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Evaluation of Anti Asthmatic Potential of Methanolic Extract of Stem Bark of *Oroxylum Indicum* Vent

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ABSTRACT

Background: *The plant of Oroxylum indicum is reported to possess anti-inflamatory activity. So, looking for dire need of a new, safe and economical anti-asthmatic agent we focused to investigate anti-asthmatic potential of Oroxylum indicum.*

Aim: *Evaluation of anti-asthmatic potential of a stem bark of Oroxylum indicum using several experimental animal models.*

Materials and Methods: *Methanolic extract of OI stem bark was prepared & subjected for various animal models to evaluate anti-asthmatic activity. Anti-asthmatic activity was evaluated by Histamine induced bronchospasm model followed by RBC membrane stabilization activity (in vitro) and histamine induced paw edema model (in vivo) were performed to ensure anti-allergic activity of same.*

Results and discussion: *Methanolic extract of Oroxylum indicum shown significant increase in PCT (Pre-convulsion Time) in Histamine induced bronchospam model as well as shown significant membrane stabilization property and reduction in Paw volume in Histamine induced paw edema.(Significance level $P < 0.005 \approx$ highly significant).*

Conclusion: *The results of performed studies clearly revealed that Methanolic extract of Oroxylum indicum possess strong anti-asthmatic activity which is possibly due to membrane stabilzation as well as anti-histaminic potential of the herb.*

Keywords: *Anti asthmatic, anti histaminic, anti inflammatory, mast cell stabilization, histamine induced, Oroxylum indicum.*

INTRODUCTION

Asthma is chronic lung diseases that inflames and narrow the airways in the lungs. The word asthma is derived from a Greek word meaning difficulty in breathing [1].

It also includes bronchial hyper-reactivity and reversible airways obstruction [2]. The clinical expression of asthma varies from a mild intermittent wheeze or cough to severe chronic obstruction that can restrict normal activity. Asthma attacks are triggered by a variety of stimuli, including exposure to allergens or cold air, exercise and upper respiratory tract infections [1]. Airway inflammation causes increase in numbers of various types of inflammatory cells eosinophils, basophils, mast cells, macrophages and certain types of lymphocytes [1].

Ethanomedicinal uses of *Oroxylum indicum* include fever, pneumonia and respiratory troubles. The plant is reported to possess anti inflamatory activity [3]. So, looking for dire need of a new, safe and economical anti asthmatic agent we focused to investigate anti asthmatic potential of *Oroxylum indicum*.

Oroxylum indicum is one of the important Rasayana herb mentioned in ayurveda [4] belongs to family Bignoniaceae [5]. *Oroxylum indicum* is a tree found in countries, such as India, Japan, China, Sri Lanka, Philippines, Indonesia, Bhutan and Malaysia. It is commonly called the tree of Damocles, Indian caper, Indian trumpet flower, Indian trumpet tree [2]. *Oroxylum Indicum* is a native tree often grown as an ornamental for its strange appearance. Mostly sighted along the river banks or slopes of the hills [6]. A tree of *Oroxylum indicum* is about 8-15 m tall, brown or grayish brown bark with lenticels and branched at top [7]. The leaves are very large, about 90-180 cm long 2-3 pinnate, cylindrical, swollen at the junction of branches [8]. The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones [7]. The flowers are reddish purple outside and pale pinkish yellow inside having large erect racemes [7]. The flowers bloom at night and emit a strong odor which attracts bats [7]. *Oroxylum indicum* lives in relationship with the *Actinomycete Pseudonocardia Oroxyli* present in the soil surrounding the roots [7].

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MATERIAL AND METHODS

Collection and authentification of plant material:

The stem bark of *Oroxylum indicum* was collected from medicinal garden of School of Pharmacy, RK University. The plant material was carried by Mrs. Trupti Marakana where the herbarium voucher (No. SOP/COG/471/2015) has been kept. The air-dried stembark of *Oroxylum indicum* were pulverized into powder by using a blender and stored in an airtight container.

