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INHIBITION OF ADRENALINE-INDUCED AGGREGATION OF HUMAN PLATELETS BY PAKISTANI MEDICINAL PLANTS

TASNEEM MARIAM SAIID, SHAHID RASHID and SHEIKH ARSHAD SAEED*

Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.
*Department of Pharmacology, Faculty of Health Sciences, Aga Khan University Medical College, Karachi, Pakistan

ABSTRACT

The effects of extracts of Pakistani plants claiming medicinal value were observed on adrenaline-induced aggregation of human platekts according to the method of Born (1962). The minds manifested significant inhibitory activity in doses of 0.625 $mgml^{-1}$ to 2.5 $mgml^{-1}$ in this *in vitro* model. The potency of the plant extracts in inhibiting platelet aggregation induced by adrenaline is in the following order:

Sida pakistanica, IC_{50} = 0.90 $mgml^{-1}$>*Tribulus terrestris*, IC_{50} = 0.97 $mgml^{-1}$ > *Solanum strattense*, IC_{50} =1.34 $mgml^{-1}$ > *Tephrosia* subtnflora, IC_{50} =1.40 $mgml^{-1}$ > *Glycyrrhiza glabra* (butanolic extract), IC_{50} =166 $mgml^{-1}$ > *Urtica dioica* IC_{50} =2.17 $mgml^{-1}$. Further studies are proposed to find out whether the inhibition of adrenaline-induced platelet aggregation is mediated through a-adrenaline blockade or suppressed synthesis of prostaglandin endoperoxides.

Introduction

Platelet aggregation is chiefly implicated in thrombogenesis and atherogenesis (Ross *et al*, 1984). Occlusive vascular disease with resultant heart attack and stroke remains the leading cause of death in industrialized countries. Of the many antithrombotic agents, only aspirin has been approved in USA as a prophylactic agent against myocardial infarction and stroke.

Adrenaline is an inducer of platelet aggregation but it does not evoke this response endogenously in normal blood concentration. It is, however, also used for testing antithrombotic agents. In *vitro* experiments it gives characteristic biphasic aggregation curve, the first phase denoting aggregation mediated by α-adrenoceptors (Hoffman *et* at, 1982). When adrenaline occupies these receptors present in the platelet membrane, adenylate cyclase activity is inhibited, CAMP production consequently decreased and platelets tend to aggregate (Hoffman and Lefkowitz, 1980). The second aggregation phase is mediated entirely through prostaglandin endoperoxides (PGG and PGH) and thromboxane A_2 (Smith *et al.*, 1974).

Herbal medicine as a system of natural cure has emerged as a new trend in the West (Phillipson, 1981). A search for agents that alter platelet function among indigenous plant drugs is undertaken on the premise that large majority of drugs currently employed in therapeutics are of vegetable origin (Phillipson and Anderson, 1989). For the present study, the following plant extracts have been selected for their reported ethno therapeutic use in heart ailments:

1.	Glycyrrhiza glabra	Butanolic extract
2.	Sida pakistanica	Aqueous extract
3.	Solanum surattense	Aqueous extract
4.	Tephrosia subtriflora	Aqueous extract
5.	Tribulus terrestris	Aqueous extract
6.	Urtica dioica	Aqueous extract

Material and Methods

Extraction from plants

The plants were collected from the vicinity of University of Karachi and other parts of Karachi during the period of March to July and identified with the help of Dr. Mansoor Ahmed of the Department of Pharmacognosy, University of Karachi. Whole plants were chopped and ground. The ground plant material was soaked in ethanol for four weeks. The alcoholic extract was evaporated under reduced pressure in rotary evaporator (Eyela). The syrupy residue thus obtained was dissolved in small quantity of water and subjected to freeze-drying. The plant extracts obtained were used for bioassay on adrenaline-induced aggregation of human platelets.

Blood collection and preparation of platelet rich plasma *(PRP) and platelet poor plasma (PPP)*

Blood was drawn by venipuncture of healthy volunteers of either sex, who denied taking any medication for a week. The blood so collected, was transferred into siliconised centrifuge tubes containing 3.8% w/v sodium citrate in ratio of blood to anticoagulant 10:1, and allowed to settle for 10 minutes. Platelet rich plasma was prepared from whole blood by centrifugation for 10 minutes at 1200 rpm at room temperature (20°C-25°C) in HIMAC centrifuge (Hitachi). The settled portion of blood from which PRP had been removed was then centrifuged at 3200 rpm for 15 minutes and the supernatant PPP was obtained.

