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Evaluating Polyherbal Compound – *Bharangyadi* for Anti-Platelet aggregating effect and Steroidogenesis activity

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ABSTRACT

Bharangyadi is a self experienced indigenous polyherbal Ayurvedic preparation use for the management of bronchial asthma. Desire for searching the probable mode of action of this drug, following study was carried out. Allergy and inflammation are two closely related as well as mutually dependent factors. Inflammation is characterized by interactions among platelets, leukocytes, and ECs. These interactions trigger autocrine and paracrine activation processes that lead to leukocyte recruitment into the vascular wall and Platelet-induced chronic inflammatory processes. Anti-Platelet aggregating effect of drug indirectly signifies anti-inflammatory activity.Antigen antibodies reaction, disruption of mast cell and liberation of inflammatory mediators are initiative in pathology of asthma. Thus in present trial anti-platelet aggregation and effect of drug on adrenal gland and spleen is use to assess the efficacy of drug in asthma. Study reveals negative result in both platelet aggregation inhibition and adrenal gland stimulation.

Key word: *Bharangyadi polyherbal compound, complementary medicine, anti-platelet aggregation, steroidogenesis activity.*

INTRODUCTION

Asthma is a chronic inflammatory disease of airways. In spite of effective contemporary medicines the incidence and severity of the disease rising day by day. An estimation says that asthma along with chronic obstructive disease become the third leading cause of death by the year 2020. Contemporary medicine either act as bronchodilator or as anti-inflammatory agent but neither available drug have dual action moreover the side effects of the drug for example the mainstay treatment of asthma i.e. inhaled corticosteroid cause adrenal suppression etc. limits the long term use of these drugs. On the other hand complementary medicines provide better tolerance but their use is still questionable because of lack of data about their action and efficacy. In the present study a self experienced polyherbal indigenous Ayurvedic drug-*Bharangyadi* is screen for its anti-asthmatic activity. Bharangyadi is a mixture of three herbs namely *Bharangi* (*Clerodendrum serratum*), *Sati* (*Hedicium spicatum*) and *Pushkarmoola* (*Inula racemosa*). All these herbs were selected on the basis of literature survey of Ayurvedic management of bronchial asthma (*Tamaka Shwasa*) and recent research proving their efficacy in asthma. *Bharangi* (*Clerodendrum serratum*) is found to have anti-inflammatory [1, 2], antihistaminic, antiallergic [3, 4], antioxidant [5] and hepatoprotective properties [6]. In Ayurvedic system of medicine, it is mainly used in respiratory tract diseases. *Sati* (*Hedicium spicatum*) is found to possess hypotensive, hypoglycaemic [7], anti-inflammatory [8], vasodilator, antispasmodic, tranquillizer [9], anti-bacterial, anti-fungal [10, 11], CNS-depressant, hypothermic, spasmolytic & analgesic effects [12]. *Pushkarmoola* (*Inula racemosa*) has been found prove beneficial for cardiovascular system, angina and dyspnoea [13-16].

MATERIALS AND METHODS

Plant material:

The plants *Clerodendrum serratum*,*Hedychium spicatum* and *Inula racemosa* were collected from local market of Varanasi. The identification of the drugs was done by Prof.A.K.Singh, Department of Dravyaguna, S.S.U. (**Identification number DG/ AKS / 604**).

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Extraction of the plant material and sample preparation:

Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug was carried out by Hot percolation method through soxhlet appratus. Thereafter extract was dried using rotatory evaporator and dried extract was put to the process of standradization. The percentage yield was noted.

Animals:

Eight week-old healthy, laboratory bred, Swiss albino rats of either sex (100 ± 20g) were maintained under standard laboratory conditions such as temperature 22–25°C, 12 hour light/dark cycle and provided with water and pellet food ad libitum. The experiments were conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals, India), Ministry of Social Justice and Empowerment, Government of India. The research protocol was approved by Intuitional Research committee of Institute of Medical Sciences, BHU- India.

Method:

Steroidal Effect:

Twelve albino rats of either sex (100± 20g) were taken and divided into six groups (n=3 for each group). Nine animals were treated with escalating doses of *Bharangyadi* compound as 500mg/kg, 1000mg/kg & 1500gm/kg b.wt respectively for two weeks. Three control animals were treated with equivalent volume of distilled water. On the 15th day the animals of all the groups were sacrificed and effect of drugs on adrenal gland, and spleen were estimated by weighting the organ.

Anti-Platelet Aggregating Factor study:

Platelets were isolated from fresh human blood by differential centrifugation. Briefly, blood from healthy volunteers was collected in citrate- phophate- dextrose adenine and centrifuged at 180x g for 10min. PRP (platelet- rich plasma) was incubated with 1mM acetylsalicylic acid for 15min at 37^{0}C. After addition of EDTA (ethylenediaminetetraacetic acid)(5mM), platelets were sedimented by cetrifugation at 800x g for 15min. Cells were washed in buffer A (20mM hepes,138mM NaCl, 2.9mM KCl, 1mM $MgCl_2$ 0.36 mM Na H_2 PO_4 ,1 mM EGTA (ethylene glycol tetraacetic acid), supplemented with 5mM glucose and 0.6 ADPase units of apyrase/ml,pH 6.2). Platelets were finally resuspended in buffer B (ph 7.4), which was the same as buffer Abut without EGTA and apyrase. The final cell count was adjusted to 0.5-0.8x 10^9/ml. All steps were carried out under sterile conditions and precautions were taken to maintain the cells in resting condition.

