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Full Length Research Paper

Antiplatelet activity of methanolic extract of *Acacia leucophloea* bark

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The anti-aggregatory property of platelets by many plant extracts and natural products are being explored as cardioprotective drugs because of their relative effectiveness, limited side effects, and low cost. Current study has been designed to evaluate platelet aggregation inhibition potential of bark of *Acacia leucophloea*, a traditional medicinal plant used by indigenous communities of Pakistan for this purpose. *A. leucophloea* bark crude methanolic extract (Al. Cr) exhibited potent platelet aggregation inhibitory activity and dose-dependent inhibition was observed against adenosine 5' diphosphate (ADP)-induced human platelet aggregation with concentration range (0.3 to 3.00 mg/ml). This preliminary screening suggests that Al. Cr may be taken as a candidate for isolation of natural compounds with beneficial effects on aberrant platelet activation mediated cardiovascular disorders.

Key words: Platelet aggregation inhibition, *Acacia leucophloea*, Pakistan.

INTRODUCTION

Blood is a vital tissue of body that transports oxygen and nutrients to every cell of the body and eliminates waste products from tissues. Platelets are cell fragments that prevent excessive bleeding by forming a clot (Campbell, 2008). Blood clot is a bulk of blood cells and blood constituents which is produced to stop bleeding resulting from blood vessel injuries. During this process, platelets in the blood become sticky and clump together at the site of the injury. Clotting is the body's normal response to prevent a person from bleeding to death (Elliot and Elliot, 2005). However, blood clot formation can be dangerous if it occur within healthy blood vessels, or if not degraded after due time. Many diseases like heart attack, stroke and pulmonary embolism are associated with inappropriate blood clot formation (Anthony, 2011; Gwala, 2011). Although, some synthetic blood thinners such as aspirin and heparin are available in market, these have side effects like cancer (Sanchez-Lamar et al., 1999). So now scientists are in search for natural anti-coagulants from plant sources that are safe, cost effective and available from indignous resources.

Like other developing countries, medicinal plant remedies prepared from indigenous flora are the only drugs available for a large number of people in Pakistan. There are at least 45,000 traditional healers in Pakistan of whom about three-quarters are practicing in rural areas. This figure has not changed significantly over the years. However, most of these traditional remedies have not been investigated scientifically. In this context, as part of our continuous studies on exploring the hidden potential of the indigenous flora of Pakistan (Zia-Ul-Haq et al., 2007a, b; 2008a, b; 2009; 2010; 2011a, b, c, d), we have evaluated platelet aggregation inhibition property of *Acacia leucophloea* bark methanolic extract (Al. Cr).

MATERIALS AND METHODS

Plant material and preparation of crude extract

Al. Cr was collected from Khanewal, a district of Punjab, Pakistan in June, 2008 and authenticated by Miss Saima Shehzadi at the institute of Pure and Applied Biology, Bahauddin Zakariya

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University, Multan. A voucher specimen of the plant (No.18-04-2008) was kept in the Herbarium of Bahauddin Zakariya University, Multan. Al. Cr were cleaned and grounded. The powdered material (1 kg) was soaked in 80% methanol for 8 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. Filtrate was evaporated on under reduced pressure to a thick, semi-solid mass of dark red color (60 g). This Al. Cr was used in current experiment.

Platelets aggregation inhibition assay

The anti-platelet aggregation activity of crude plant extract material on human platelets was assessed by procedure as described previously (Shah et al., 1999).

Preparation of human platelets

Venous blood was taken from healthy human subjects of either sex, aged (25 ± 4) years and a body weight of 60 ± 7 kg, reported to be free of medication for 1 week. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at 1000 rpm for 15 min at 20°C to obtain platelet rich plasma (PRP) and at 4000 for 10 min to obtain platelet poor plasma (PPP). Aggregation studies were carried out at 37°C with PRP having platelet counts between 2.5 and 3.0 $\times$ 10^9/L of plasma. All experiments were performed within 3 h of PRP preparation.