Platelet aggregation

Platelet aggregation was performed in NICK Hematracer (Nitro Platelet, Inc.) equipped with a four-channel automatic blood platelet aggregometer with a four channel chart recorder, by the method of Born (1962). It gives a measure of platelet aggregation in citrated PRP by photometric recording of light transmission.

Incubation of PRP with test solutions and aggregation were carried out at 37°C with continuous stirring at 100 rpm. 10 ml of plant *extract* solutions a (15 mg ml^{-1}) b (30$mgml^{-1}$) and c (60 $mgml^{-1}$) in 0.2% w/v sodium carbonate (E. Merck) was added to cuvettes containing 225 *µl* PRP. They were allowed to incubate for 2 minutes before challenge with 5 id of 4 mM adrenaline (Sigma). The control with adrenaline was also measured along with responses of concentrations A (0.625 mg ml"), B (1.25 $mgml^{-1}$) and C (250 mgm^{-1}) for a period of 5 minutes.

Calculation of results

For each experiment, the height in chart units measured at the end of the fifth minute was determined. Percent inhibition of adrenaline-induced platelet aggregation was calculated as follows.

$$\% \text{ inhibition} = \frac{C—T}{C} \text{ X } 100$$

where:

C = Percent aggregation of the control.
T = Percent aggregation after 2 minute incubation with test solution.

Statistical analysis

Values were expressed as mean f S.E.M. calculated from a minimum of 5 observations. The significance of difference between means was determined by student's t-test. Values of $P < 0.05$ were considered significant.

Median inhibitory concentration, IC_{50}, i.e. the dose which produced 50% inhibition of adrenaline-induced platelet aggregation was determined from dose-response regression lines and used to assess the relative potency of the plant extracts.

All statistical procedures were performed according to the method of Telluride and Murray (1984).

Results and Discussion

Aggregometer tracings from representative aggregation experiments are shown in Fig. 1. The summary of data obtained from the above experiments is given in Table 1.

Table 1

Effects of various plant extracts on adrenaline induced aggregation of human platelets.

Plant Extract	% Inhibition of Adrenaline Induced platelet aggregation			IC_{50} $mgml^{-1}$
	Dose A ($0.625\ mgml^{-1}$)	Dose B ($1.25\ mgml^{-1}$)	Dose C ($2.50\ mgml^{-1}$)	
Glycyrrhiza glabra (butanolic) (n = 5)	$20.29 \pm 5.58^{***}$	$46.71 \pm 5.16^{***}$	$59.28 \pm 5.17^{***}$	1.66
Sida Pakistania (aqueous) (n = 5)	$39.37 \pm 3.53^{***}$	$58.09 \pm 5.42^{***}$	83.90 ± 6.14	0.90
Solanum surattense (aqueous) (n = 5)	$29.10 \pm 2.47^{***}$	$48.39 \pm 4.03^{***}$	$66.85 \pm 3.80^{***}$	1.34
Tephrosia subtriflora (aqueous) n = 5)	$22.97 \pm 5.08^{**}$	$46.84 \pm 10.73^{**}$	$68.99 \pm 7.37^{***}$	1.40
Tribulus terrestris (aqueous) (n = 5)	$34.83 \pm 6.77^{***}$	$59.30 \pm 9.25^{**}$	$83.64 \pm 4.95^{***}$	0.97
Urtica dioica (aqueous) (n = 5)	$21.50 \pm 7.34^{**}$	$34.36 \pm 6.96^{***}$	$54.61 \pm 10.11^{***}$	2.17

The results were expressed as mean + standard error (S.E.M.)
**Significantly different from control ($p < 0.05$)
*** Significantly different from control ($p < 0.01$)

The potency of the tested plant extracts (aqueous) in inhibiting adrenaline-induced platelet aggregation is in the following order:

Sida pakistanica, IC_{50} = 0.90 $mgml^{-1}$ > *Tribulus terreshis*, IC_{50} = 0.97 mg ml^{-1} > *Solanum surattense*, IC_{50} =1.3 $mgml^{-1}$ > *Tephrosia subtriflora*, IC_{50} =1.40 $mgml^{-1}$ > *Glycyrrhiza glabra* (butanolic) IC_{50} =1.66 $mgm1^{-1}$ > *Urtica dioica*, IC_{50}= 2.17 $mgml^{-1}$.

