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Ethnomedicinal Plants for the Treatment of Platelet Dysfunction Allied Diabetes

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Abstract

Diabetes mellitus is associated with oxidative stress, which could be a consequence of either increased production of free radicals, or reduced antioxidant defenses. Oxidative stress is not only associated with complications of diabetes, but has been linked to Insulin Resistance in vivo. Abnormalities in platelet function are associated with insulin-resistance. Insulin is a key regulator of glucose metabolism. Platelets have been shown to be targets of insulin action because they retain a functional insulin receptor capable of insulin binding and autophosphorylation. The hypothesis that platelets behave differently in type I and type II diabetes determines the role of IR in platelet hyperaggregability in diabetes. Reduced insulin sensitivity may account for platelet hyperactivity in type 2 diabetes. Since antiquity, diabetes has been treated with plant medicines. The third world nations of Asia are rich in biodiversity and indigenous knowledge particularly ethnomedicinal practices. Among them, India is endowed with a rich biological heritage. Historical reports indicate that before the discovery of insulin, Jamun was used in the treatment of diabetes both in India and other countries. In this present study, six different ethnomedicinal plants *Gymnema sylvestre, Momordica charantia, Syzygium cumini, Pterocarpus marsupium, Trigonella foenumgraecum* and *Cinnamomum tamala* were evaluated, of which *Syzygium cumini* and *Gymnema sylvestre* were found to be more effective in the regulation of PMA (Phorbol Myristic Acid) induced formation of ROS (Reactive Oxygen Species) in the platelets obtained from the diabetic subjects. The observed health benefits may be credited to the presence of the various phytochemicals like polyphenols, terpenes, anthocyanins and flavonoids. Future studies should be on validating the mechanism of action responsible for the various beneficial effects and also on understanding which compound/s are responsible for the reported effects.

Key words: Medicinal Plants, Platelet, Diabetes, Insulin.

Introduction

Diabetes is a complex metabolic disorder characterized by defects in the body's ability to regulate glucose and insulin homeostasis. Diabetes has become an epidemic and remains a major public health issue across the nations of the world. An estimate suggests that diabetes would affect more than 210 million people worldwide by 2010 and the number has been reported to increase by two fold by the year 2030, posing a tremendous economic burden on individuals and healthcare systems worldwide (WHO, 2003). With the rising cost and escalating incidence of diabetes, it is important to understand the mechanism that leads to the onset of the disease.

Diabetes is divided into two main types, type 1 and type 2. Type 1 diabetes occurs when the system impedes to produce or produces little amount of insulin. On the other hand, type 2 diabetes results when the body fails to produce enough or has complications in utilization of the insulin available inside the system. Beside, these two major types of diabetes, others result from specific conditions - genetics, environment, lifestyle, dietary, surgery, medications, infections, including pancreatic disease, and other illnesses (National Diabetes Fact Sheet, 2011).

Oxidative stress is thought to be a major risk for the onset and progression of diabetes and its associated complications (Kyrou *et al*., 2006). Furthermore, risk factors such as age, diet, lifestyle, either individually or in combination with genetic factors contribute to an oxidative environment that may alter insulin homeostasis. A common result of both types of diabetes is hyperglycemia, which in turn contributes to the progression and maintenance of an overall oxidative environment.

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Formation of ROS in hyperglycemic condition plays an important role in the activation of platelets through various mechanisms. Platelets are small anucleate discoid cells that circulate in the bloodstream and participate in homeostasis (Ashby *et al*., 1990). Platelet regulatory factors, such as nitric oxide (NO), prostacyclin (PGI2), and adenosine, reactive oxygen species (ROS) participate in the regulation of platelet activation. The increased platelet activity is emphasized to play a role in the development of vascular complications of this metabolic disorder (Demirtunc *et al*., 2009). Platelets have been shown to be targets of insulin action because they retain a functional insulin receptor capable of insulin binding and autophosphorylation (Falcon *et al*., 1998). Insulin is generally thought to reduce platelet responses to the agonists ADP, collagen, thrombin, arachidonate, and platelet-activating factor (Trovati *et al*., 1988).

Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (Barry, 1991). The currently available antidiabetic agents including sulfonylureas, biguanide, thiazolidinedione and a-glycosidase inhibitors are widely used to control the hyperglycemia and hyperlipidemia, but these drugs fail to significantly alter the course of diabetic complications and have limited use because of undesirable side effects and high rates of secondary failure (Babu *et al*., 2010). Thus, it is essential to look for more effective antidiabetic agents with fewer side effects.