Platelet Aggregation: Platelets were stirred (1200 rpm) at 37^0 C for 2min in a Chronolog Whole Blood/Optical Lumi-Aggregometer (model 700-2) prior to the addition of agonists. Wherever indicated, cells were stimulated stirring to prevent aggregation. Aggregation was induced with the agonist thrombin (1U/ml). Aggregation was measured as percent change in light transmission, where 100% refers to transmittance through blank sample.

RESULT

The study shows that *Bharangyadi* compound has no endogenous steroidogenesis effect neither it has any role in platelet aggregation inhibition. As no significant change was found in the weight of adrenal gland after two week treatment with drug it can be concluded that the anti-inflammatory (as reported in previous studies) effect of the *Bharangyadi* compound is not due to increase synthesis of steroids.

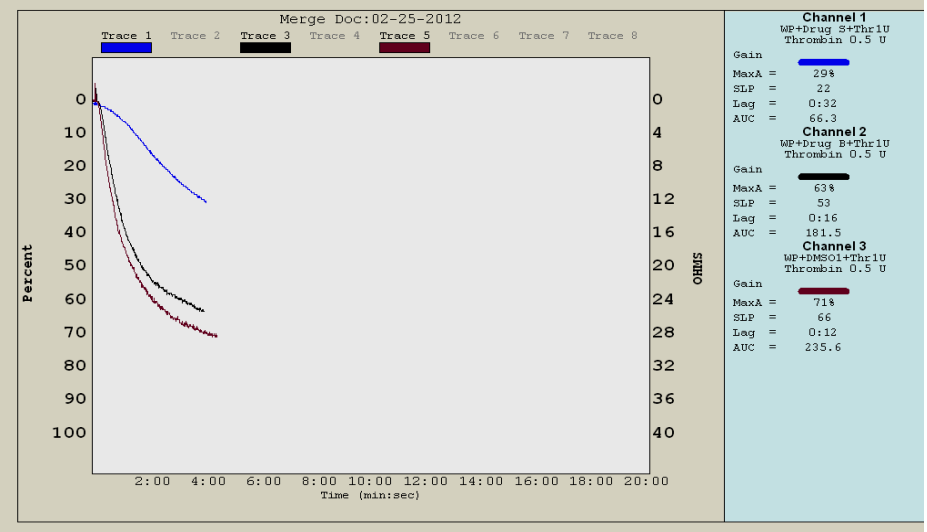

Fig. 1: Showing Inhibition of Platelet Aggregation by *Bharangyadi* Compound

Table No. 1: Effect of Hydroethanolic extract of *Bharangyadi* Extract on various organ weights after 2 week treatment

Dose /100g of animals	Lung/ 100gbwt N=3	Liver/ 100gbwt N=3	Stomach/ 100g bwt N=3	Kidney/ 100gbwt N=3	Heart/ 100gbwt N=3	Adrenal/ 100gbwt N=3	Testis/ 100gbwt N=3	Spleen/ 100gbwt N=3
Control	948mg ± 1.24	3200mg± 1.76	1078mg ± 2.76	350mg ± 1.22	330mg± 1.55	7.1mg ± 1.28	884mg ± 1.78	281mg ± 0.87
200mg	890mg± 0.87	3000mg ± 2.09	1178mg± 3.21	320mg± 2.56	300mg± 2.54	4.8mg ±0.45	880mg ± 0.77	415mg ± 1.65
500mg	800mg ± 0.56	2980mg ± 1.87	1090mg ± 1.22	298mg± 3.65	280mg ± 2.98	3.5mg ± 0.78	826mg ± 2.45	200mg ± 0.87
1gm	750mg ± 1.09	3800mg ± 2.57	1150mg ± 0.45	250mg± 1.98	268mg± 1.69	8.5mg ± 1.78	798mg ± 2.65	234mg ± 0.67
2gm	900mg± 0.98	4000mg ± 0.99	1200mg ± 1.67	348mg± 1.54	310mg± 1.11	8.6mg± 1.65	850mg ± 2.22	195mg ± 1.43

All the values are Mean ± SDE , where n=3.

DISCUSSION

Recent advancement shows that asthma is a multifactorial complex disease involving many factors like inflammation, allergy and various inflammatory mediators like histamine, interlukines, cytokines and more recently and most concern factor i.e. Platelet activating factor []. Role of platelet in the pathogenesis of asthma draw attention of scientist and medical professionals to search advancement in the treatment strategy. In recent years our understanding of the involvement of coagulation and anticoagulant pathways, the fibrinolytic system and platelets in the pathophysiology of asthma has increased considerably. Asthma is associated with a procoagulant state in the bronchoalveolar space, further aggravated by impaired local activities of the anticoagulant protein C system and fibrinolysis. Protease activated receptors have been implicated as the molecular link between coagulation and allergic inflammation in asthma [17]. Thrombin- induced platelet aggregation was not significantly inhibited by 15 min pre-incubation with the *Bharangyadi* extract at different concentrations. *Bharangyadi* showed insignificant reduction in aggregation.

Fourteen days treatment produces no significant change in weight of adrenal gland and spleen, proving that there are no evidences that the drugs in any way stimulates the adrenal gland. Thus it can be said that anti-anaphylactic activity of the drug is not attributed to endogenous stimulation of adrenal gland.

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