Measurement of platelet aggregation

Aggregation was monitored in 0.45 ml aliquots of PRP using a Dual-channel Lumi-aggregometer (Model 400 Chronolog Corporation, Chicago, USA) based on turbidmetric principle (Born, 1962). The final volume was made up to 0.5 ml with crude extract in defined proportion dissolved either in normal saline or appropriate vehicle known to be devoid of any effect on aggregation. Aggregation was induced with adenosine 5' diphosphate (ADP) (5 µM). The anti-aggregatory effect of Al. Cr was studied by pretreating PRP with crude extract for 1 min, followed by addition of 5 µM of ADP. The resulting aggregation was recorded for 5 min after challenge with the aggregating agent by change in light transmission as a function of time (Shah et al., 1999; Hussain et al., 2009). Once the antiplatelet activity of anti-aggregatory plant extract was established, dose-response curves were constructed to calculate 50% inhibitory concentration of the inhibitors.

Statistical analysis

All the data expressed are the mean of three experiments ± standard error of the mean (SEM, n = number of experiments) and the median effective concentrations (EC_{50}) with 95% confidence intervals. P-value <0.05 was considered statistically significant. Concentration-response curves were analyzed by non-linear regression (Graph PAD prism 5.04).

RESULTS AND DISCUSSION

Cardiovascular system diseases are major causes of death globally (Ulrichts et al., 2004). It is now believed that many pathologic conditions of the cardiovascular system are affected by an increase or dysfunction of the blood platelet activity, mainly in arterial thrombi (Golino et al., 2005), since these play a major role in thrombotic disorders (Andrioli et al., 1996, Hernandez et al., 1997). It is therefore important to prevent platelet dysfunctions that could lead to cardiovascular events. Despite the progress made in finding better and effective anti-platelet aggregation agents, cardiovascular diseases are still responsible for a large number of deaths and morbidities. Several medicinal ,aromatic and food plants, trees and crops have the reputation being used to prevent or at least to decrease the incidence of different vascular diseases based on their ability to prevent platelet aggregation. So, current study was designed to authenticate the traditional use of bark of Al. Cr for platelet aggregation inhibition. *A. leucophloea* was tested for inhibitory effects on human platelets aggregation induced by ADP. The extract caused the concentration dependent inhibition of ADP-induced aggregation at concentration range (0.3 to 3.00 mg/ml) with half maximal inhibitory concentration (IC_{50}) values of 0.8340 mg/ml (0.7681 to 0.8999, 95% CI, n = 3) (Figures 1 to 3*).* The ADP-induced platelet activation is autocatalytic as upon activation by ADP, platelets release other ADP molecules that act on near platelets, amplifying the reaction. ADP acts through G-protein coupled receptors P2Y1 and P2Y12 as both receptors work closely together to ensure a complete activation and aggregation of platelets. The platelet activation and aggregation is initiated through P2Y1, amplified and sustained through P2Y12. The activity of ADP-induced platelet activation requires the availability of Ca^{2+} and it is inhibited by cAMP. Therefore, increased intracellular Ca^{2+} and a decrease in cAMP level are crucial for ADP-induced platelet activation and aggregation (Anthony, 2011; Gwala, 2011).

The anticoagulant or anti-platelet aggregation activity of phenolic compound and flavonoids are reported previously. The phytochemical composition of Al. Cr indicated presence of tannins, flavonoids and phenolic compounds. Thus, the anti-platelet aggregation activity of Al. Cr could partly be attributed to their relatively high tannin, phenolic and/or flavonoid content. These bio-active compounds present in extract might have prevented the adhesion and aggregation of platelets, besides the release of cytoplasmic calcium that stimulates the release of ADP and 5 HT. *A. leucophloea* extract may have beneficial effects in primary prevention of cardiovascular disease by reducing platelet activation, which may contribute to a reduction in thrombotic events. Detailed *in vivo* studies on platelet aggregation are required before any conclusion on effects of Al. Cr 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. The results suggested that Al. Cr, can

