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STUDY OF THE ANTIASTHMATIC ACTIVITIES ON *A. MARMELOS, S.URENS* & V. *NEGUNDO* IN ANIMAL MODELS

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Abstract:

The present study was undertaken to evaluate the effects of antiasthmatic on A. marmelos, S.urens and *V. negundo. Leaves of alcoholic extracts of* A. marmelos, S. urens, *V. negundo were subjected for the antiasthmatic evaluation for Histamine induced paw edema (HIPE), Milk Induced leucocytosis (MIL), Clonidine Induced Catalepsy (CIC). Adult albino mice (25-30gms) of either sex were used for the study. The animals were divided into three groups containing of 6 animals of each group-1 received normal saline, group-2 received standard drug and group-3 received test drug aqueous extract of Plant extract maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Treatments were administered and observed for specific periods, all results showed significant value when compared to standard treatment group. From the above findings it can be confirmed that* A. marmelos, S.urens and *V. negundo has antiasthmatic activity. However further studies are required to know the exact mechanism of actions.*

Keywords: *Leucocytosis, Catalepsy, A.* marmelos, S.urens & *V. negundo*

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INTRODUCTION:

The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy [1]. Asthma is a inflammatory disease of respiratory system mainly in chronic form which is characterized by acute exacerbation of hacking, dyspnoea, and wheezing and chest tightness mainly at evening or in the early morning. Patients usually have decreased expiratory volume and airflow reduction. It also causes hyper-responsiveness of bronchi, irritation of respiratory track. These are non specific to asthma. Asthma is generally identified as chronic disorder of lungs characterized with reversible broncho-constriction, eosinophils increase, raised basic tone of airway, lymphocyte deposition, submucosal fibrosis, dysfunction of activated epithelial cell, damage to smooth muscles, hypertrophy of submucosal gland, edema in airway wall, overproduction of mucus and non-specific hyper responsiveness of airway to produce spasmogens.

Herbal drugs against asthma are well known to India at the time of Atraya and Dhanvantari. In writings of Charaka and Shushruta, we find many herbs claimed to possess anti-asthmatic activity which is being used by practitioners of Indian medicine even today. Some of the important traditional herbs used as anti-asthmatics are – *Atropa belladonna, Ephedra sinica, Albizzia lebbeck, Allium sativum. Clerodendron serratum, Piccrorrhiza kurroa, Tylophora asthmatica, Solanum xanthocarpum, Ficus racemosa* etc.

A.*marmelos,* commonly known as bael is a species of tree native to the India. The tree is considered to be sacred by hindus. The leaf is trifoliate, alternate, each leaflet 5-14 x 2–6 cm, ovate with tapering or pointed tip and rounded base, untoothed or with shallow rounded teeth. Young leaves are pale green or pinkish, finely hairy while mature leaves are dark green and completely smooth[2-3]. Each leaf has 4-12 pairs of side veins which are joined at the margin. *S.urens,* belongs to malvaceae native to the India. A small to medium-sized tree with a pale-coloured trunk and known for its medicinal properties. V.*negundo* belongs to lamiaceae, is erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. The bark is reddish brown. Its leaves are digitate, with five lanceolate leaflets. The fruit is a succulent drupe, 4 mm (0.16 in) in diameter, rounded to egg-shaped. It is black or purple when ripe. It is used for treating cough in countries and also used to control mosquitoes.

MATERIALS AND METHODS:

Plant Material:

Plant materials were collected and authenticated from the department of Pharmacognosy, GCOP, R.R.Dist. The Leaves of the plants were dried under shade at room temperature, later chopped and grounded into coarse powder. The powdered materials were used for extract preparations. All chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

Animals:

Adult albino mice (25-30gms) of either sex were used for the study. The animals were divided into three groups containing of 6 animals of each group-1 received normal saline, group-2 received standard drug and group-3 received test drug aqueous extract of Plant extract maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed and drinking water was provided *ad libitum.* Animals were acclimatized to laboratory conditions one week prior to initiation of experiments and the protocol was approved by the institutional animal ethical committee.

EXPERIMENTAL

Leaves of alcoholic extracts of *A. marmelos, S. urens,* V. negundo were subjected for the antiasthmatic evaluation for Histamine induced paw edema (HIPE), Milk Induced leucocytosis (MIL), Clonidine Induced Catalepsy (CIC).

Histamine induced paw edema (HIPE): Group I was treated as control administered with vehicle only 0.5 ml of 1% Tween 80 solution. Group II was treated with Cyproheptadine, at a dose of 10mg/kg orally, treated as positive control. Other groups of Animals were received alcoholic extracts of *A. marmelos, S.urens* and V. negundo. 30 minutes after drugs treatment rats were treated with 1% histamine (50µg/paw) in isotonic solutions in to sub plantar region of hind paws. With help of plethysmometer paw volume was measured at 0, 0.5, 1, 2, 3, 4th hrs, after the injection of histamine into planter region of the right hind paw [2-4].

