REPORT

Platelet aggregation inhibition activity of selected legumes of Pakistan

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Abstract: The current study was designed to explore the platelet aggregation activity of methanolic extracts of green gram, lentil, mash bean and soya bean. The extracts dose-dependently inhibited platelet aggregation initiated by arachidonic acid (AA) and platelet activating factor (PAF). Extract of green gram was effective only against AA mediated activity while mash bean and soya bean extracts were effective against both AA and PAF mediated activity. But lentil extract has no activity. The order of activity based on IC_{50} value is, Mash bean> Soya bean> Green gram. This preliminary result suggests that legume seed extract may be taken as a candidate lead natural compound to be considered in the search for natural products with beneficial effects on aberrant platelet activation mediated cardiovascular disorders.

Keywords: Platelet aggregation inhibition, legumes, Pakistan.

INTRODUCTION

It is well-established that blood platelet activation has a pivotal part in haemostasis and in pathological development of several many severe ailments (Kroll and Schafer, 1989; Duttaroy *et al.*, 1991; 2002). Platelets are also involved in tumor growth. Due to these important roles of platelets, researchers are in search of new medicines having platelet aggregation inhibition capacity. Legumes are an important food commodity and staple diet of Pakistani population of all age and income groups. These are cheap source of important constituents necessary for various biochemicophysiological functions of human body. Besides having greater protein contents, legumes have many other characteristics like plenty of complex carbohydrates and fiber content, ability to decrease serum cholesterol, low oil quantity (with some exceptions), rich in polyunsaturated fatty acids and variety of food items that may be attained from this family. Despite their nutritional and compositional studies (Zia-Ul-Haq *et al.*, 2007a,b; 2008a,b; 2009; 2010; 2011), very little work exists on their pharmacological studies. So current investigation has been carried out to evaluate platelet aggregation inhibition potential of these legumes.

MATERIALS AND METHODS

Mash bean, soybean, green gram and lentil seeds were obtained from local market, identified by Prof. Dr. Shakeel Ahmd, Department of Agronomy, BZU-Multan, and voucher specimen numbers 078-081 were deposited in museum of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. The said seeds were macerated in methanol for two weeks and the filtrate obtained was concentrated by using rotary evaporator. Resulting extract was used current research. Healthy human individuals, who had not taken any drug two weeks prior to participate in the study, were used for blood collection. Plasma rich in platelets was obtained by mixing blood with sodium citrate solution (3.8 w/w) in 9:1 proportion and centrifugation for fifteen minutes at 260 g at 20^{o}C. Plasma having fewer amounts of platelets was obtained from The residual blood by centrifugating for ten minutes at 1200 g. Phase contrast microscopy was carried out to determine Platelet count. Aggregation was carried out in platelet rich plasma at human body temperature. Aggregation was monitored by Dual-channel Lumi aggregometer- 400. Platelet activating factor and arachidonic acid (0.8 µM and 1.8 mM) were used to initiate aggregation. Anti-coagulation effects of crude extracts of green gram, mash bean, lentil and soybean were studied by addition of platelet activating factor and arachidonate (aggregation agents). For five minutes, aggregation produced was recorded as previously mentioned (Hussain *et al.*, 2009).

RESULTS

Green gram extract inhibited AA induced aggregation (fig. 1**)** but no inhibition was recorded in PAF induced aggregation. The inhibition is dose dependent *i.e.* was

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greater at 75µg/ml and less at 10 µg/ml. Median inhibitory concentration (IC_{50}) was determined from dose response regression line for green gram extract (IC_{50} value= AA=57 µg/ml). Lentil extract did not show any activity against AA and PAF. Mash bean extract produced significant results against AA and PAF induced aggregation, the inhibitory effect observed was decreasing with the decrease in dose 25, 10, 5 µg/ml for AA induced aggregation and 75, 25, 10 µg/ml for PAF induced aggregation. Figs 2-3 the median inhibitory concentration was determined from the dose response regression curve of mash bean (IC_{50} value= AA=14 µg/ml, PAF=40 µg/ml). Soya bean extract was tested following the protocol as for the above legumes and the results were obtained which also showed dose dependent inhibitory activity. Figs. 4 & 5 The IC_{50} value were determined, IC_{50} value= AA=24 µg/ml, PAF=61 µg/ml. comparatively mash bean extract had the most potent activity because it had the least IC_{50} value. The potency order based on IC_{50} value is, Mash bean> Soya bean> Green gram

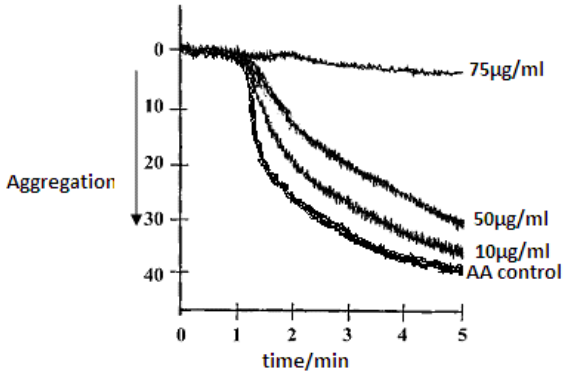

Fig.1 green gram

Fig. 1: Tracings of inhibition of the platelet aggregation by green gram against AA. Control is aggregation curve obtained by adding AA (1.8mM) and was taken as 100% aggregation. (IC_{50} value= AA=57 µg/ml)