Ethno medicinal plants having antidiabetic properties can provide a useful alternative source for the development of safer and effective oral hypoglycaemic agents. Around the world, they have identified more than 1,200 species of plants with hypoglycemic activity.

The production of drugs and other preparations based on indigenous systems of medicine in India has increased many folds during the past few decades. Based on this background, the present study was aimed to evaluate the role of phytochemicals against ROS formation.

Six ethnomedicinal plants *Gymnema sylvestre; Momordica charantia; Syzygium cumini; Pterocarpus marsupium; Trigonella foenumgraecum* and *Cinnamomum tamala* were evaluated for their activity against platelet dysfunction associated with diabetes.

Materials and Methods

The study group comprised of diabetic and non-diabetic (control) subjects recruited from NRMC, Periayakulam, Theni.

Clinical parameters

Clinical and biochemical parameters were followed by Standard methods such as age and duration of diabetes were recorded and a complete clinical examination was done. Blood pressure and Body Mass Index (BMI) was calculated.

Biochemical parameters

A fasting blood sample was collected after an overnight fast of at least 10 h for biochemical investigations. Fasting and 2h plasma glucose estimations (GOD method), serum cholesterol (CHOD-PAP method) and serum triglycerides (GPO-PAP method) were measured. HDL cholesterol was estimated by CHOD-PAP method after precipitating low-density lipoprotein (LDL) and chylomicron fractions by the addition of phosphotungstic acid in the presence of magnesium ions and very low-density lipoprotein (VLDL). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald formula. Glycated hemoglobin (HbA1c) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, California, USA). Serum creatinine was measured using Jaffe's method. Approximately about 250 µl volume of reagent mixture from the kit is mixed with 150 µl of plasma.

Estimation of Glucose

Glucose is oxidized by glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen-peroxide which is converted to H_2 and O_2 by peroxidases. (The reaction mix contained phosphate buffer 200mM, pH 7.5, glucose oxidase ≥183µkat/L; peroxidase POD (horse radish) ≥0.33 µkat/L, 4-aminophenazone (O_2 acceptor) 0.77 mm/L, phenol 11 mM/l). 4 aminophenazone with phenol forms a pink color which can be measured at 515mm.

Estimation of Serum Cholesterol

Serum total cholesterol was estimated using CHOD-PAP method, and read at 505 nm. The

color intensity is directly proportional to the concentration of cholesterol.

Estimation of Serum Triglyceride

Triglyceride was estimated using GPO-PAP method, and read at 505 nm.

Estimation of Serum HDL cholesterol

HDL-cholesterol in serum was measured using polyanion-metal combinations, polyethylene glycol (PEG) with anti-apoprotein CIII antibodies.

Estimation of serum LDL cholesterol

LDL cholesterol was calculated using the friedwald formula, which is given below:

LDL Cholesterol = Total cholesterol - (HDL - Cholesterol + Triglyceride/5)

Estimation of serum Urea levels

The estimation of blood urea was based on the coupled Urease/ glutamate dehydrogenase (GLDH) enzyme system. The decrease in absorbance at 340 nm due to consumption of NADH is measured kinetically.

HbA1c estimation

HbA1c possesses less charge positivity and hence elutes faster from a cation exchange column. Hba1c levels were expressed as % age of glycated hemoglobin (HbA1c %).

Creatinine Estimation by Jaffes Method

Determination of the creatinine concentration was derived by the Jaffes caloric method in which yellow/orange coloration appears when the urine is treated with alkaline picrate. To one ml of alkaline picric acid, 0.1 ml of serum was added and the color development was read at 498 nm.

Preparation of Platelets

Whole blood from the fasting and post-prandial state was collected from the study subjects in Acid Citrate and Dextrose (ACD) tube containing acid-citrate and dextrose (BD Vacutainer) and centrifuged at 800 rpm for10 min, platelet rich plasma (PRP) was collected. The platelet rich plasma (PRP) was pelleted at 2000 rpm for 10 min and the pellet is suspended in HEPES buffer without $CaCl_2$ and $MgCl_2$ containing 100 μM Aspirin and 20 μM EGTA to prevent the activation of platelets. The suspension was pelleted and washed twice with HEPES buffer without $CaCl_2$ and $MgCl_2$ containing 0.1% BSA.

