



Received on 28 October, 2013; received in revised form, 22 January, 2014; accepted, 24 March, 2014; published 01 April, 2014

EVALUATION OF EFFECTS OF *PARTHENIUM HYSTEROPHORUS L.*, LEAVES EXTRACTS AGAINST MILK-INDUCED EOSINOPHILIA IN MICE

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Keywords:

Parthenium hysterophorus L., Milk, Eosinophilia, Leucocytosis, Dexamethasone

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ABSTRACT: *Parthenium hysterophorus L.*, compositae, also known as congress weed, carrot weed, star weed, reaching a height of 2 m in weeks of germination, is believed to have entered India accidentally in the mid 1950's and is now available abundantly all over the India. Leaves are traditionally used in the treatment of asthma and allergy, leavas of the plant were extracted successively using aqueous and ethanol solvents to obtain the respective extracts. In the present study, we have investigated the role of aqueous and ethanol extracts of *Parthenium hysterophorus* against milk-induced leukocytosis and eosinophilia in albino mice. The results of the study revealed that pretreatment with both the extracts caused significant reduction in the total leukocyte and eosinophil counts in animals in dose-dependent manner. From these results, it can be concluded that the plant *Parthenium hysterophorus* is having antieosinophilic activity.

INTRODUCTION: *Parthenium hysterophorus L.*, compositae, also known as congress weed, carrot weed, star weed, white top, chatak chandani, bitter weed, ramphool, and gajar grass, reaching a height of 2 m in weeks of germination, is believed to have entered India accidentally in the mid 1950's and is now available abundantly all over the India¹.

All parts of the plant are reported to be used as bitter tonic, febrifuge, emmenagogue, anti-dyscentric, etc.² Antimicrobial spermicidal activities³ and Hypoglycemic effect⁴, Antifungal activity⁵. The plant is used by some people in the state of Maharashtra and Gujarat (India) in the treatment of diabetes mellitus.

However, no scientific data is available regarding the effect of *Parthenium hysterophorus L.* on blood glucose level. In the present investigation, we have evaluated effect of aqueous and ethanol extracts of *Parthenium hysterophorus* against milk-induced eosinophilia in albino mice.

MATERIALS AND METHODS:

Plant material: Fresh plant of *Parthenium hysterophorus L.* was collected in the month of July-August and was authenticated at the Plant Anatomy Research Center, Chennai. (Reference No: PARC/203/2012)

Preparation of the extract/ drug: The fresh leaves of *Parthenium hysterophorus* collected and dried under shade, sliced into small pieces with mechanical grinder. The powder was passed through sieve no.30 and stored in a container. Then the marc was defatted with Ethanol 95% (75-78oC) by using hot percolation method (Soxhlet apparatus).

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.5(4).1303-06
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(4).1303-06	

The marc was then subjected to cold maceration using distilled water for 72hrs. The extracted was concentrated using a rotary vacuum evaporator and then dried under reduced pressure and kept in the desiccator.

The extracts were suspended in Tween80 for present study. The extract obtained was subjected to various Preliminary Phyto-chemical Screening tests as per the procedure mentioned in the standard reference books ^{6, 7}. The extract was used for pharmacological evaluation.

Experimental animals: Swiss albino mice (20-25 g) were fed with a standard diet and water ad libitum. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27°C and 12 hours light / dark cycle) throughout the experimental period.

Animal experiments were carried out following the guidelines of the animal ethics committee of the institute no (P.COL/64/2012/IEAC/VMCP).

Acute toxicity ⁸: The ethanol and aqueous extract of *Parthenium hysterophorus L.*, was screened for acute toxicity, following the standard method (OECD/OCDE No: 423). Albino mice of female sex weighing 20-25 gm were used in this study. Animals were maintained on normal diet and water prior to and during the course of experiment. The dose of ethanol and aqueous extract was prepared with saline and was administered by intubations. The acute toxicity was tested at the doses of 5, 50 300 and 2000mg/kg.

Studies on milk induced Eosinophilia and Leucocytosis in mice: Mice were divided into seven groups, six animals in each group. Animals belonging to group-I received distilled water (DW) 10 ml/kg, (p.o.). Animals belonging to group II, III, IV, V, VI, VII received boiled and cooled milk injection in dose of 4 ml/kg, (s.c.).

Animals belonging to groups III, IV, V, VI, VII received ethanol and aqueous extract of *Parthenium hysterophorus* in dose 200 and 400 mg/kg, p.o. respectively.

Blood samples were collected from each mouse from the retro orbital plexus, under light ether anaesthesia. Total leukocyte count and total eosinophilia count was done in each group before drug administration and 24 hr after milk injection. Difference in Total leukocyte count and total eosinophilia count before and 24hr after drug administration was reported.

Statistical Analysis: The results of various studies were expressed as mean \pm SEM and analyzed statistically using one way ANOVA followed by Dunnett's Test to find out the level of significance. Data were considered statistically significant at minimum level of $p < 0.05$.

