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Anti-inflammatory and anti-platelet aggregation activity of human placental extract¹

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ABSTRACT

AIM: To find the anti-inflammatory and anti-platelet aggregatory activity of human placental extract (HPE, Placentrex). **METHODS:** The HPE was studied for anti-inflammatory effect in Wistar rats on carrageenin, serotonin (5-HT), and prostaglandin E₁ (PGE₁) induced edema in acute model and cotton pellet induced granuloma on sub-acute model. Anti-platelet aggregation was studied against protection of adenosine diphosphate (ADP)-induced aggregation of human platelet through *in vitro* study. **RESULTS:** HPE showed positive results both in acute and sub-acute models of inflammation. Highly significant ($P<0.01$) results were obtained against 5-HT induced acute inflammation and cotton pellet induced sub-acute inflammation in comparison with standard (diclofenac sodium) and control (normal saline) drugs. The anti-inflammatory property of HPE in animal model was well supported with clinical study of platelet aggregation. There was highly significant ($P<0.01$) inhibition of platelet aggregation with HPE at different doses against ADP. **CONCLUSION:** Our data suggest that human placental extract may be useful in suppressing inflammation and platelet aggregation.

INTRODUCTION

The variety of biological actions of human placental extract (HPE) is a matter of increasing interest. Recent research studies reveal that HPE is the rich resources of various bio-active substances like polydeoxyribonucleotides (PDRN), RNA, DNA, peptides, amino acids, enzymes, trace elements, *etc*^[1]. Therapeutic properties in the treatment of patients with wounds have been described^[2]. It is reported that human placental extract has corticotropin releasing factor (CRF)-like action^[1]. Enzyme-linked immunosorbent assay (ELISA) studies revealed that human placental cytotrophoblasts which expressed interleukin-8, a known mediator of inflammation, was suppressed by glucocorticoid^[3].

The current study was aimed to find the anti-inflammatory activity of HPE in both acute and sub-acute experimental models. Platelet aggregation is an important pathogenic marker of inflammation. Hence, one rational approach in the research of anti-inflammatory drugs is to search for compounds causing inhibitions of platelet aggregation. Although there are some reports of placental extract for their anti-platelet aggregation activity^[4,5] but the observations not correlated with its anti-inflammatory activity. In the current research programme, anti-inflammatory effect of human placental extract was observed in experimental animal model

while platelet aggregation activity was studied in clinical cases.

MATERIALS AND METHODS

Test drug Human placentas weighing between 400-600 g collected at the time of full term spontaneous delivery were immediately placed under ice, then the amniotic membrane and umbilical cord were removed, minced into small pieces and washed with cold normal saline. Aqueous extract with these pieces of placenta was prepared, sterilized, and sealed in ampules (2 mL) under inert condition. The extract 1 mL in the ampule was derived from 0.1 g of placenta. This extract contains protein (0.95 g/L), DNA (2.8 mg/L), RNA (1.6 mg/L), Na⁺ ion (27.9 mmol/L), K⁺ ion (3.07 mmol/L), and Cl⁻ ion (15.1 mmol/L).

Acute toxicity (LD₅₀) This test was performed to evaluate the therapeutic dose as well as for screening of the CNS toxicity. HPE was administered for this purpose at 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL per 20 g of mice in intra peritoneal route. The studies were carried out continuously for 72 h.

Animals Male Wistar rats weighing 150-180 g were used for present research programme. They were housed in groups under 12:12 h regime (lights on from 7:00 h to 19:00 h) at (23±2) °C prior to the experiments. They were supplied pellet diet and water *ad libitum*.

Drugs Carrageenin (Sigma), serotonin hydrochloride (Sigma), prostaglandin E₁ (Sigma), and adenosine diphosphate (Sigma) were used in this study.

Anti-inflammatory activity

Acute inflammation Acute paw edema was induced in groups of ten rats, each using three different experimental models. The rats were deprived of food for 24 h before the induction of inflammation, but water was allowed *ad libitum*. The HPE was administered at dose of 300 mg/kg intramuscularly^[6]. Diclofenac sodium (10 mg/kg, im) and 0.9 % NaCl (5 mL/kg, im) were used as reference drugs. After each treatment paw volume of the animals were measured plethysmometrically.