Measurements of ROS production

To measure intracellular ROS production, cell suspension in HEPES buffer saline were loaded or inoculated with 10 μM Dichlorofluorescein acetate (DCFH-DA) for 45 min at room temperature. Aliquot containing approximately 200,000 cells were centrifuged to remove the extracellular dye and the pellet was suspended in 200 μl HEPES buffer which was injected into microplate well. The ROS production was measured in MicroMax 384 fluorescence microplate connected to Fluoromax-3 reader with an excitation at 485 nm and emission at 530 nm, due to the change in the fluorescence by the conversion of non-fluorescent dichlorofluorescein diacetate to the highly fluorescent compound 2',7'-dichlorofluorescein (DCF) in the cells by the superoxide. To measure SIN-1 induced ROS generation DHR123 was used as detecting agent since it also measure peroxynitrite concentration in the cell system. Dihydrorhodamine 123 (DHR 123) is the uncharged and non-fluorescent reduction product which passively diffuses across most cell membranes where it is oxidized to fluorescent cationic rhodamine 123, by peroxynitrite, hydrogen peroxide etc., Aliquots containing 200,000 cells in 200 μl HEPES buffer was injected into microplate well and 10μM concentration DHR123 dye was loaded and activated with the agonist PMA (500nM).

Preparation of Herbal extracts

The roots, fruits, seeds, leaves, wood and whole plant of natural authentic herbs herbs/medicinal plants collected from different natural sources. The plant materials were cut into small pieces of about 12 cm, dried at 45°C crushed and powdered to 350-mesh. The dried powder of Gymnema sylvestre, Momordica charantia, Syzygium cumini, Pterocarpus marsupium, Trigonella foenum-graecum, Cinnamomum tamala were used for the preparation of crude extracts.

Data Analysis

Unless otherwise mentioned, data are presented as mean±SD. Comparisons between groups were performed using ANOVA. Two tailed P values ≤0.05 was considered statistically significant. All calculations and analysis were performed SPSS.

Results and Discussion

Based on the clinical characteristics features of the subjects (Table -1), it could be observed that age is a non significant criterion among the diabetic subjects. The values obtained for the diabetic subjects were almost similar and comparable to the normal are non diabetic subjects.

Further, it could be inferred that occurrence of diabetes is more common in children and in young adults. Comparison of body mass in index of the subjects used in the study shows that there is no significant correlation between the diabetic and the non diabetic individuals. Hence body mass index is a non significant parameter. Statistical evaluation of the data and the p value show that this factor has less influence on the onset of diabetics in the population.

Table -1: Clinical record of the study subjects

Parameters	Non-diabetic (n=15)	Diabetic (n=15)	P value
Age (y)	47.0 ± 7.0	50.0 ± 14.0	NS
Duration (y)	-	5.0 ± 2.5	-
Body mass index (Kg/m^2)	23.6±6.4	26.4±5.3	NS
Systolic blood pressure (mm Hg)	115.0 ± 8.0	127.0 ± 16.0	0.029
Diastolic blood pressure (mm Hg)	76.0 ± 9.0	78.0±9.0	NS
Fasting plasma glucose (mg/dl)	84±9	151±60.0	0.001
Glycated hemoglobin (HbA1c) (%)	5.7±0.4	8.3±2.5	0.001
Serum triglyceride (mg/dl)	120±69	153±65.0	NS
Serum cholesterol (mg/dl)	174±30	176±33.0	NS
HDL cholesterol (mg/dl)	45±10	46±12.0	NS
LDL cholesterol (mg/dl)	108±27	128±41.0	NS
Serum creatinine (mg/dl)	0.9±0.13	0.95±0.10	NS
Urea (mg/dl)	25 ± 5.0	26±10.0	NS
Creatinine (mg/dl)	0.9±0.13	0.95±0.3	NS

Mean± SD; NS- Non-Significant

Analysis of systolic blood pressure in the study subjects showed that this factor significantly contributes to the onset of diabetes. A p value of 0.029 indicates that systolic blood pressure is the most significant characteristic feature that contributes to the onset of diabetes. It is higher in the diabetic patients when compared to the non diabetic subjects. The higher value of 127.0 ± 16.0 among the diabetic patients clearly indicates that systolic blood pressure significantly increases in the subjects as against the normal value of 115.0 ± 8.0 in the non diabetic subjects. However comparison of diastolic blood pressure was almost the same (78.0 ± 9.0) in the both diabetic and non diabetic individuals used in the study this indicates that the disease is not significantly influenced by this factor. Further, statistical analysis and non significant nature of p value also supports this observation.

