardial infarction more than six months) were selected who were stable in their symptoms and receiving isosorbide-5-mononitrate and aspirin. They were divided into two groups:

Group IIa: Five individuals were administered *P. tuberosa* tuber powder in different doses of 0.5, 0.75, 1.5, 3, and 5 grams. Blood samples were collected initially and after four hours of its administration. They were off the drug for seven days before the next dose was administered.

Group IIb: Twenty individuals were randomly administered either 1.5 g doses (1 capsule BID) or 3 g doses (2 capsules BID) tuber powder in two divided doses for 4 weeks. Blood samples were collected initially and at the end of one month. Then they were put on matched placebo for another one month and blood samples were again collected.

During the entire study period, they were not allowed to take any medicine except isosorbide-5-mononitrate and aspirin. Also, they were not allowed to alter their dietary and exercise schedule which they were following for the last 3 months.

Blood chemistry: All the blood samples were subjected to estimation of fibrinolysis [14] and fibrinogen [15] within half an hour of taking the blood samples. Plasma was separated from blood cells by centrifugation at 3000 rpm for 10 minutes. Plasma fibrinolytic activity (Units) was assessed as euglobulin lysis time (ELT) in minutes. Euglobulin fraction of plasma contains plasminogen activator, plasminogen and fibrinogen. Normally occurring inhibitors of conversion of plasminogen to plasmin are not present in this plasma fraction. The euglobulin fraction is clotted with thrombin and the time taken for clot lysis is estimated and expressed in units by multiplying the reciprocal of lysis time in minutes by 10000. Fibrinogen was measured by a chemical method as described by Nath and Debnath [15].

Statistical analysis: All the data were expressed as mean ± standard error of mean (SEM). The results were analyzed with Student's *t* test for paired data and a 'p' value less than 0.05 was considered as significant difference in the analysis.

RESULTS

Administration of 3 g tuber powder of *P. tuberosa* as a single dose to healthy subjects (50 to 60 Kg/

Table 1: Effect of one month administration of *Pueraria tuberosa* (PT) tuber powder on fibrinogen and fibrinolytic activity in patients with coronary artery disease (CAD). Values are expressed as Mean ± SEM, N- Number of subjects. p values: < 0.01: * II v/s I, e III v/s II. < 0.05 : b II v/s I, d III v/s II. NS : a II v/s I, c III v/s I, f III v/s II.

Dose	Parameters	Initial (I)	One month after PT (II)	One month after placebo (III)
3 G (N = 10)	Fibrinogen (Mg %)	305.8 ± 34.87	217.66 ± 32.62*	307.19 ± 25.91 c, d
	Fibrinolytic activity (Units)	63.62 ± 6.42	156.19 ± 11.04*	62.28 ± 5.58 c, e
1.5 G (N = 10)	Fibrinogen (Mg %)	233.2 ± 26.81	214.39 ± 22.43 a	228.75 ± 24.43 c, f
	Fibrinolytic activity (Units)	63.00 ± 5.83	101.69 ± 14.72b	64.23 ± 5.92 c, d

Fig. 1: Twenty four hours profile of fibrinolytic activity in male healthy volunteers administered 3 g *Pueraria tuberosa* (PT) and placebo (Values are mean from 5 subjects in each group.)