Carrageenin-induced paw edema Acute inflammation was induced by carrageenin according to the model of Winter *et al*^[7]. For this purpose 0.1 mL of 1 % suspension of carrageenin in normal saline was injected into the sub-planter tissues of right hind paw in rats. The paw volume was measured plethysmometrically at 0 h and 3 h after carrageenin injection. The treated drugs were administered intramuscularly 1 h prior to carrageenin injection.

5-HT-induced paw edema In this experiment 0.1 mL of 5-HT (1 g/L) in sterile saline was injected into the sub-planter tissue of the right hind paw of rats. The paw volume was measured plethysmometrically before and after 30 min of the 5-HT injection^[8,9]. HPE, diclofenac sodium, and 0.9 % NaCl were administered 1 h before 5-HT treatment.

Prostaglandin-induced paw edema Prostaglandin E₁ (1 mg/kg) was administered into the sub-planter region of the right hind paw of rats, in accordance with the method of Willis and Cornelsen^[10]. The paw volume up to the ankle joints were measured plethysmometrically before and after 30 min of the prostaglandin E₁ injection.


Sub-acute inflammation Sub-acute inflammation was produced by cotton pellet induced granuloma in rats^[11]. Sterile cotton (15±1) mg was implanted sub-cutaneously bilaterally in axilla under ether anesthesia. The treated drugs were administered for consecutive 6 d in the same dose as mentioned earlier. The animals were sacrificed on d 7. The granulation tissues with cotton pellet were dried at 60 °C overnight and then the dry weight was taken. The weight differences were considered as the weight of granulation formation.

Platelet aggregation study

Selection of subject Total 15 volunteers of either sex were selected from the medical out patients' department of Bangladesh Institute of Research and Rehabilitation on Diabetes, Endocrine & Metabolic Disorders (BIRDEM), Dhaka, Bangladesh. A careful drug history was taken from the subjects. Patients not receiving for last two weeks the drugs like aspirin, sulfinpyrazone, chlorpromazine, amitriptyline, furosemide, penicillin and its derivatives, dextran, which interfere with the platelet aggregation activity, were selected for the present research programme.

Collection of blood Specimens of blood samples were collected using 3.2 % sodium citrate at the ratio 1:9 with the blood in plastic container with minimum trauma or stasis at the venipuncture site. Testing was performed 30 min after venipuncture at the room temperature.

Preparation of plasma Samples of blood and anticoagulants were gently inverted up and down, avoiding shaking. Platelet rich plasma (PRP) was prepared by centrifuging at 100×g under 4 °C for 15 min. PRP thus prepared were collected by careful pipetting in a sterile polypropylene tube and closed. Platelet poor plasma (PPP) was prepared by centrifuging at approximately 2400×g for 20 min. PPP was collected in a polypropylene plastic tube.

Study methods of platelet aggregation The aggregation was measured on a dual channel Chrono-Log Optical Platelet Aggregometer (Chrono-Log Corporation, Havertown PA 19083-4691) at constant stirring of 1200 rpm (1000 rpm in 50 Hz Units) and electronically controlled temperature (37±2) °C. The light transmission was set at 0 % with PRP and at 100 % with PPP^[10]. Aggregation was induced with aggregating agent ADP at a concentration of 1 mmol/L. HPE was added at different doses of 2.5, 5, 10, and 20  L/mL, 5 min before addition of ADP.

Statistical analysis The results of animal experiments were analysed by unpaired Student's *t* test. Paired *t* test method was applied for the analysis of the clinical data.

RESULTS

Acute toxicity (LD₅₀) From this study it was observed that the HPE is safe upto 45 mL/kg body weight in intra peritoneal route on mice.

