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EVALUATION OF ANTIULCER ACTIVITY ON VIGNA MUNGO AND MUNG BY USING ALBINO RATS

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Abstract:

A thrombus formation in the circulatory system due to failure of haemostasis causes vascular blockage and leads to serious consequences such as myocardial or cerebral infarction. Antipltelet drugs are used to prevent formation of unwanted blood clot but their uses are associated with serious side effects. In association of efforts to develop natural products from plant origin as antiplatelet agent, Vignamungoseeds were investigated for same. The well known antiplatelet drug aspirin was taken as standard drug and activity was compared with it. Adenosine phosphate induced platelet aggregation method was used. 0.4 ml of 0.9mg/dl aspirin solution was used as standard dose. The antiplatelet activity of vignamungo was relatively high ($p<0.05$; ANOVA) compared to aspirin. In conclusion, these observations suggest that both 100 and 200 mg/kg body weight exert different levels of inhibitory action on platelets in vitro (secretion and platelet aggregation suppression) due to differences in concentration.

Keywords: *Vignamungo, Aspirin, Anti-platelet activity.*

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INTRODUCTION:

Atherothrombotic coronary artery diseases are one of the most common causes of death worldwide.Atherothrombotic diseases such as myocardial or cerebral infarction are serious outcome of the unwanted thrombusformed in blood vessels.Platelet plays a key role in the physiological hemostatic process and pathologic thrombosis [1,2]. 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, collegen, thrombin and ADP.6 Adenosine 6- diphosphate (ADP) is an important agonist for platelet aggregation. ADP induces platelet change of shape, granule secretion, and mobilization of intracellular calcium and inhibition of stimulatedadenylylcyclase. Antiplatelet or anti thrombotic drugs are those, that interferes with platelet function and may be useful as a prophylactics in thromboembolic disorders [3-5]. 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. 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. 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. Antiplatelet drugs are used to prevent unwanted thrombus formation [6,7]. However, these drugshave certain limitations which cause serious and sometimes fatal consequences.A blood clot (thrombus) developed in the circulatory system due to failure of haemostasis causes vascular blockageand leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction.Antiplatelet drugs are used to prevent formation of unwanted blood clot and thrombolytic agents that include tissueplasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc are used for the treatment of these diseasesbut their uses are associated with hyper risk of GI irritation , gastric erosions with bleeding, neutropenia,hemorrhage, severe anaphylactic reaction and lacks specificity. Many antithrombotic drugs may have a deleteriouseffect on normal haemostasis leading to bleeding complications. Therefore it is necessary to find out a new drugwhich shows less adverse effect. Considerable efforts have been directed towards the discovery and development of natural products from variousplant and animal sources which have antiplatelet, anticoagulant, antithrombotic, and thrombolytic activity.Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could reduce risk of thrombosis. Herbs showing antithrombotic and thrombolytic activity have been studied and somesignificant observations have been reported [8-10]. The *in vitro* effects of *A. cepa*extract have beenexamined by monitoring the metabolism of arachidonicacid (AA) from the platelets. *A. cepa*extract seems toreduce the formation of thromboxane A2 (TxA2) and ofthe products resulting via lipoxygenase pathway, fromexogenous AA; it does not inhibit AA incorporation intoplatelet phospholipids. Thus, it has been shown thatthe extract inhibits the formation of AA metabolites,acting one step after its release. At extract concentrationsthat suppress the formation of AA (inducer of plateletaggregation), only a partial inhibition of aggregation inthe presence of ADP and epinephrine has been observed [11-13]. These results suggest that the inhibition of plateletaggregation in the presence of the *V.mungo*extract ismediated by its effects on the synthesis of TxA2. It isalso known that platelet response induced by the *V.mungo*extract is time and temperature dependent, with themention that the maximum effect is observed when theextract is preserved over 30 minutes at a temperatureof 25°C. Although the antioxidant characterof *V.mungo*extracts is well known, there are no studiesthat link the antioxidant and antiplatelet activities withparameters describing the quality of the extract.Black gram is a type of bean grown in the Indian subcontinent, which has a surprising number of health benefits, including its ability to boost energy, protect cardiovascular health, reduce pain and inflammation, improve immunity, aid in maintaining skin health, prevent diabetes, build strong bones, strengthen the nervous system, and optimize digestion [14].

MATERIALS AND METHODS:

EXTRACTION PROCESS:

The dried plant material was mixed and macerated with absolute ethanol at a 2:5 ratio (100 g in 1 L solvent) for 7 days. Then the extract was filtrated through What man No 1 filter paper and then followed by rotor- evaporated the supernatant by using the Rotary Evaporator to remove the ethanol and to obtain concentrated.