Fig. 1: Plant of *Oroxylum indicum*

Extraction of plant material:

The finely ground crude drug was placed in thimble which was made of filter paper and placed in soxhlet apparatus. The extracting solvent Methanol was heated in round bottom flask and

its vapors condense in condenser. The condensed extract drops into the thimble containing the crude drug. When the level of liquid in soxhlet apparatus rises to the top of siphon tube, the liquid contents of apparatus siphon in to the round bottom flask. The process is continous and carried out till green pigments disappear. The methanolic extract was used as test extract. All doses were expressed in terms of crude extract (mg/kg body weight).

Fig. 2: Extraction by Soxhlet Appratus

Preparation of the drug:

The fraction of extract was weighted (200 mg) and dissolved in 1 ml of distilled water to prepare required concentration in mg/kg and administered by gavage. Fraction was stored and protected from direct sunlight until use.

Drugs and chemicals:

Histamine dihydrochloride and mepyramine meleate were purchased from chitichem. Histamine solution was freshly prepared in Phosphate buffer (pH 7.4). Test extract dissolved in 1% PEG solution prior to use.

Experimental Animals:

Wistar albino rats of either sex weighing 220-280 g were used for the study. The animals were procured from animal house, Department of Pharmacology, School of Pharmacy, RK University, Rajkot, India. Guinea pig (350-400 gm) and albino wistar rats (200-250 gm) were used in this study. Animals were housed at a temperature of 24±20˚C and relative humidity of 30-70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water. The animals were used after getting approval from Institutional Animal Ethics Committee (IAEC) of School of Pharmacy, RK University. (Protocol no. RKCP/COL/RP/15/60)

***In Vitro* Study:**

Stabilization study of red blood cell membrane:

Approximately 2 ml of blood collected from a healthy rat (300-350 gm) and mixed with 2 ml of Alsever's solution (2 ml distilled water + 1 ml phosphate buffer + 2 ml of hyposaline). This mixture was centrifuged at 3000 rpm for 3 minutes and the packed cells washed with iso saline and 10% W/V solution was made by iso-saline acting as RBC suspension. Three sample tubes were prepared and treated as follow:

Tube I: Normal control: 2 ml distilled water + 1 ml phosphate buffer + 2 ml of hyposaline + 0.5 ml RBC suspension.

Tube II: Standard: 2 ml Ketotifen (1 mg/100ml) + 1 ml phosphate buffer + 2 ml of hyposaline + 0.5 ml RBC suspension.

Tube III: Test: 2 ml MEOI (200mg/100ml) + 1 ml phosphate buffer + 2 ml of hyposaline + 0.5 ml RBC suspension.

Prepared tubes were incubated for 30 minutes and centrifuged for 3000 rpm – 3 minutes. Supernant was collected and absorbance measured at 560 nm. RBC membrane stabilization or % protection was calculated using following formula:

$$\% \ Protection = \frac{100 - OD \text{ of control}}{OD \text{ of drug treated sample}} X\ 100$$

Parameters to be measured: This experiment will be performed thrice and mean value of % protection will be measured.

***In Vivo* Study:**

Histamine induced bronchospasm in guinea pig:

Eighteen guinea pigs of either six were exposed to aerosol of 1.0 % Histamine diphosphate using nebuliser with constant pressure 40mm/Hg in histamine chamber and time for preconvulsion dyspnoea (PCD) was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of asphyctic convulsions i.e. Pre-convulsion time (PCT). As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. All the animals were randomly divided into 3 groups each containing six animals. Two and a half hours later, the animals of groups I received distilled water (vehicle) and served as a control. Group II received Dexamethasone 0.5 mg/kg, p.o. and served as standard. Group III received methanolic extract of *Oroxylum indicum,* 200 mg/kg, p.o. One and half hour after the treatment, animals were exposed to Histamine diphosphate aerosol and time taken for onset of PCT was noted. Animals which withstood exposure to histamine aerosol for 15 min considered to be completely protected. The percentage protection offered by the treatment was calculated by the formula,

Percentage protection = {1- T1/T2} × 100

Where, T1 = time in second for PCD before treatment; T2 = time in second for PCT after treatment. Statistical analysis was done by ANOVA.