Anti-inflammatory activity

Acute inflammation From the study it was observed that there was significant (*P*<0.01) inhibition of paw edema in the animals treated with HPE both on carrageenin (54.3 %) and PGE₁ (39.7 %) induced inflammation. These results are almost same as in the case of diclofenac sodium (57.1 % and 44.4 % respectively) treated group. However, the rate of inhibition (*P*<0.01) of edema in 5-HT induced acute inflammation was even better (49.0 %) than diclofenac sodium (39.4 %) treated group (Tab 1).

Tab 1. Effect of human placental extract (HPE) and diclofenac sodium on carrageenin induced rat paw edema. Mean±SD. *n*=10. ^c*P*< 0.01 vs control.


Experimental model	Control	Paw volume increase/mL	HPE	Paw volume increase/mL	Diclofenac sodium
	Paw volume increase/mL		Inhibition/%		Inhibition/%
Carrageenin induced	1.0±0.5	0.5±0.4 ^c	54.3	0.45±0.25 ^c	57.1
5-HT induced	1.04±0.16	0.53±0.25 ^c	49.0	0.63±0.09 ^c	39.4
PGE ₁ induced	0.63±0.09	0.38±0.22 ^c	39.7	0.35±0.19 ^c	44.4

Sub-acute inflammation In cotton pellet induced sub-acute inflammation model, there was highly significant ($P<0.01$) decrease of the weight of granuloma tissue (39.5 %) in HPE treated group. However, the rate of inhibition of granuloma tissue weight in diclofenac sodium treated group (52.1 %) was found to be better (Tab 2).

Tab 2. Effect of human placental extract (HPE) and diclofenac sodium on sub-acute inflammatory model in rat. Mean±SD. n=10. ^cP< 0.01 vs control.

Experimental model	Control	HPE	Diclofenac Sodium		
	Weight of granuloma tissue/mg			Weight of granuloma tissue/mg	Inhibition/%
Cotton pellet induced granuloma	53±19	32±10 ^c	39.5	25±0 ^c	52.1

Anti-platelet aggregation activity Results of platelet aggregation were expressed as a percent of aggregation at a given time interval (5 min) from reagent addition. Cent per cent aggregation was defined as the differences between the 0 % baseline (PRP) and 100 % baseline (PPP). Highly significant responses ($P<0.01$) of anti-platelet aggregation were observed with all the doses of HPE. There were 83.9 %, 76.6 %, 59.2 %, and 60.2 %

aggregation of platelet with HPE at the doses of 2.5, 5, 10, and 20  L/mL of plasma (PRP) respectively with respect to 100 % PPP control (Tab 3, Fig 1).

Tab 3. Effects of human placental extract (HPE) against ADP-induced platelet aggregation in human PRP. Mean±SD. n=10. ^cP< 0.01 vs control.

Treatment groups and dose	Platelet aggregation against ADP/%
Control	100
HPE	
2.5 µL/mL (n=6)	84±13 ^c
5.0 µL/mL (n=6)	77±16 ^c
10.0 µL/mL (n=12)	59±33 ^c
20.0 µL/mL (n=6)	60±14 ^c

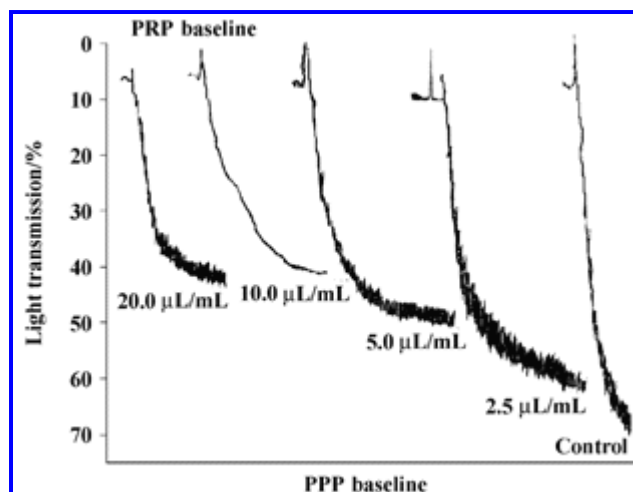


Fig 1. Effect of different doses of HPE for anti-platelet aggregation activity.