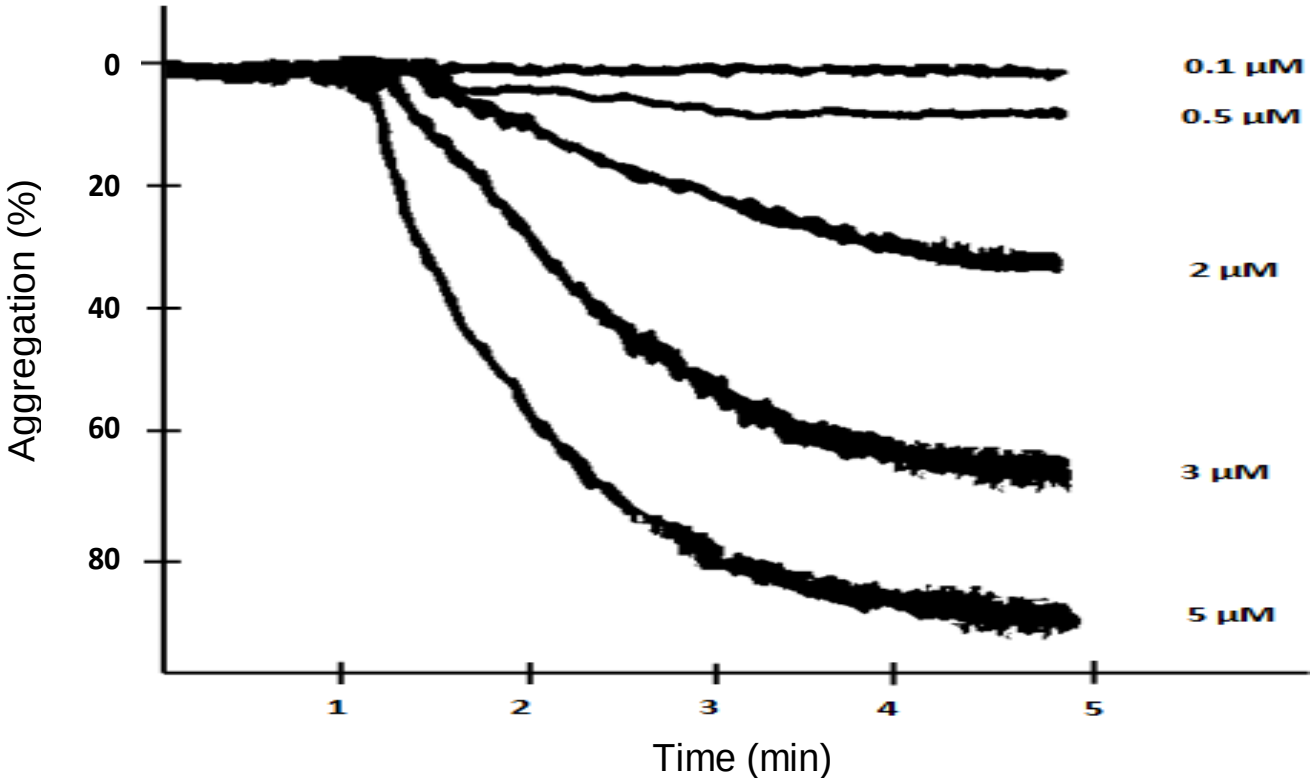

Figure 1. Effect of ADP at different concentration on human platelet aggregation.