Milk Induced leucocytosis (MIL): Group I Animals were treated with only vehicle (Tween 80 solution in distilled water at concentration of 1 %) at a dose of (5 ml per kg, Orally) The animals of this group were not treated with Milk. It is served as positive control. Animals of Group II considered as negative control, treated only with vehicle 1%Tween 80 solution, at dose of 5 ml/kg, by oral route. Group III Animals were served as standard given dexamethasone at dose of 50 mg/kg, i.p. Group IV, V, VI Animals were received alcoholic extracts of *A. marmelos,*

S.urens and V. negundo [5-7]. All groups were exposed to the previously boiled and cooled milk at a dose of (4 ml/kg, s.c.) before the administration of standard drug and extracts. Blood were collected from the retro orbital plexus. Further diluted with WBC diluting fluid by sucking in WBC pipette up to the mark. The pipette was shaken properly to mix the blood and WBC diluting fluid and kept aside for few minutes. The fluid was mounted on Neubaur's chamber and total leucocyte count was done. Every animal was injected with milk after one hour after administration of the different extracts. The total leucocytes count was calculated for before and after 24 hours of drug treatment and difference in leukocytes were calculated [8-10].

Clonidine Induced Catalepsy (CIC): Group I Animals were named as control group and these were treated only with Tween 80 in distilled water as vehicle orally at 5 ml per kg dose. Group II animals were classified as standard group and treated with Chlorpheniramine maleate at a dose of 10 mg per kg, i.p. other group of Animals were received alcoholic extracts of *A. marmelos*, *S.urens* and V. negundo[11-12]. All the animals were pretreated with 1 mg per kg, dose of Clonidine by subcutaneous route 1 hr after doses of different extracts and standard drug were given. The catalepsy was determined at initial (0 min) after administration of Clonidine. The catalepsy time was determined at 0 to 90 min. Forepaws of mice were kept on horizontal bar and (1 cm in diameter, 3 cm above the table) time was observed till animals remove paws from bar.

Statistical analysis:

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance was carried out.

Results and Discussion

A. marmelos contains the potential chemical constituents such as Aeglemarmelosin, Beta-sitosterol, Amino Acids, Dictamnine, Marmesin, Skimmianine, xanthotoxol and furocoumarins. *Sterculia urens is gum karaya has* leaves are alternate palmate lobes. It has many important chemical constituents like D-galactose, L-rhamnose, D-galactouronic and aldobiuronic acid etc.

Histamine induced paw edema (HIPE) test: All test groups showed significant results when compared with standard group 0.18±0.02 (70.96) (Table 1).

Table 1 Effects of extracts –HIPE test

GROUPS	Change in paw volume (ml) mean ±SEM & % inhibition		
	1 hr	3 hr	6 hr
Control	0.49±0.02	0.54±0.02	0.62±0.03
Reference	0.19±0.01 (61.22)	0.17±0.02 (68.51)	0.18±0.02 (70.96)
A. marmelos	0.29±0.02 (40.81)	0.32±0.02 (40.74)	0.30±0.02 (51.61)
S.urens	0.25±0.02 (48.97)	0.31±0.02 (42.59)	0.24±0.01 (61.29)
V. negundo	0.29±0.01** (40.81)	0.28±0.01 (48.18)	0.29±0.01* (53.22)
Values are mean ±SEM, *p<0.05, ** p<0.01			

Milk Induced leucocytosis (MIL) test: All test groups showed results when compared with standard groups (Table 2 & fig. 4.12).

Table 2 Effects of extracts –MIL test

GROUPS	Before Treatment	After Treatment	Difference
Positive control	6560±170	6821±280	261±250
Negative control	6580±155	12962±160	6382±270
reference	6665±220	9236±155*	2571±180
A. marmelos	7129±190*	9899±250**	2770±170
S.urens	7149±240	9786±150	2637±240
V. negundo	6243±160*	9156±270*	2913±270
Values are mean ±SEM, *p<0.05, ** p<0.01			

Clonidine Induced Catalepsy (CIC) test: All test groups showed significant results with that of standard group 94.15±0.65 at 90 min (Table 3).

Table 3 Effects of extracts –CIC test

Treatment	Control	Std	*A. marmelos*	*S.urens*	V. negundo
0 min	15.64±0.85	10.99±0.20	21.69±0.48	12.64±0.69	22.47±0.65
15 min	70.91±0.62	21.84±0.36	32.39±0.65	19.98±0.25	37.94±0.46
30 min	120.49±0.16	46.27±0.65	45.87±0.17	26.88±0.45**	46.91±0.62
45 min	145.61±0.27	59.18±0.85	59.61±0.25	49.68±0.45	59.71±0.62
60 min	171.76±0.34	66.87±0.24	68.14±0.32	65.94±0.45	71.98±0.82*
90 min	180.25±0.35	94.15±0.65	72.94±0.75	75.44±0.54	82.01±0.34
Values are mean ±SEM, *p<0.05, ** p<0.01					

In HIPE method, % edema inhibition was observed between the test group and standard groups. Similarly in MIL method also leucocyte count was recorded for plant extract groups along with standard/control groups, significant results observed prominent values after induction of leucocytosis with milk. Milk found to cause some allergic reaction when given to parentally by increase in the leucocyte count. This in turn causes inflammation and stress induced by oxidation. In CIC method test groups produced appreciable results with standard i.e., Clonidine which produce dose dependant catalepsy in animals, as it belongs to the agonist of adrenoreceptor (α_2).

CONCLUSION:

From the above findings it can be confirmed that *A. marmelos, S.urens and* V. negundo has antiasthmatic activity. However further studies are required to know the exact mechanism of actions.

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