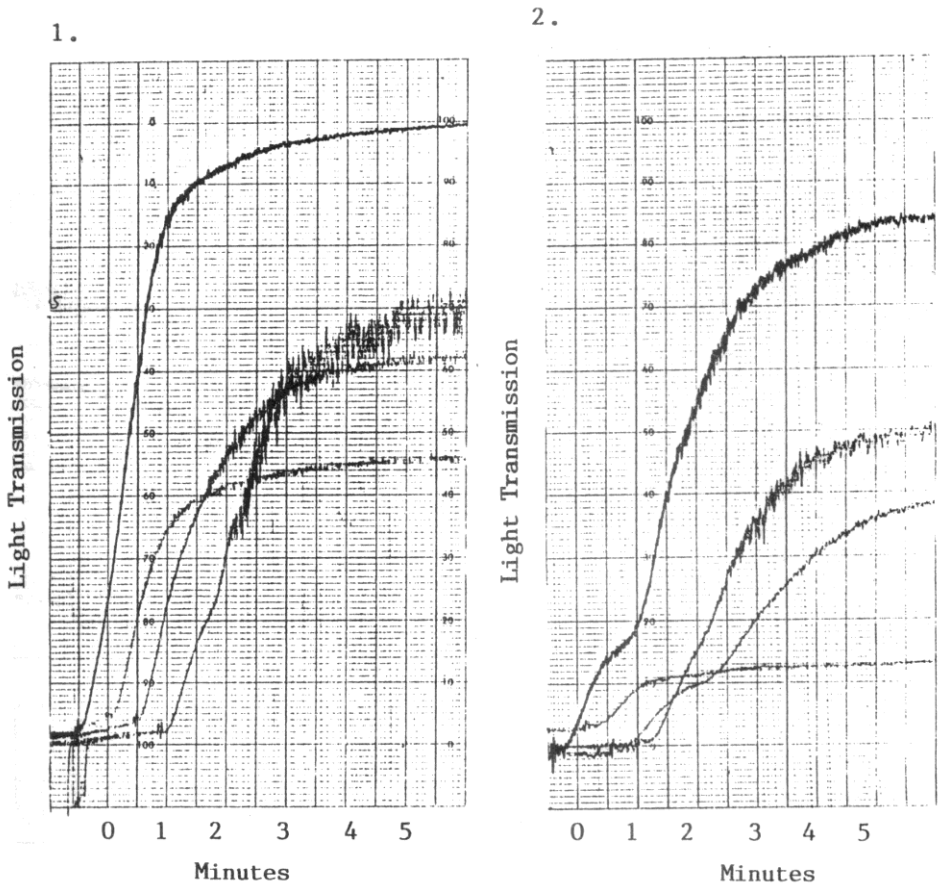
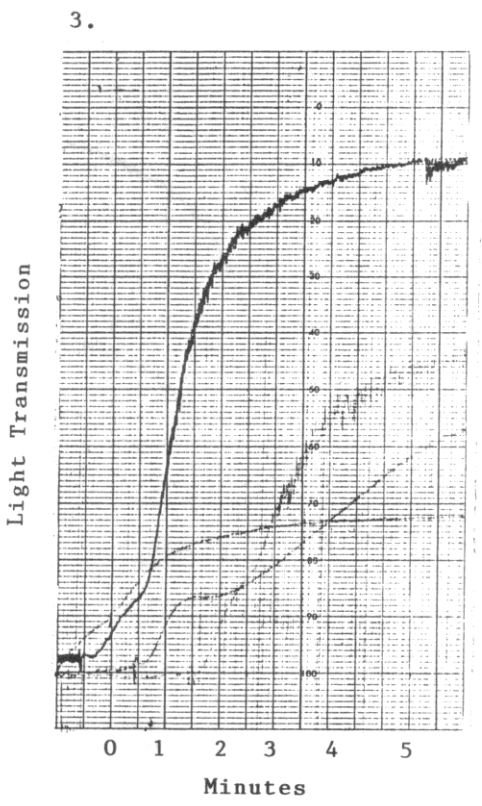
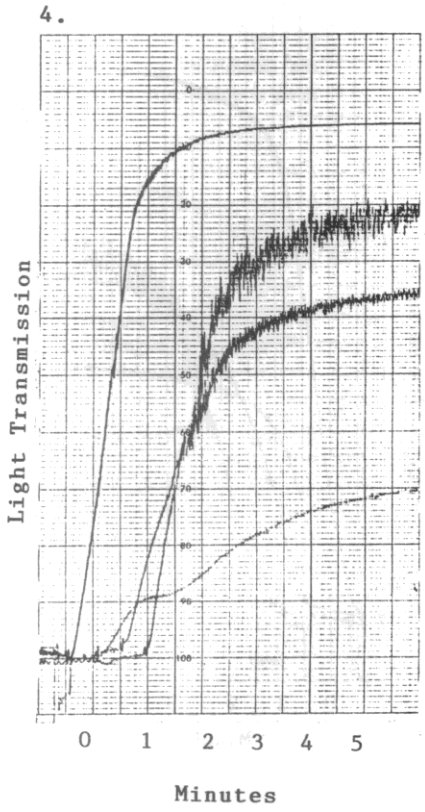