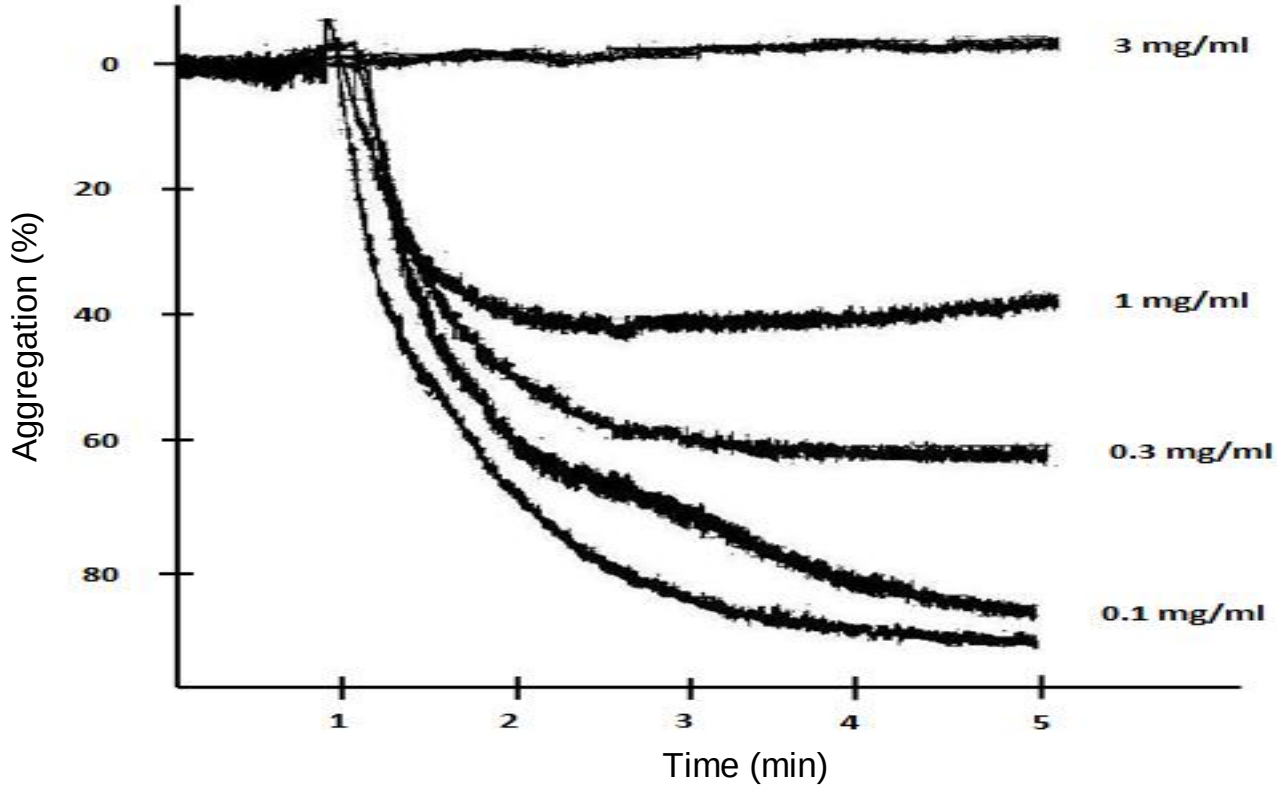

Figure 2. Effect of crude extract of *A. leucophloea* against human platelet aggregation induced by ADP.

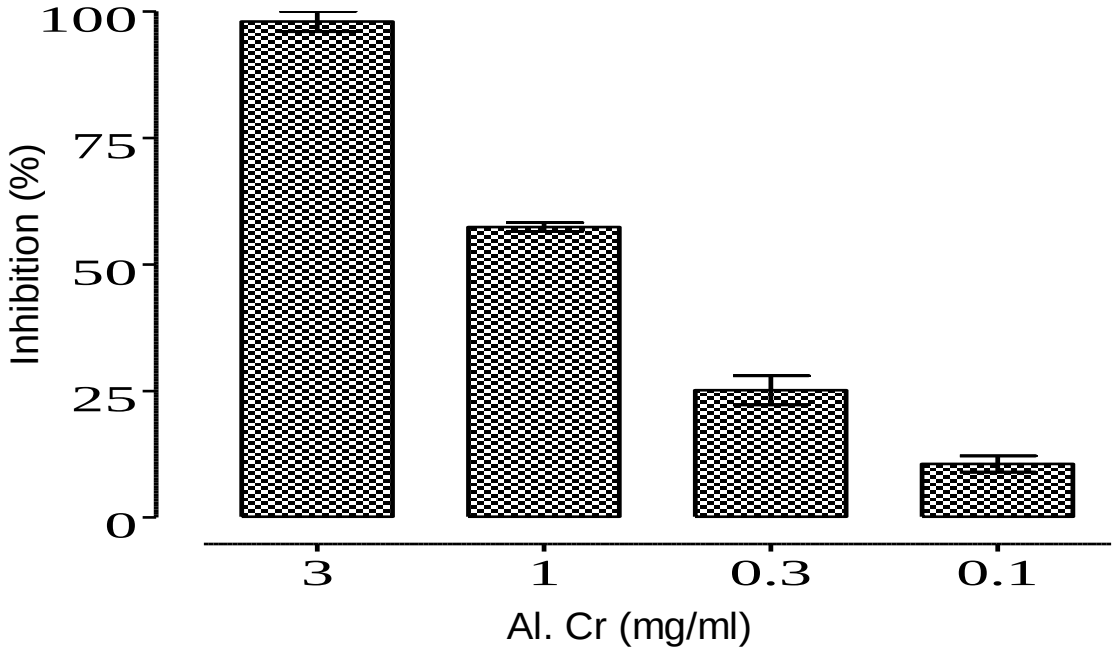

Figure 3. Dose dependent inhibitory effect of Al. Cr on ADP induced aggregation of human platelets.

be considered as herbal treatment for disease associated with blood clotting.

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