DISCUSSION

Inflammation covers a series of reparative and protective responses in tissue injury, whether caused by infection, auto-immune stimuli or mechanical injury. Several classes of compounds such as plasma proteins, vasoactive amines, tissue digestive enzymes, biologically derived oxidant and eicosanoids are all associated with inflammatory response^[13,14]. Most of all investigators have reported that inhibition of carrageenin-induced inflammation in rats is one of the most suitable test procedure to screen anti-inflammatory agents^[9]. The development of carrageenin-induced edema is bi-phasic, the first phase is attributed to the release of histamine, 5-HT, and kinins, while, the second phase is related to the release of prostaglandins^[7,15]. The inhibitory action observed after HPE treatment (Tab 1) on carrageenin-induced paw edema in rats may be mediated through either any of these mediators alone or in combination. Hence, HPE was further studied against paw edema induced by individual agent like 5-HT or PGE_1 . 5-HT is present in mast cells and is considerably more potent than histamine in increasing vascular permeability in rats^[16]. As there was considerable reduction (49.0 %) of inflammation in 5-HT pre-treated rats by application of HPE, it might be conjectured that the inhibitory action was 5-HT mediated. Prostaglandins (PGs) biosynthetic pathway or the cyclooxygenase (COX) activity of the enzyme is the site of action of non-steroidal anti-inflammatory drugs or NSAIDs^[17,18]. Diclofenac sodium is a widely used potent NSAIDS with pronounced analgesic and anti-pyretic activity. It is used mainly for long term symptomatic treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis^[19,20]. Therefore, diclofenac sodium was selected in this study as positive control (Tab 1, 2). Hence it may be assumed that HPE exhibits their anti-inflammatory responses either through inhibition/inactivation of chemical mediators or by directly modulating PG production by suppression of COX. It has been recognized recently that mammalian cells explain two forms of COX activity. COX-1 is the major form present in platelets, while COX-2 is only found in elevated levels in inflammatory exudates^[20,21]. The action of HPE on PGE_1 induced edema (Tab 1) explains that it may modulate PGs production by inhibition of COX.

The sub-acute anti-inflammatory activity of HPE was studied by investigation of its inhibitory effect on the granuloma formation (Tab 2). Cotton pellet granuloma is a model of non-immunological type of inflammation mediated by the activation of the chemical mediators of inflammation, mostly kinins^[23]. The action of kinin involves the activation of two membrane receptors, B_1 and B_2 . B_1 -receptor plays an important role in inflammatory processes^[24,25]. In this present research programme highly significant ($P < 0.01$) result was obtained with HPE in cotton pellet induced sub-acute inflammation model indicating that it may act by the way of inhibiting the B_1 -receptor.

Platelets are essential for normal haemostasis. Activation of the clotting cascade by trauma results in platelet


activation, which is followed by aggregation. The major COX metabolite in platelets is thromboxane A₂ (TXA₂), which is capable of initiating aggregation. NSAIDS inhibit TXA₂ production and thus inflammation [26,27]. The aggregation of human platelets induced by ADP was used^[12] to study the anti-platelet effect of HPE (Tab 3). There was 84 %±13 % aggregation of platelet against ADP with a dose of 2.5 L/mL HPE which gradually decreased (77 %±16 % for 5 L/mL) with the increase in dose, reaching minimum (59 %± 33 %) with a dose of 10 L/mL. There was no appreciable change on further increase of the dose (60 %± 14 % for 20 L/mL). ADP is contained within the platelet in storage organelles and released from the platelet during formation of the primary haemostatic plug and thereby induce further platelet aggregation^[28,29]. There are so many activation pathways leading to platelet aggregation. PGs and 5-HT are considered as the major chemical mediators of platelet aggregation^[30,31]. The clinical study of platelet aggregation reflects that HPE can either inhibit PGs synthesis pathway or 5-HT release.

These data indicate that the anti-inflammatory effect of HPE might be mediated through the suppression of chemical mediators. Further experiments are needed to confirm the mode of action of anti-inflammatory and anti-platelet effect of HPE.

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