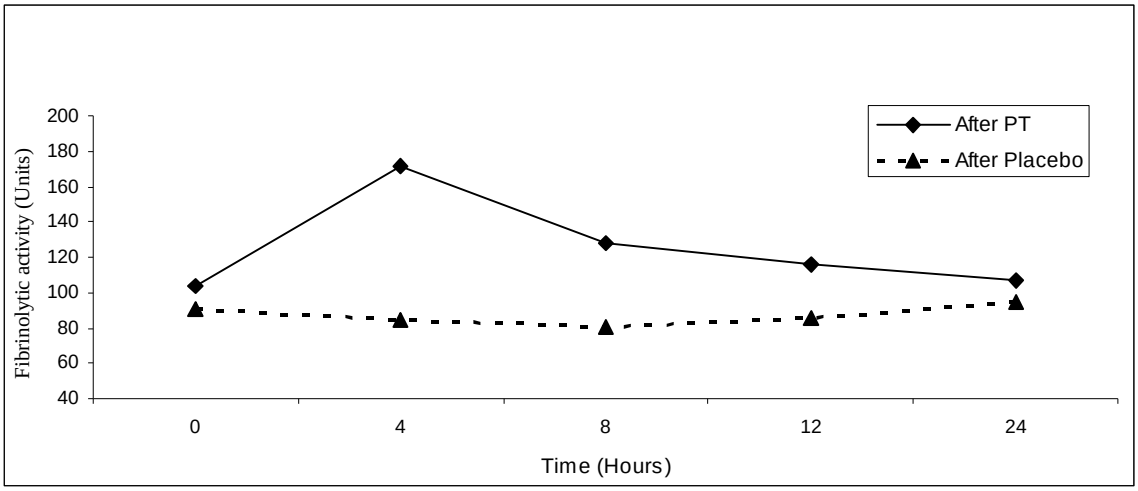
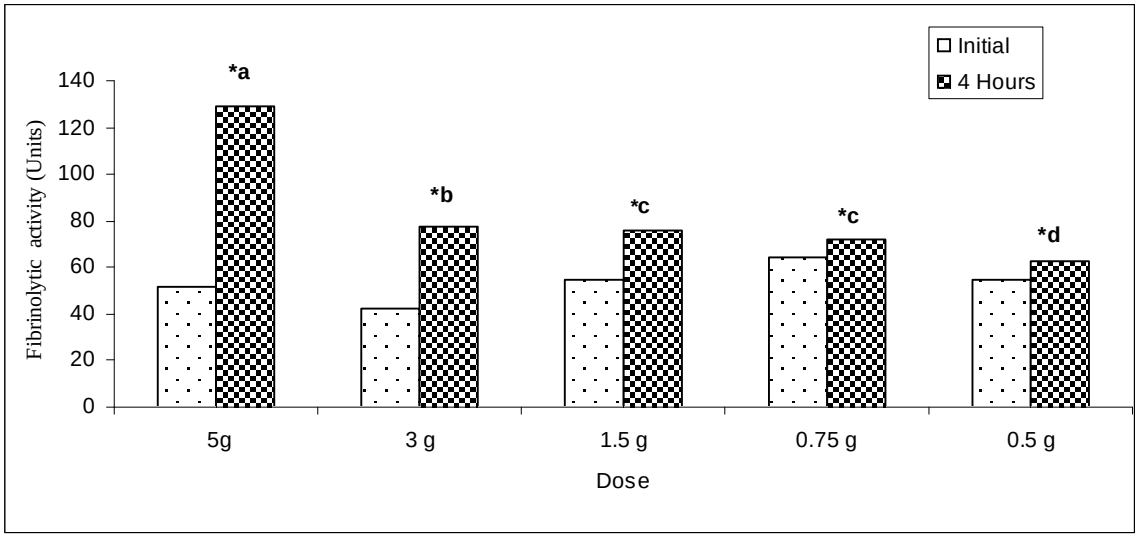


Fig. 2: Acute effect of *Pueraria tuberosa* (PT) on fibrinolytic activity in patients with coronary artery disease. 5 CAD patients took part in the study. They were off the drug for 7 days before the next dose was administered. *=p values, a: < 0.01, b: < 0.02 , c: < 0.05, d: NS.



body weight) significantly increased fibrinolytic activity to the tune of 66 % at the end of 4 hours and the rise was constantly observed even at the end of 8 hours (24 percent). Thereafter, study shows a progressive decrease in the activity of fibrinolysis at the end of 12 and 24 hours. The placebo group on the other hand showed normal variation in the fibrinolytic activity (Fig. 1).

Acute effect of different doses of *P. tuberosa* on fibrinolytic activity in patients with coronary artery disease reveals significant enhancement of fibrinolysis with 5 ($p<0.01$), 3 ($p<0.02$), 1.5 ($p<0.05$) and 0.75 ($p<0.05$) grams at the end of four hours which was of 150%, 83%, 40% and 12% respectively. However, in 0.5 gram dose, the effect was statistically insignificant (Fig. 2).

There was a significant rise in fibrinolytic activity and fall in fibrinogen levels ($p<0.01$) in patients with coronary artery disease after administration of 3 g *P. tuberosa* for one month. Fibrinogen levels did not decrease significantly after administration of 1.5g dose while fibrinolytic activity increased significantly ($p<0.05$) at the end of one month. However, administration of placebo to both the groups in the last four weeks of the study again increased fibrinogen levels ($p<0.05$) and decreased fibrinolytic activity significantly ($p<0.01$) bringing back the levels to the initial values (Table 1).

DISCUSSION

The pattern of enhancement of fibrinolytic activity after a single dose of 3 gram in healthy individuals (50 to 60 Kg body weight) corresponds with pharmacokinetic profile of puerarin extract in human volunteers. Puerarin was found to be rapidly absorbed via the oral route, reach peak levels at two hours and has a half life of approximately 4.3 hours. However, the plasma concentration remains at a level that is biologically active even 8 hours after the last dose [16].

It is interesting that even with the use of a crude drug, the fibrinolysis corresponds with the puerarin pharmacokinetics. It reached the maximum at 4 hours and the effect is still significant at the end of 8 hours. As the drug doesn't accumulate, repeated doses are recommended.

