

Research Article



In Vitro Antiplatelet Activity of a Polyherbal Formulation – Diabet

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Accepted on: 23-04-2014; Finalized on: 30-06-2014.

ABSTRACT

Diabet is a proprietary ayurvedic polyherbal formulation consisting of dried powdered extracts of *Tribulus terrestris*, *Commiphora mukkal*, *Cyperus rotundus*, *Phyllanthus reticulatus*, *Tamarindus indica*, *Red ochre*, *Curcuma longa*, *Coscinium fenestratum*, *water lily* and *Strychnos potatorum*, used in the supportive therapy of diabetes mellitus. It also possesses diuretic, antioxidant, anti-inflammatory, anthelmintic and cardiotoxic activities. As diabetic condition is closely associated with coronary vascular diseases, an attempt has been made to investigate efficacy of Diabet on platelet aggregation. The present study was designed to evaluate the efficacy of polyherbal formulation - Diabet on in vitro antiplatelet activity. Antiplatelet activity of Diabet was evaluated using ADP induced platelet aggregation by *in vitro* method. Under the *in-vitro* condition, Diabet in various concentration (125, 250, 500, 1000, 1250 µg/ml), alleviated ADP induced platelet aggregation in a dose dependent manner. Aspirin (100 µg/ml) was used as standard anti platelet drug. Anti-platelet activity was observed and was significant at 1000 µg/ml and 1250 µg/ml. The *in vitro* study shows that the polyherbal formulation Diabet possesses anti-platelet activity.

Keywords: ADP induced platelet aggregation, Antiplatelet, Diabet, Polyherbal formulation.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by altered carbohydrate, protein and lipid metabolism leading to pathological changes like cellular proliferation resulting in vascular complications like peripheral vascular insufficiency, lumen narrowing, sclerosis of glomerular capillaries, retinopathy and neuropathy. Diabetes mellitus is one of the most common chronic diseases across the world and number of diabetic patients is on rise.¹ Statistical data across the world reveal that nearly 366 million are diagnosed with diabetes in 2011 and it is expected to affect 552 million people by 2030.² The prevalence of diabetes is increased in India. At present, approximate diabetes patients in India was found to be 67 million, with another 30 million in pre diabetes group. It is expected that by 2030, India will have the largest number of diabetic patients in the world. Diabetes is a condition where there will be an increased blood sugar level that leads to other complications also.³ The increased risk of death due to myocardial infarction is three times in diabetic patients when compared with non-diabetic patients. The management of the disease is considered to be a global problem and long term treatment with modern drugs including insulin and other oral hypoglycemic agents⁴ control the blood sugar as long as they are regularly administered but also produce a number of undesirable effects. Plants have been used by man, from time immemorial for their extraordinary healing properties and pain relieving properties. Presently the medical fraternity has increasingly started using plants to overcome various illness and sufferings mainly to obviate the profound side effects encountered in usage of modern drugs. In recent study, the polyherbal

formulation Diabet was found to exert potent antioxidant activity on alloxan induced oxidative stress in rats.⁵

Platelet plays a key role in the physiological hemostatic process and pathologic thrombosis. They are essential for the maintenance of cardiovascular integrity, control bleeding through the formation of blood clot. Platelet activation is central to the pathogenesis of hemostasis and arterial thrombosis. Platelet aggregation plays a major role in acute coronary artery diseases, myocardial infarction, unstable angina, and stroke. In vitro platelet aggregation can be induced by Adrenaline, collagen, thrombin and ADP.⁶ Adenosine 6- diphosphate (ADP) is an important agonist for platelet aggregation. ADP induces platelet change of shape, granule secretion, mobilization of intracellular calcium and inhibition of stimulated adenylyl cyclase.⁷

Antiplatelet or anti thrombotic drugs are those, that interferes with platelet function and may be useful as prophylactics in thromboembolic disorders. They also decrease the formation of platelet, beneficial in the prevention and treatment of occlusions, cardiovascular diseases and as an adjuvant to thrombolytic therapy in myocardial infarction.⁸ Platelets stick to the damaged vessel wall and stick to each other and release ADP, thromboxane A2 which promote further aggregation. In arteries, platelet mass is the main constituent of the thrombus. Hence antiplatelet drugs are useful in arterial thrombosis, reduce mortality and prevent reinfarction, reocclusion after thrombolytic therapy, reduces the risk of myocardial infarction and sudden death of patients with unstable angina.⁹ Aspirin and Clopidogrel are the commonly used effective antiplatelet drugs, but their continuous use produces series of side effects, such as,



vomiting, headache, gastric ulceration and abdominal cramps.^{10,11} As the polyherbal formulation consists of phytoconstituents such as flavones, resin, alkaloid, organic acids, the experiment was designed to evaluate the antiplatelet activity of the formulation at various concentrations.