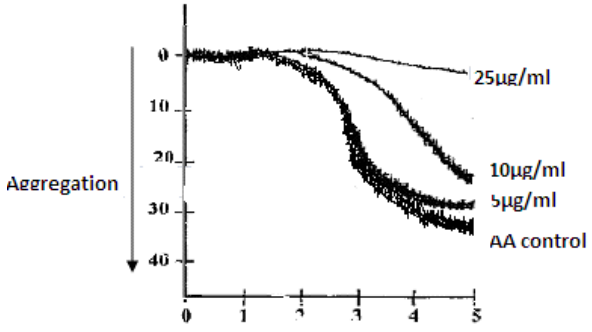

Fig.2 Mash bean

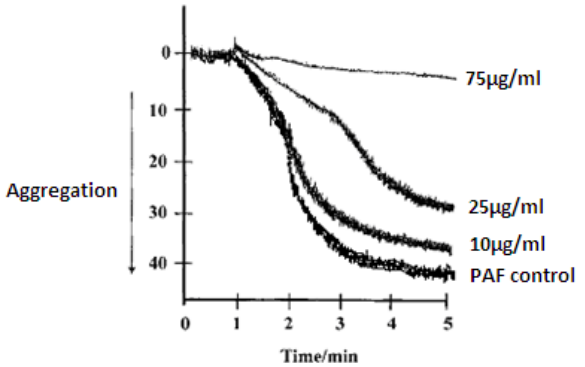

Fig. 3 Mash bean

Figs. 2 & 3: Tracings of inhibition of the platelet aggregation by black gram, against PAF and AA. Control is aggregation curve obtained by adding AA (1.8mM) or PAF (0.8µM) and was taken as 100% aggregation. (IC_{50} value= AA=14 µg/ml, PAF=40 µg/ml)

DISCUSSION

The anti-occlusion property of many plant extracts and dietetic alimental constituents are reported because of their relative effectiveness, limited side effects, and low cost. Consumption of food legumes reduces mortality from ischemic heart disease (IHD) (Rajaram 2003). During injury or trauma platelet clumps together to form clot to stop bleeding. Various agents, both natural and synthetic, are discovered targeting different steps to inhibit platelet aggregation to manage cardiovascular conditions such as arterial thrombosis (Geoffrey 2007). *In*

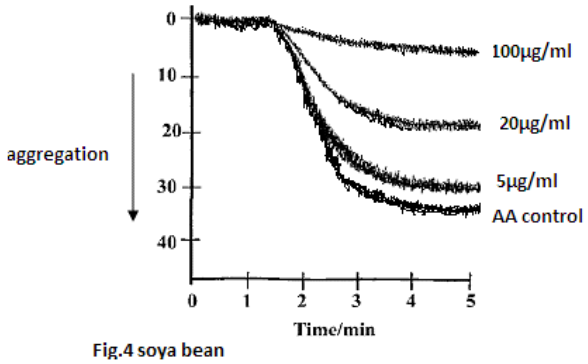

Fig.4 soya bean

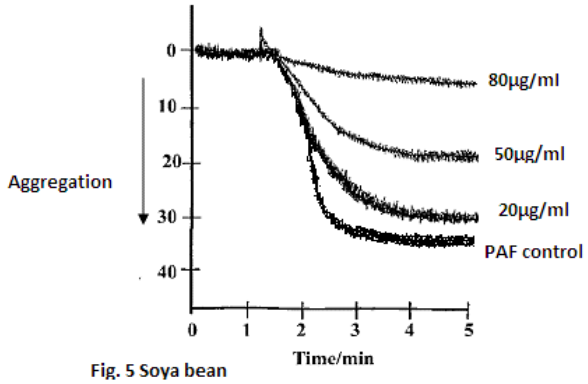

Fig. 5 Soya bean

Figs. 4 & 5: Tracings of inhibition of the platelet aggregation by soybean against PAF and AA. Control is aggregation curve obtained by adding AA (1.8mM) or PAF (0.8µM) and was taken as 100% aggregation. (IC_{50} value= AA=24 µg/ml, PAF=61 µg/ml).

vitro platelet aggregation can be induced by platelet-activating factors, like ADP and PAF. ADP can mainly bind to G protein-coupled P2Y1 receptor and activates phospholipase C, and thus resulting in the elevation of intracellular calcium concentration [$Ca2^{+}$]i. Our results indicate that some of investigated legumes have excellent platelet aggregation inhibition activities. Detailed *in vivo* studies on platelet aggregation are required before any conclusion on effects of legume diet and lower risk of cardiovascular diseases. The reason is that *in vitro* environment as in present study is different from in vivo environment and this may be achieved by using human volunteers. Further studies should be carried out to characterize compounds responsible for such an excellent activity and to ascertain the mechanism of action of extracts.

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