Figure 1. Representative tracings showing the effects of Concentration A (0.625 $mgml^{-1}$), B (1.25 $mgml^{-1}$) and C(2.5 $mgml^{-1}$) of extracts of 1. *Glycyrrhiza glabra* (butanolic) 2. *Sida Pakistanica* 3. *Solanum surattense,* 4. *Tephrosia subtriflora,* 5. *Tribulus terrestris,* 6. *Urtica dioica* on adrenaline-induced aggregation of human platelets. PAP was incubated for two minute with the plant extract before adding adrenaline (4 mM).


3.
Light Transmission
0 1 2 3 4 5
Minutes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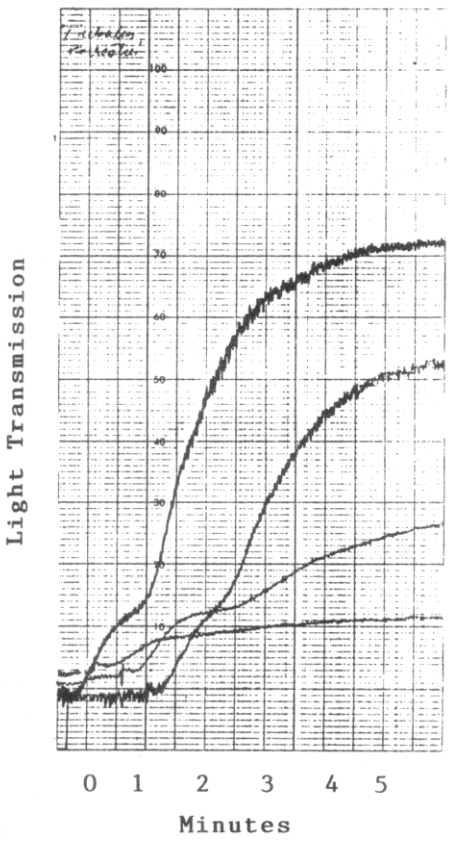
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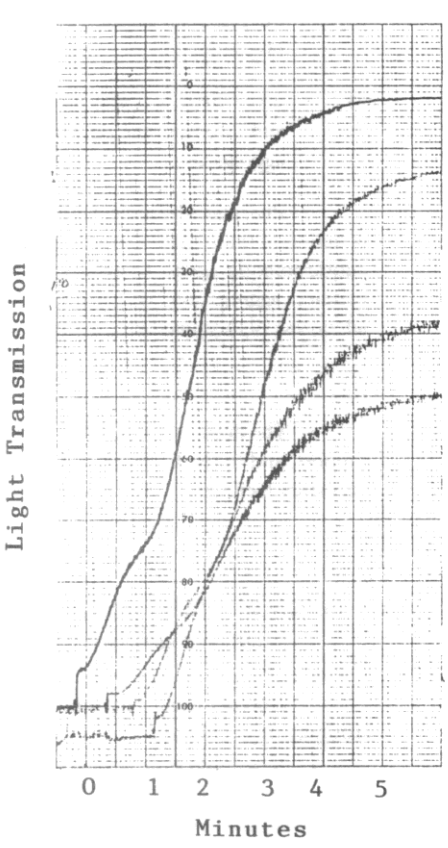
Light Transmission
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Minutes

Inhibitory activity on adrenaline-induced platelet aggregation by these plant extracts indicates one of the followings,

1) the plant extract may have exerted its inhibitory action by α-adrenoceptor blockade.

2) like NSAIDs, it may have suppressed the synthesis of PG endoperoxides and thromboxane Az which play an important role in the second phase of aggregation.

Pharmacodynamic studies should be undertaken to establish the mechanism of action of these plant extracts. In order to claim that a plant extract has potential as antithrombotic agent, screening against pathologically more important inducers of platelet aggregation viz platelet activating factor, collagen, arachidonic acid, adenosine diphosphate etc., is proposed.

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