It has been well established by several studies that glucose level is a key factor is used to differentiate diabetic subjects from normal individuals. Comparative analysis of fasting plasma glucose level indicates that it is almost twice in the diabetic patients (151.0 ± 60.0) as against the normal value of 84.0 ± 9.0 in the non diabetic individuals.

Comparative analysis of the % glycated hemoglobin content among the diabetic and non diabetic individuals revealed that this parameter is also one of the significant factors that influence the onset of diabetics. It could be observed from the Table 1 that the normal level of % glycated hemoglobin content (5.7 ± 0.4) increased to 8.3 ± 2.5 in the diabetic subjects. Further statistical analysis of this parameter revealed that % glycated hemoglobin content is serves an indicator to diagnose of diabetics.

The p value of 0.001 indicates that it is one of the key factors which could be used to determine the clinical manifestation of diabetic patients. Likewise, comparison of cholesterol related parameters such as serum triglycerides

(ST), serum cholesterol level (SCL), high density lipo-protien (HDL) and low density lipoprotein (LDL) indicate that these parameters have least influence on the onset / progression / persistent nature of diabetics among the sufferers when compared to the normal individuals. The lower level of ST (120.0 ± 69.0) against its higher level (153.0 ± 65) in diabetic patients indicates that this is the non significant parameter. Similarly the lower values 174, 45, 108 as against the higher value 176, 46, 128 for SCL, HDL and LDL respectively clearly indicates that these factors do not play any significant role to distinguish diabetic patients from non diabetic individuals. Likewise statistical analysis of serum creatinine, creatinine and urea indicates that these parameters do not have a significant role to distinguish diabetic patients from non diabetic individuals.

From the comparative analysis of various parameters that have been reported to contribute to the onset of diabetics it is clear that systolic blood pressure, blood glucose level and the percentage glycated hemoglobin content of the serum play a major role.

Further these parameters significantly affect the level of glucose in the blood as determined by the level of insulin. When compared to control subjects, diabetic patients exhibited high systolic blood pressure and poor glycemic control (high fasting plasma glucose and HbA1c levels).

Effect of different herbal extracts on formation of ROS of the different herbal extracts used in the study GS, MC, SC, PM, TF and CT it was found that extracts of SC and GS were more effective in the regulation of PMA inducted formation of ROS in the platelets obtained from the diabetics patients. This was followed by the extracts from other herbs used in the study (Table 2; Fig. 1, 2).

The mean value for the herbal treatment for the diabetic patients was 47.16 as against the calculated mean value for the control subjects and total subjects as 62.5 and 54.83 respectively (Table 3a).

Table -2: PMA induced ROS by different herbal extracts

Plant source	Control (n=15)	Diabetes (n=15)
Gymnema sylvestre (GS)	86	59
Momordica charantia (MC)	76	54
Syzygium cumini (SC)	60	60
Pterocarpus marsupium (PM)	46	38
Trigonella foenumgraecum (TF)	58	41
Cinnamomum tamala (CT)	49	31

Table -3a: ANOVA for the selected diabetic and non-diabetic subjects

	Samples		
	Control	Diabetes	Total
N	6	6	12
$\sum X$	375	283	658
Mean	62.50	47.16	54.83
$\sum X^2$	24653	14083	38736
Variance	243.1	146.96	241.42
Std. Dev	15.59	12.12	15.53
Std. Err.	6.36	4.94	4.48

Un-weighted -means analysis

Analysis of variation between the treatment groups had a P value of 0.0862. This indicates that the data is statistically significant (Table 3b).

In diabetes oxidative stress plays a key role in the pathogenesis of vascular complications, and an early step of such damage is considered the development of an endothelial dysfunction. Present study provides direct evidence that insulin, insulin sensitizers and herbals reduce intracellular formation of peroxynitrite. Insulin exerts vasodilatory, antiplatelet, and anti-inflammatory effects at the cellular level in vitro and in humans in vivo. The mean platelet volume (MPV) is an indicator of the average size and activity of platelets. The mean platelet counts and MPV were higher in diabetics compared to the non-diabetic subjects [277.46 ± 81 X 109/l vs. 269.79 ± 78 X 109/l (P= 0.256)], 8.29 ± 0.74 fl versus 7.47 ± 0.73 fl (P = 0.001), respectively (Kodiatte *et al*., 2012).