Preliminary Phytochemical Screening

The aqueous extract of VMEE was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds (Kokate, 1986).

i. Test for Alkaloids

The extract was treated with diluted HCl and filtered. The filtrate was treated with various alkaloidal agents.

Mayer's Test: Sample was treated with Mayer's reagent, appearance of cream colour indicates presence of alkaloids.

**Dragendroff`s Test**: Sample was treated with Dragendroff`s reagent, appearance of reddish brown precipitate indicates presence of alkaloids.

Hager's Test: Sample was treated with Hager's reagent, appearance of yellow colour indicates presence of alkaloids.

Wager's Test: Sample was treated with wager's reagent, appearance of brown precipitate indicates presence of alkaloids.

ii.Test for Phenols

The extract was treated with neutral ferric chloride solution, appearance of violet colour indicates presence of phenols. The extract was treated with 10%sodium chloride solution, appearance of cream colour indicates presence of phenols.

iii. Test for Flavanoids:

5ml of the extract solution was hydrolyzed with 10%sulphuric acid and cooled. It was then extracted with diethyl ether and divided in to 3 portions in three separate test tubes .1ml of diluted sodium carbonate, 1ml of 0.1 n sodium hydroxide and 1 ml of diluted ammonia solutions was added to the first second and third test tube respectively. Development of yellow color in each test tube indicates presence of flavanoids.

Shindoas test: The extract was dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of Conc. HCL and heated. Appearance of magenta color indicates presence of flavanoids.

iv. Test for Saponins

Foam test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes presence of saponins.

v. Test for Terpenes : The extract was treated with tin and thionyl chloride, appearance of pink colour indicates presence of terpenes.

ANIMALS

Albino rats weighing around 200g – 300g of either sex were obtained from Nirmalacollege of pharmacy, mangalagiri. Animals had free access to food and water and maintained under standard laboratory conditions with a natural light and dark cycle. The animals were acclimatized for at least five days before behavioural experiments. Experiments were carried out between 9.00 and 15.00 hrs. Experimental protocol was approved by the institutional animals' ethics committee before the start of the study.

Chemicals

Aspirin (Sigma Aldrich), distilled water, Normal saline and other chemicals were of analytical grade.

Acute Toxicity Studies

Aqueous extract of V.mungo (was studied for acute oral toxicity as per revised OECD (2002) guidelines No. 423. Animals were observed for four hours hourly for behavior changes and daily for fourteen days. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 100 and 200 mg/kg doses of extract were used.

Antiplatelet Activity

Platelet aggregation was monitored at 37 °C by the turbidimetric method of Born and Cross using a Chrono-Log aggregometer model 490 (Harvertown, PA, USA). Blood was collected from anesthetized rats by aorta puncture using syringes containing 3.8% trisodium citrate (9:1 v/v). Plateletrich plasma (PRP) was prepared by centrifugation at 300 ×g for 10 min at room temperature. The platelet-poor plasma (PPP) was prepared by centrifugation of the pellet at 1600 ×g for 10 min at room temperature. In a cuvette, PRP (400 µL) was pre-incubated with compounds (150 µM), acetylsalicylic acid (150 µM; positive control) or vehicle DMSO (0.1% v/v) at 37 °C for 3 min with continuous stirring at 1000 rpm. Platelet aggregation was induced by ADP (10 µM) and arachidonic acid (AA) and the aggregation curve was determinated for 5 min after agonist addition. The results were expressed as mean ± SD of three independent experiments. The inhibition percentage and were calculated as follow: The inhibition ratio % = [(platelet aggregation of control group − platelet aggregation of treated groups)/platelelet aggregation of control group 100%] × 100. The Statistical analysis was performed with ANOVA followed by Tukey's test.

Platelet Aggregation Studies

The Chronolog platelet aggregometer was used to study platelet aggregation. Turbidimetricaggregometry is based on the concept