Histamine Induced Paw Edema In Rat:

Wistar rats (200-250 gm) of either sex were divided into three groups containing six animals each. Disease control group received 0.1 ml histamine (1% w/v) in right paw, Standard control group received Mepyramine (50 mg/kg, p.o.) and Test group received Methanolic extract of *Oroxylum indicum* (200 mg/kg, p.o.) Test and standard drugs were administered 30 minutes prior to histamine injection into planter region. The paw volume was measured prior, at 1 and 3 h using plethysmometer. Statistical analysis will be done by ANOVA.

Statistical Analysis:

Statastical analysis of results was done by ANOVA for determination of variance. Data were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.001$.

RESULT

***In Vitro* Study:**

Stabilization study of red blood cell membrane:

Administration of MEOI (200mg/kg, p.o.) was done, which results in significant increase in membrane stabilization. From the results it is clear that MEOI has significant anti allergic activity. Ketotifen was the standard drug used in the study. Histamine is mediator of allergic response. The amount of histamine released depends on the number of mast cells that are degranulated.

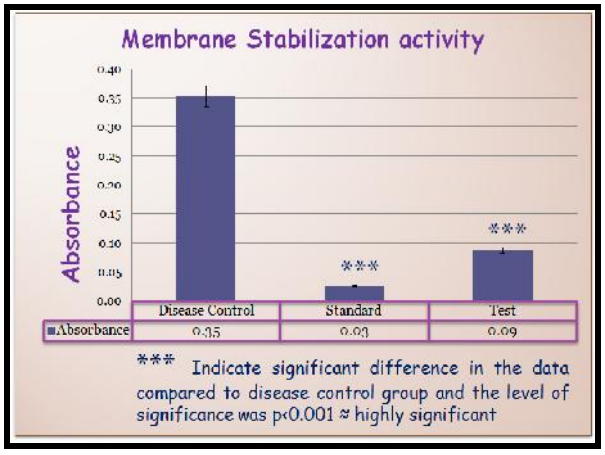

Fig. 3: Test control shows significant membrane stabilization activity as compared to disease control group

***In Vivo* Study:**

Histamine-Induced Bronchospasm:

In the histamine aerosol model, the control animals showed convulsion in 42 seconds of the experiment. Prior treatment of MEOI (200mg/kg, p.o.) protected the animals to a significant extent from development of dyspnoea produced by histamine aerosol confirming that it has antihistaminic activity. Guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstrictor including histamine and leukotrines. Dexamethasone (0.5mg/ml, p.o.) acts as standard anti-inflammatory agent in the model.

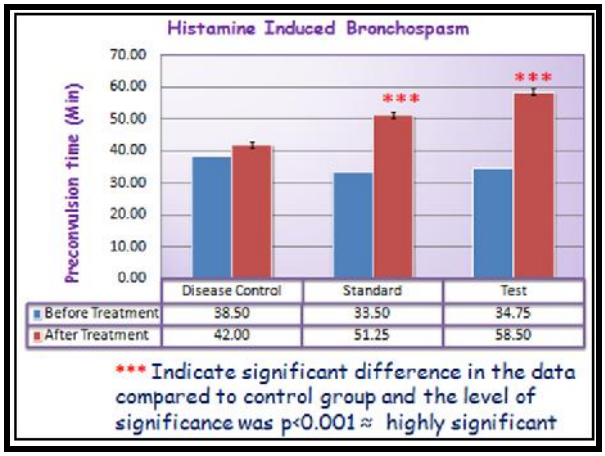

Fig. 4: Test control shows significant bronchodilatory activity as compared to disease control group

Histamine induced paw edema in rat:

Histamine induced paw edema, an inflammatory model, involves several mediators released in sequence. Initial phase of inflammation release histamine and serotonin which is followed by release of bradykinin and prostaglandins. MEOI showed decrease in paw edema in comparison with Mepyramine (50mg/kg, p.o.), an anti histaminic agent. Flavonoids of plant are known to possess anti-inflammatory properties. Flavonoids in MEOI may be responsible for the reduction osf inflammation.