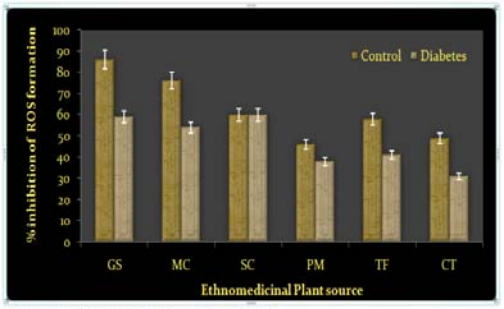

Fig. 1 Level of ROS inhibition by different herbal extracts

Table- 3b: ANOVA Summary for the selected diabetic and non-diabetic subjects

Source	SS	df	MS	F	P
Treatment (between groups)	705.33	1	705.33	3.62	0.0862
Error	1950.33	10	195.03		
Total	2655.66	11			

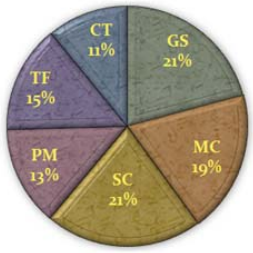

Fig. 2 % PMA induced ROS by different herbal extracts

It is stressed that the metabolic abnormalities associated with uncontrolled diabetes go well beyond just hyperglycemia, and that these additional abnormalities undoubtedly contribute to the enhanced micro and macro-vascular complications associated with the diabetic state. Knowledge of the mechanisms of ROS damage is the first step for development of new therapeutic molecules and for rationalizing the use of existing drugs (Penckofer et al., 2002). Future studies should focus on validating the mechanism of action responsible for the various beneficial effects and also on understanding which compound/s are responsible for the marked effects on management of diabetic complications.

References

Ashby, B., Daniel, J.L. and Smith, J.B. 1990. Mechanisms of platelet activation and inhibition. *Hematol. Oncol. Clin. North Am*., 4:1-26.

Babu, R.K., Dilip, R.M., Vinay Kumar, K., Shaik Sameena, F., Tiruvenkata Kumar, E.G., Swapna, S., Ramesh, B. and Rao, C.A. 2010. Antihyperglycemic and antihyperlipidemic activities of methanol : water (4:1) fraction isolated from aqueous extract of Syzygium alternifolium seeds in streptozotocin induced diabetic rats. *Food Chem Toxicol.*, 48: 1078-1084.

Barry, H. 1991. Antioxidant affects a basis for drug selection. *Drugs*, 42: 569.

National Diabetes Fact Sheet, 2011. National estimates and general information on diabetes and prediabetes in the United States. Centers for Disease Control and Prevention.

Demirtunc, R., Duman, D., Basar, M., Bilgi, M., Teomete, M., Garip, T. 2009. The relationship between glycemic control and platelet activity in type 2 diabetes *mellitus*. *J.Diabetes Complications*, 23: 89-94.

Falcon, C., Ptliegler, G., Deckmyn, H. and Vermylen, J. 1988. The platelet characterization and search for a function. *Biochem. Biophys. Res. Commun.,* 157: 1190-1196

Kodiatte, T.A., Manikyam, U.K., Rao, S.B., Jagadish, T.M., Madhavi Reddy, Lingaiah, H.K.M. and Lakshmaiah, V. 2012. Mean Platelet Volume in Type 2 Diabetes Mellitus. *J. Lab. Physicians*, 4(1): 5-9

Kyrou, I., Chrousos, G.P. and Tsigos, C. 2006. Stress, visceral obesity and metabolic complications. *Ann NY Acad Sci.*, 1083: 77-110

Penckofer, S., Schwertz, D. and Florczak, K. 2002. Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and pro-oxidants. *J. Cardiovasc. Nurs.*, 16: 68-85

Trovati, M., Anfossi, G., Carabot, F., Massucco, P., Mularoni, E. and Emankelli, G. 1988. Insulin directly reduces platelet sensitivity to aggregating agents: studies *in vitro* and *in vivo*. *Diabetes*, 37: 780-786.

WHO. 2003. Screening for Type 2 Diabetes. Report of a World Health Organization and International Diabetes Federation meeting, Geneva. pp 1-54.