Acute study also demonstrates the dose dependent rise in fibrinolytic activity after 4 hours in patients with coronary artery disease. The minimum effective dose is 0.75 gram and the maximum affectivity in a reasonable amount is 3 grams. In order to further increase fibrinolysis, the dose has to be increased to 5 grams which is not practical in the form of crude powder. However, extract may be used for such purposes. From the point of view of its effect and suitability, 1.5 and 3 g doses were selected for further study.

Long term administration of *P. tuberosa* in both the doses of 3 g and 1.5 g, enhanced fibrinolytic activity significantly ($p<0.01$), which reduced after administration of placebo. Fibrinogen significantly ($p<0.01$) decreased with 3 g dose but not with 1.5 g of the drug. The effect on fibrinolysis was there even when the drug was given in a twice daily schedule. Pharmacokinetically, we expect more enhancement if it would have been given in 8 h dose schedule.

The fibrinolysis enhancement activity of *P. tuberosa* needs attention as it has been demonstrated for the first time in human volunteers, with crude tuber powder in a 12 h dose schedule, in a well planned dose dependent study and 24 hours fibrinolysis profile.

Pueraria root is known in traditional Chinese medicine as an important medicament. Puerarin and puerarin-xyloside, isolated from the roots, were the first isoflavone glycosides reported to occur in nature. It is used in traditional medicine as a fertility enhancing agent and as an aphrodisiac, cardiotonic, diuretic and galactogogue. Its crude powder, ethanolic and butanolic extracts possess significant estrogenic activity. In addition to this, significant progestational and mild antiprogestational activity of different fractions has been demonstrated [17]. Genistein and daidzein both have shown phytoestrogenic activities [18] and exhibited effective antioxidant properties [19].

Puerarin induces an endothelium independent relaxation in rat aortic rings [12], while daidzein demonstrates effectiveness in preventing ventricular fibrillations induced by chloroform in mice [20]. Puerarin does augment coronary collateral circulation in dogs with experimental acute myocardial infarction [21]. Cardioprotective effect of the combined use of puerarin and danshensu has also been demonstrated on acute ischemic myocardial injury in rats [22] Not only this, *Pueraria* isoflavone significantly decreases blood viscosity and platelet adhesion and inhibits thrombosis and ADP induced platelet aggregation in rats [23]. Numerous experimental studies have also

demonstrated its hypolipidaemic [10,11], antihyperglycemic [9] and antioxidant [8] properties of *P. tuberosa*.

Fibrinolytic activity is considered to be a major physiological means of disposing the fibrin after its haemostatic function has been fulfilled. The process is of great importance in wound healing and recanalization of thrombosed vessels. Fibrinolysis is accomplished by a proteolytic enzyme plasmin (Fibrinolysin) which is formed from an inert precursor in the plasma called plasminogen (pro-fibrinolysin). Plasmin digests fibrin fibers and some other protein coagulants, causing lysis of a clot. In healthy individuals, there exists a dynamic equilibrium between fibrin deposition and its cleaning by fibrinolytic process. If the fibrin is not removed properly then its organization and fatty deposition on the artery involved results in atheroma formation [24]. The role of fibrinogen in atherosclerotic coronary artery disease and stroke cannot be overemphasized. It has been documented as an independent risk factor for both coronary artery disease and stroke [25].

There are many factors that influence fibrinolytic activity such as age, gender, physical exercise, stress, pregnancy, menstruation etc. Drugs, disease, diet and diurnal variations also affect this system [26,27]. Many of the plants for example, *Bombax ceiba* [28], *Fagonia arabica* [29], *Ganoderma lucidum* [30], *Commiphora wightii* [31] and plant derived dietary supplements such as garlic [32], amala [33], ginger [34], cardamom [35], saffron [36], asafaetida [37] etc. have also been shown to have significant fibrinolysis enhancing activity.

Reduced fibrinolytic activity has been reported in ischemic heart disease, hypertension and diabetes in many of the scientific studies [38,39]. In view of this, the present observation is an important addition to the list of plant derived fibrinolytic agents, which can be utilized to treat patients with a tendency of reduced fibrinolysis.

During the past decade, interest in polyphenols including isoflavanoids has increased considerably because of its beneficial effects in cardiovascular diseases [40]. In this context, fibrinolysis enhancing effect of isoflavanoids of *P. tuberosa*, is quite promising as evident in the present communication. It would be interesting to further investigate the effect of its active component on the mechanism of clot lysis with respect to its effect on plasminogen, plasmin and antiplasmin.

On the basis of its significant effect on fibrinolytic activity in patients with coronary artery disease, *P. tuberosa* may be incorporated as an addition to the list of thrombolytic agents of plant origin, with negligible side effects and inexpensive too. However, further studies are warranted to evaluate its effects on other coronary risk factors in a well controlled study, with inclusion of large number of patients for a longer duration. The work in this respect is in progress.

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