Polyherbal formulation - Diabet

In ayurvedic formulation combination of substances is used to get the enhanced desired action and to eliminate unwanted side effects. The diabet is an ayurvedic polyherbal formulation consisting of dried powdered extracts of *Tribulus terrestris*, *Commiphora mukkal*, *Cyperus rotundus*, *Phyllanthus reticulatus*, *Tamarindus indica*, *Red ochre*, *Curcuma longa*, *Coscinium fenestratum*, *water lily* and *Strychnos potatorum*. The table 1 shows the details about the composition of Diabet.

Table 1: Shows the details about the constituents of polyherbal formulation Diabet

Constituent of polyherbal Formulation- Diabet	Family	Name			Parts used	Uses
		English	Tamil	Sanskrit		
<i>Tribulus terrestris</i>	Zygophyllaceae	Land Caltrops	Nerunji	Gokshura	Flowers & fruits	Diuretic, Kidney stone Digestant
<i>Commiphora mukkal</i>	Bursweaceae	Gum-gugul	Gukkulu	Gukkulu	Resinous gum	Demulcent, Carminative, Astringent, Antispasmodic, Diuretic
<i>Cyperus rotundus</i>	Cyperaceae	Nul grass	Kavai	MustaMu rtaka	Tuber on bulbous root	Demulcent, Diuretic, Anthelmintic, Astringent
<i>Phyllanthus reticulatus</i>	Euphorbiaceae	Black-honey shrub	Kattu-killanelli	Pulika, Krishna-kamboji	Whole plant	Diuretics stomachic Burns Obesity
<i>Tamarindus indica</i>	Caesalpinaceae	Tamarind tree	Puli Amilam	Cinta	Roots Leaves Fruits Seeds	Astringent, Anti inflammatory, Anti fungal, Hemorrhoids, Diabetes
<i>Curcuma longa</i>	Zingeberaceae	Turmeric	Manjal	Haridra, Nisa	Rhizomes & Tubers	Anti-inflammatory Antiseptic, Anthelmintic Laxative Diuretics, Ulcer Wounds, Jaundice, Diabetes
<i>Coscinium fenestratum</i>	Menispermaceae	Tree Turmeric	Mara-Manjal	Daru-haridrakam	stem	Anti-inflammatory, Ulcer, Wounds, Diabetes, Thermogenic
<i>Strychnos potatorum</i>	Soganiaceae	Clearing nut tree	Tattamaram	katakah	Seeds, Roots Fruits, Pulp	Diuretic, Digestive, Hepatopathy, Nephropathy, Diabetes

Method - *In-vitro* platelet aggregation study¹²

To determine the *in vitro* antiplatelet aggregation property, 100 µl of the various concentrations of Diabet (250, 500, 750, 1000, 1250 µg/ml) respectively was added to 1 ml of platelet suspension and incubated for 1 minute exposure at 37°C and then 1.5 ml buffer, 0.5 ml ADP were added and the transmittance was measured at 600 nm. The percentage transmittance was taken immediately and every one minute for up to five minutes. Aspirin at 100 µg/ml was used as standard antiplatelet drug.¹³ A graph was plotted between percentage transmittance and time in minutes is given in (Figure 1).

MATERIALS AND METHODS

1. Diabet, the polyherbal formulation was obtained from M/S Herbal Galenicals, Purasaiwakkam, Chennai.
2. Tyrode buffer – Prepared using sodium chloride 149mM, potassium chloride 2.6mM, sodium bicarbonate 9.5mM, glucose 5.5 mM, sodium dihydrogen phosphate 0.5mM, magnesium chloride 0.6 mM and gelatin 0.25%.
3. Platelet rich plasma (PRP) - For assay PRP is suspended in Tyrode buffer and pH adjusted to 7.4 with 0.25M HCl.
4. ADP
5. Aspirin (standard antiplatelet drug)

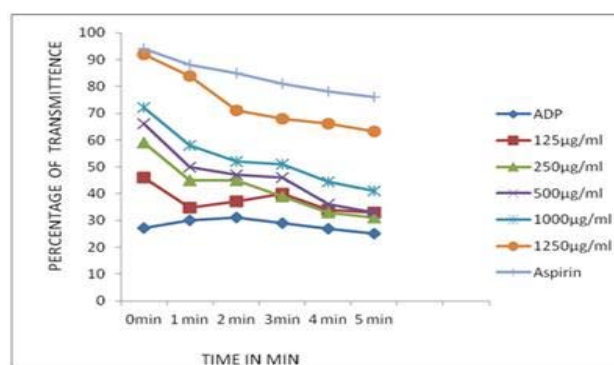


Figure 1: *In-vitro* Antiplatelet activity of polyherbal formulation - Diabet

RESULTS

In the present study, *in vitro* antiplatelet activity of Diabet was evaluated against ADP induced platelet aggregation model. A dose dependent inhibition of platelet aggregation was observed. Diabet showed antiplatelet activity in all five concentrations (250, 500, 750, 1000, 1250 µg/ml). The maximum antiplatelet activity was observed at the concentration of 1250 µg/ml in comparison with standard antiplatelet drug Aspirin (100 µg/ml).