of passing light through a stirred turbid suspension of platelets. The presence of platelets in suspension causes the light to be scattered in such a manner that a reduced proportion of light passes through the platelet suspension unobstructed. The amount of transmitted light is recorded and gives a measure of optical density of the platelet suspension. On addition of an aggregatory agent, platelets form clumps, as a result of which the amount of light that is scattered is reduced since it passes unobstructed through the suspension. Thus, as platelets aggregate, the optical density of the suspension is reduced. The unstimulated suspension of Platelet Rich Plasma (PRP) (Photoplate 1C) has a relatively high optical density and represents 0% aggregation. Autologous Platelet Poor Plasma (PPP) provides the measured optical density equivalent to 100% aggregation. Platelet aggregation was performed using the turbidimetric method (Born, 1962). The sample size was chosen to be 12. Platelet rich plasma was obtained from the collected blood as a supernatant by centrifugation (1000rpm for 10 min at 250 C) and the remaining blood was centrifuged (4000 rpm for 15 min at 250 C) to obtain platelet poor plasma (PPP). Platelets in the PRP were counted using a platelet counter and the count was adjusted to 2x105 /µl using autologous PPP for dilution (working plasma). The working plasma (440µl) was incubated with 10µl of distilled water (vehicle), positive control (Aspirin-5µM) and the test drugs of different concentrations for 3 mins at 37oC. Adenosine Diphosphate was then added and platelet aggregation was studied using platelet aggregometer. The percentage inhibition shown by Aspirin and the different concentrations of tea extract was then calculated by the formula:

% Inhibition = 1 - MPA of test drug or aspirin /MPA of Vehicle X 100

STATISTICAL ANALYSIS:

All the results are expressed as Mean ± SEM. All the groups were analysed using student's 't' test.

RESULTS AND DISCUSSION:

The observation of acute toxicity study indicated that there was no death in 400 mg/kg dose after 72hr.In the present experiment, the impactof four different concentrations (i.e. 50%,25%, 12.5% and 6.25%) of *Vignamungo*seeds ethanolic extract has been assessed on inhibition ofplatelet aggregation in ratsblood. Theresults obtained are discussed below.

Table 1: Percentage aggregation & inhibition results

Study groups	% aggregation	% inhibition
Normal	53.83 ± 29.23	
Reference drug (Aspirin)	27.75 ± 17.90	50.14 ± 12.52
50%	38.58 ± 28.09	35.69 ± 20.34
25%	35.83 ± 27.16	42.02 ± 20.97
12.5%	32.33 ± 26.19	48.71 ± 22.36
6.25%	28.58 ± 24.55	56.10 ± 22.37

Results expressed as Mean ± SD

Platelets play a pivotal role in Homeostasis. Natural products including polyphenols and flavonoids have beenreported 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 andobesity related disorders. Many cardiovascular diseases can be attributed to excessive platelet aggregation, whichhas a critical role in thrombus formation. A dose dependent inhibition of platelet aggregation was observed, in comparison to the standard aspirin. Platelet dysfunction contributes to the development and progression of many cardiovascular diseases such as arterial hypertension, atherosclerosis and thrombosis. It has been reported that patients with hypertension or coronary heart disease tend to have increased platelet reactivity. Therefore, many investigations were carried out towards the prevention of the abnormal hyperactivity of platelets reported in cardiovascular disorders employing different therapies, including use of medicinal plants (Durairaj and Dorai, 2010). The objective of the present study was to determine whether different concentrations of the black gram extracts would affect platelet aggregation. The results obtained indicated that all the concentrations of tea

leaves extracts showed effective antiplatelet activity in a reverse dose-dependent manner with maximum activity at 6.25% concentration (Table-1). At lower concentrations, *Vignamungo*extract was inhibitory. 50% concentration showed around 36% inhibition in platelet aggregation, followed by 25% concentration giving 42% inhibition, 12.5% concentration reveals around 49% inhibition and 6.25% concentration exhibits the highest i.e., 56% inhibition in platelet aggregation (Photoplate 1D and 1E). Furthermore, the antiplatelet activity of 6.25% extract was relatively high (6% higher) compared to the standard drug (aspirin). The polyphenols and flavonoids present in the aqueous extract might have prevented the adhesion and aggregation of platelets besides release of cytoplasmic calcium that stimulates the release of ADP. These results are in accordance with those of other studies demonstrating that flavonoid compounds isolated from many plants inhibit platelet aggregation (Durairaj and Dorai, 2010). Antiaggregant activity has previously been demonstrated for some known medicinal plants. For example, platelet antiaggregant properties *in vivo* and *invitro* for the whole fruit of *PaullinіaVignamungo* In an *in vitro* and *ex vivo* study, it was observed that *Camellia sinensis*catechins inhibits *in vitro* platelet aggregation induced by COL, AA and U46619 (9, 11-dideoxy-9α, 11α-methanoepoxy-prostaglandin F2α) and *ex vivo* induced by AA (Son *et al*., 2004).

CONCLUSION:

Vignamungo 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*Vignamungo*which may be beneficial in various cardiovascular ailment, but it should be used cautiously in patients withbleeding 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 *Vignamungo*the herbal extract has antiplatelet activity.

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