

*Full Length Research Paper*

## **Anti-platelet activity of methanolic extract of *Grewia asiatica* L. leaves and *Terminalia chebula* Retz. fruits**

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**There is resurgent interest in exploring the anti-platelet activity of medicinal plant extracts because these are cost effective, easily available form of indigenous resources and are comparable to synthetic blood thinners in this prospective. Current investigation has been carried out to assess the anti-platelet activity of crude methanolic extracts of *Grewia asiatica* L. leaves and *Terminalia chebula* Retz. fruits. Both crude extracts exhibited potent platelet aggregation inhibition activity in a dose-dependent manner at concentration range (1 to 10 mg/ml). This preliminary screening suggests that *G. asiatica* L. leaves and *T. chebula* Retz fruits crude extract can be considered as herbal treatment for disease associated with blood clotting.**

**Key words:** Anti-platelet activity, *Grewia asiatica* L., *Terminalia chebula* Retz.

### **INTRODUCTION**

For blood coagulation, interaction between platelets and blood vessels is necessary. Platelets play an important role to maintain cardiovascular (CV) integrity and to regulate bleeding by blood-clot formation. However, uncontrolled platelet aggregation is dangerous in arterial blockage and may lead to life threatening disorders (Davies and Thomas, 1985). Anti-platelet agents are therefore considered as a significant tool in the treatment and/or prevention of cardiovascular thrombotic diseases (DeMeyer et al., 2008). Despite well-known role of synthetic blood thinning agents to manage efficient secondary deterrence of cardiovascular complaints, these drugs may lead to hemorrhagic events and upper gastrointestinal bleeding as main drawbacks (Johnson, 2008). There is increasing interest in natural products isolated from plants to suppress platelet aggregation.

Pakistan harbours the richest array of cultivated and wild medicinal plants due to its distinct, diverse and majestic geographic and climatological conditions. Thus native approaches of herbal treatment are part of the culture and the prevailing method of curative therapy.

These remedies, with a substantial magnitude of efficacy, are socially recognized, cost effective and in some cases are the only accessible sources. However, most of these traditional remedies have not been validated scientifically. *Grewia asiatica* L. locally known as phalsa is a large bushy shrub considered horticulturally as a small fruit crop and having height of 4 m or even more. Its leaves possess antimicrobial potential and are therefore used to treat skin rashes and pustular besides their tagged antiemetic activity. Similarly, *Terminalia chebula* Retz., locally known as Hardad is a large tree with juvenile spreading branches and may have height up to 30 m (Zia-UI-Haq, 2011a). Its seeds are commonly used to cure various ailments for household remedies in Pakistan. As part of our

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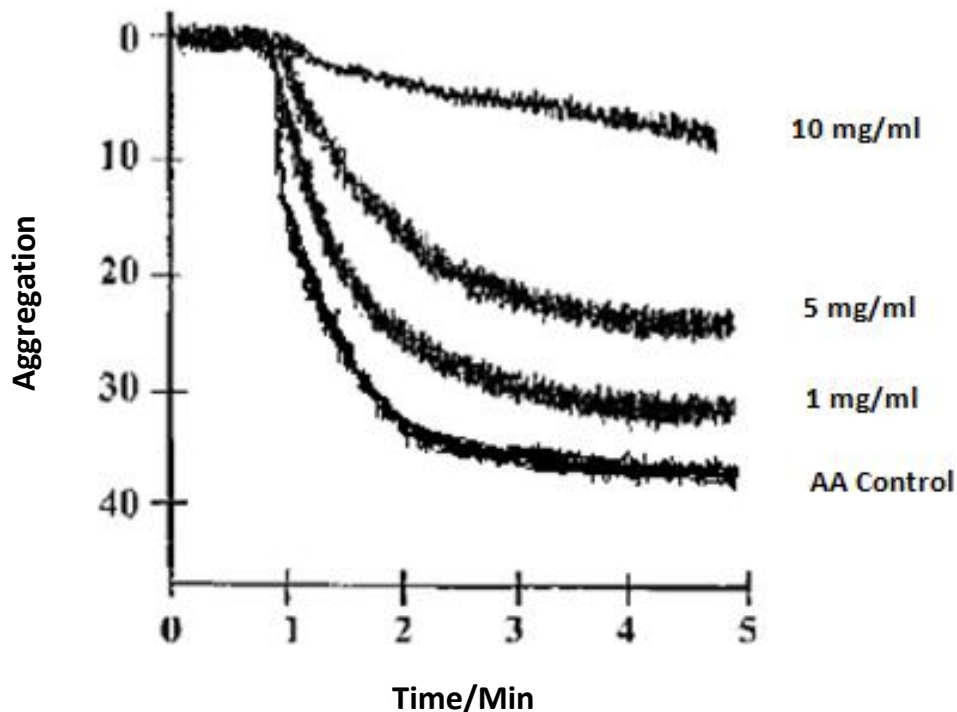


Figure 1. Effect of *G. asiatica* leaves extract on AA-induced platelet aggregation.

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), we have evaluated anti-platelet potential of methanolic extracts of *G. asiatica* L. leaves and *T. chebula* Retz., fruits.

## MATERIALS AND METHODS

### Plant material and preparation of crude extract

*G. asiatica* L. leaves and *T. chebula* Retz., fruits were obtained from Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan and authenticated by Dr. Shakeel Ahmad, Assistant Professor of same department. Plant material was cleaned and soaked in methanol for 15 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. Filtrate was evaporated under reduced pressure to a thick, semi-solid mass. This methanolic extract of each plant was used in the current experiment.