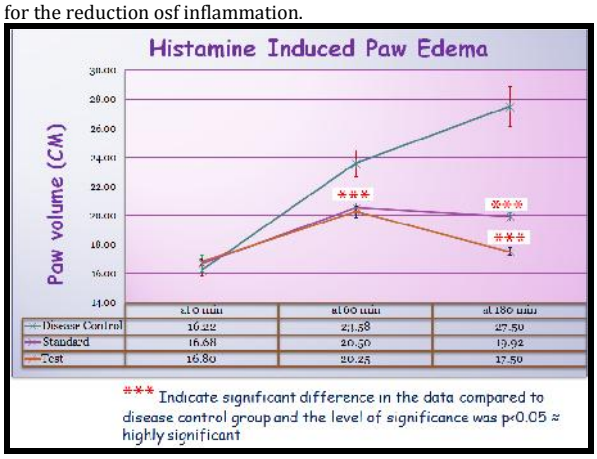

Fig. 5: Test control shows significant bronchodilatory activity as compared to disease control group

DISCUSSION

Table No. 1: Benificial effects of Methanolic Extract of *Oroxylum indicum* (MEOI) on various models of Asthma

Group Name/Model	Membrane stabilization	Histamine Induced Bronchospasm (PCT)	Histamine Induced Paw Edema (At 120 Minutes)
Disease Control	0.14	5.62	1.37
Standard Control	0.01	4.81	0.30
Test Control	0.04	4.94	0.29
F-value	56.74	35.56	40.03
dF	17	17	17
p-value	0.0000001010	0.000002030	0.000002010

Asthma is chronic disease states that inflames and narrow the lung airways. It also includes bronchial hyper-reactivities and reversible airways obstruction. Airway inflammation causes increase in numbers of various types of cells eosinophils, basophils, mast cells, macrophages and lymphocytes. Available beta adrenergic blocker drugs may lead to increased heart rate, cardiac arrhythmias and central nervous system (CNS) effects. Corticosteroids cause mood disturbances, increased appetite, impaired glucose control in diabetic and candidiasis. Mast cell stabilizer causes bronchospasm, cough or wheezing, laryngeal edema, joint swelling and pain, angioedema, headache, rash, and nausea.

The results demonstrate that methanolic extract of *Oroxylum indicum* (MEOI) exhibited anti asthmatic activity in experimental animal models.

In stabilization study of red blood cell membrane *In vitro* model, MEOI showed significant effect in stabilization of membrane. From the results it is clear that MEOI has significant membrane stabilization activity. This beneficial effect may lead to decrease in release of inflammatory mediators from mast cells. The amount of histamine released depends on the number of mast cells that are degranulated.

MEOI showed increase in bronchodilatory effect in histamine-induced bronchospasm in guinea pig. The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes and their ability to be sensitized to foreign proteins. Although there are various model of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans.

Histamine induced rat paw edema is a suitable experimental model that represents a classical tool to estimate acute inflammation and anti-inflammatory potential. This model is widely used to evaluate an antiedematous effect of herbs having anti-inflammatory properties. The paw edema developed serves as an index of acute inflammatory changes, which is determined from differences in the paw volume measured immedialtely after histamine injection. Paw volume were compared with the anti-inflammatory drug Mepyramine. Results obtained from in vivo study showed significant anti-inflammatory activity between 1-3 hours against histamine induced rat paw edema.

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