DISCUSSION

Platelets play a pivotal role in Homeostasis.¹⁴ Natural products including polyphenols and flavonoids have been reported to have antiplatelet activity. Plant preparations containing polyphenols and flavonoids have been used for several decades as herbal remedies for a variety of diseases and found to have an impact on diabetes and obesity related disorders. Many cardiovascular diseases can be attributed to excessive platelet aggregation, which has a critical role in thrombus formation. A dose dependent inhibition of platelet aggregation was observed, in comparison to the standard aspirin. The activity may be due to the presence of oleo resin – 2 – guggulusterone in *Commiphora mukul*, alkaloids and essential oil with pinene and cineole in *Cyperus rotundus*, tartaric acid, citric acid, maleic acid, vitamin A and niacin in *Tamarindus* and carotenoids in *Curcuma*.

CONCLUSION

Diabet, the polyherbal formulation possesses very good antiplatelet activity in *in-vitro* model. Therefore, it may be used to treat or prevent some cardiovascular diseases. The present study shows the antiplatelet activity of *Diabet* which may be beneficial in various cardiovascular ailment, but it should be used cautiously in patients with bleeding or other hematological disorders as it may increase the risk of bleeding and other complications. Hence it can be concluded that the polyherbal formulation has antiplatelet activity. Hence it can be concluded that Diabet the polyherbal formulation has antiplatelet activity.

Acknowledgement: The authors thank Dr.Panicker, incharge, Blood Bank, SRMC and RI for providing PRP and

the management of SRMC and RI (DU) for encouragement and support.

REFERENCES

1. Viswanathan Mohan, Siddharth Shahanshi, Sabo Whiting Dr, Guariguata L, Weil C, Shaw j, IDF Current Glycemic Status and Diabetes Related Complications Among Type 2 Diabetes Patients in India: Data from the A1chieve Study 2013, 61.
2. Diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030, Diabetes Res Clin Pract, 94, 2011, 311-321.
3. Ismat Tahseen, Diabetes epidemic on the rise in India, The times of India, 2013, Nov 14, 02.40PM IST.
4. Prasad R, Bobby P, Arch Plant Res, 14(6), 2001, 473.
5. Umamaheswari S, Prathiba D, Srikanth J, Umamaheswara Reddy, Antioxidant potential of a polyherbal formulation (Diabet) on Alloxan induced oxidative stress in rats, Drug invention Today, 1(1), 2009, 46-49.
6. Viswanathan GL, Mohamed Rafiq, Rajesh S, Sandeep Rao KS, Mohammed Azeemuddin, Anturlikar SD, Rangesh P, Patki PS, Anti-platelet and Anti-Thrombotic Effects of a Poly-ingredient formulation: *In-vitro* and *in-vivo* experimental evidences Oman Medical Journal, 2012, 27.
7. Puri RN, Colman RW, ADP-induced platelet activation, Crit Rev Biochem Mol Biol, 32(6), 1997, 437-502.
8. Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 10, Mc Graw Hill Medical Publishing division, New Delhi, 2001, 1534-1536.
9. Tripathi KD, Essentials of Medical Pharmacology, 5, Jaypee brothers medical publishers (p) Ltd, New Delhi, 557-574.
10. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, Costa MA, Variability in individual responsiveness to Clopidogrel: clinical implications, management, and future perspectives, Journal of the American College of Cardiology, 49, 2007, 1505-1516.
11. Eun Ji Seo, Dong-Ung Leee, Jong Hwan Kwakf, Sun-Mee Leef, Yeong Shik King, Yi- Sook Jung, Antiplatelet effects of *Cyperus rotundus* and its component (+)-nootkatone, Journal of Ethnopharmacology, 135, 2011, 48-54.
12. Iman RA, Lakshmi Priya B, Chitra R, Shalini K, Sharan V, Chamundeeswari D, Vasantha J, *In Vitro* Antiplatelet Activity – Guided Fractionation of Aerial Parts of *Melothria maderaspatana*, Indian Journal of Pharmaceutical Sciences, 2006, 668-670.
13. Patrnoc C, New Eng J Med, 330, 1994, 1287.
14. Sathyapriya E, Velpandian V, Anbu, Ashwini Anjana, *In-Vitro* Anti Platelet Aggregation Activity And Thrombolytic Activity Of Cheenalinga ChendhuRAM, International journal of life science & pharma research of, 2(4), 2012, 127.

Source of Support: Nil, Conflict of Interest: None.

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