### Preparation of platelets

Blood was taken via venepuncture from normal volunteers who were reported to be free of medication for 7 days. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at 260 g for 15 min at 20°C to obtain platelet rich plasma (PRP). The remaining blood samples were centrifuged at 1200 g for 10 min to obtain platelet poor plasma (PPP). Platelet count was determined by phase contrast microscopy and all aggregation studies were carried out at 37°C with PRP having platelet counts between 2.5 and 3.0 × 10<sup>8</sup> ml<sup>-1</sup> of plasma (Shad

and Saeed, 2007).

### Measurement of platelet aggregation

Aggregation was measured by Dual-channel Lumi- aggregometer (Model 400 Chronolog Corporation, Chicago, USA) using 0.45 ml aliquots of PRP (Shah and Saeed, 1995; Shah et al., 1996). The final volume was made up to 0.5 ml with crude extract, dissolved either in normal saline or appropriate vehicle known to be devoid of any effect on aggregation. Aggregation was induced by using arachidonic acid (AA). The anti aggregatory effects of extract was studied by incubating PRP with crude extract for 1 min followed by the addition of aggregating agent (AA). The resulting aggregation was recorded for 5 min after the challenge, by the change in light transmission as a function of time (Shah et al., 1999; Hussain et al., 2009) Figures 1 and 2.

### 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<sub>50</sub>) with 95% confidence intervals. P-value <0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

Platelets activation do not play key role in homeostasis, moreover their hypersensitivity is reported to be related with development and progression of atherosclerosis.

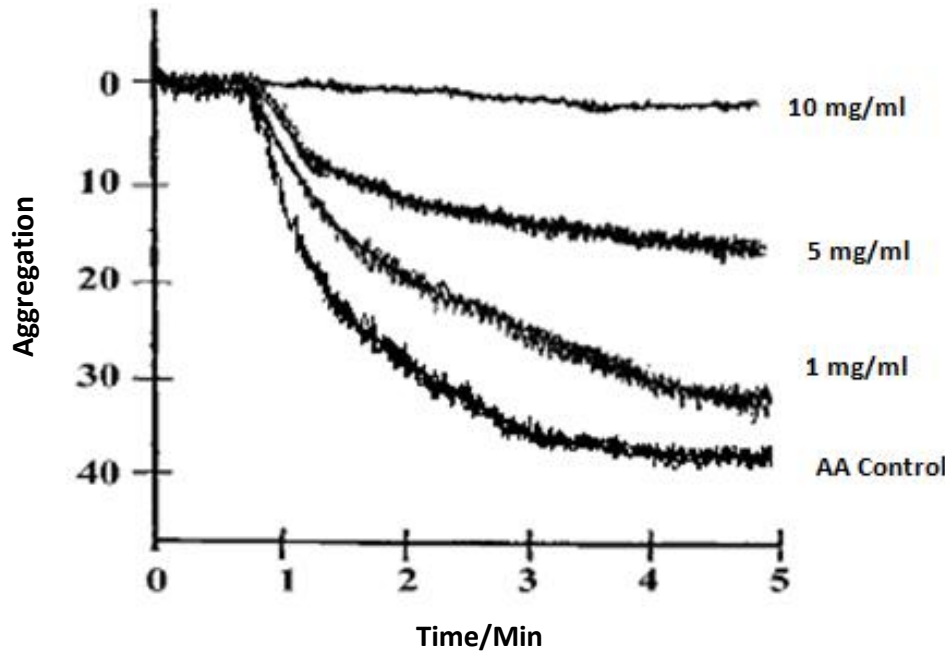


Figure 2. Effect of *T. chebula* fruit extract on AA-induced platelet aggregation.

Mechanistic studies indicate that anti-platelet agents significantly reduce the prevalence of primary and secondary coronary events related to cardiovascular disease (CVD). Administration of anti-platelet agents along with lipid-lowering measures and blood pressure monitoring, to all persons having age > 55 years can decrease the number of heart attacks and strokes by up to 80% (Law et al., 2003; Wald and Law, 2003). So, current study has been designed to find some natural anti-platelet agents. *G. asiatica* L. leaves extract showed dose dependent inhibition of AA-induced human platelet aggregation and more than 20% inhibition were observed at the initial dose of 1 mg/ml (Figure 1).

The effects at all three doses were significantly higher than the saline effect. More than 50% inhibition was observed at 5 mg/ml dose while maximum inhibition of 93% was observed with 10 mg/ml dose. ( $IC_{50}$ : 4.85 mg/ml). *T. chebula* Retz fruit extract showed dose dependent inhibition of AA-induced human platelet aggregation and only 5% inhibition was observed at the initial dose of 1 mg/ml (Figure 2). The effects at next two doses were significantly higher than the saline effect. More than 30% inhibition was observed at 5 mg/ml dose while maximum inhibition of 95% was observed with 10 mg/ml dose ( $IC_{50}$ : 6.74 mg/ml). It is reported previously that secreted Adenosine diphosphate (ADP) leads to AA-stimulated aggregation, however other studies believe this response as being ADP-independent. Release of AA is a vital controlling factor in platelet adhesion and aggregation and interference with the arachidonate cascade is extensively used in hyperactive platelets therapy (Hashizume et al., 1997; Nosal and Jancinova,

2001). Therefore, investigated extracts may be a good source for development of antithrombotic agents targeting the inhibition of the AA cascade. However *G. asiatica* leaves extract was more potent than *T. chebula* fruits extract. Due to country-wide awareness of the importance of medicinal plants, there is need to ink-down a national policy on traditional medicinal knowledge and a development plan to uplift the system of traditional medicine as it is more culturally acceptable. There is dire need to build up more and better organized interdisciplinary studies among farmers, scientists, scientific research institutes and herbal industries of Pakistan to add value to medicinal plants from cultivation to marketing by processing, chemical analysis and bioactivity-guided fractionation to rationalize their folk uses.

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