Phytochemical Analysis: The ethanol and aqueous extract of *Parthenium hysterophorus* leaves was concentrated under vacuum using rotary vacuum evaporator to a dry residue and kept in a desiccator. The percentage yield was 11.4%w/w and 16.6 %w/w.

The Phytochemical screening of the percentage crude yields of extracts studied has shown that the leaves of *Parthenium hysterophorus* were rich in lignands, flavonoids, glycosides, sterols, sugars, amino acids and triterpenoids.

Acute toxicity study: The mice treated with oral administration of *Parthenium hysterophorus*. Aqueous and ethanol extract upto 2000mg/kg did not produce any toxic effects in rats. No mortality was observed and *Parthenium hysterophorus*. Ethanol and aqueous extract was found to be safe at given doses. Dose selected for pharmacological evaluation were 200 mg/kg and 400 mg/kg.

Effect on milk induced Eosinophilia and leukocytosis in mice: After 24hrs mice were challenged with milk, in the milk injection significant increase in the differential total leucocytes and differential in eosinophil count were observed.

Ethanol and aqueous extract of *Parthenium hysterophorus*. (200 and 400mg/kg p.o for 24hrs) significantly and dose dependently decrease differential total leucocytes and differential in eosinophil count was observed in group IV, V, VI, VII compared to group II (**Table 1, Figure 1, 2**).

TABLE 1: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACTS OF LEAVES OF *PARTHENIUM HYSTEROPHORUS* EXTRACTS ON MILK INDUCED LEUCOCYTOSIS AND EOSINOPHILIA

Treatment	Dose	Difference in number of leucocytes count	Difference in number of Eosinophil count
		Before and After treatment	Before and After treatment
Normal		222.66 ± 6.1	20.16 ± 5.01
Negative control (milk)	4ml/kg (s.c)	4301.83 ± 16.19 ^{###}	133.50 ± 8.9 ^{###}
Positive control (dexamethasone + milk)	1mg/kg (s.c)	476.16 ± 3.54 ^{***}	25.16 ± 6.25 ^{***}
Aqueous extract + milk	200mg/kg	2317.00 ± 7.73 ^{***}	52.33 ± 5.3 ^{***}
Aqueous extract + milk	400mg/kg	1463.66 ± 16.12 ^{***}	39.50 ± 2.31 ^{***}
Ethanol extract + milk	200mg/kg	2248.50 ± 6.78 ^{***}	68.00 ± 13.15 ^{***}
Ethanol extract + milk	400mg/kg	1556.66 ± 25.48 ^{***}	48.33 ± 10.26 ^{***}

normal control group compare with milk treated group. * compare with milk treated group

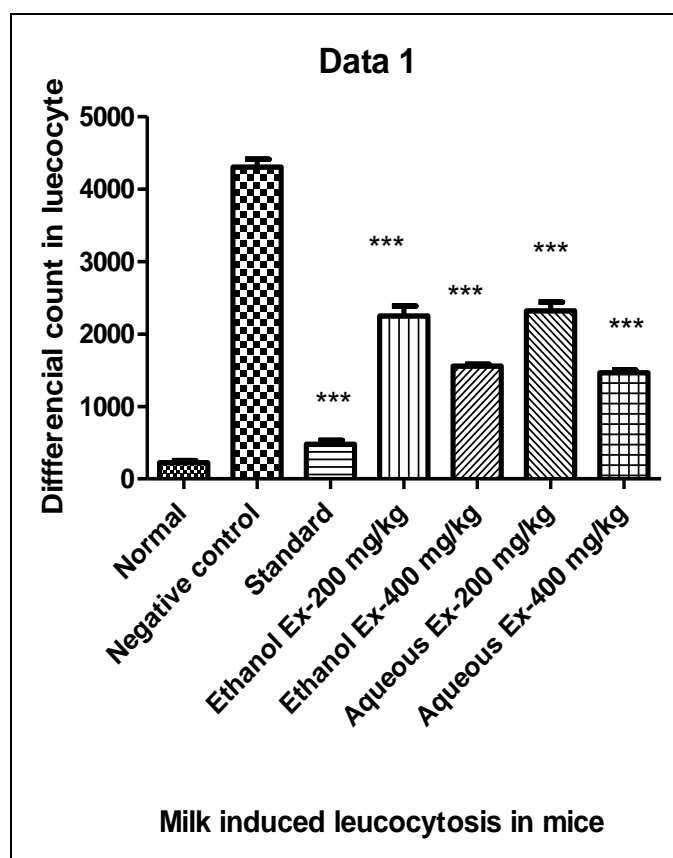


FIGURE 1: EFFECT OF ETHANOL & AQUEOUS EXTRACT OF *PARTHENIUMHYSTEROPHORUS* ON LEUCOCYTES LEVEL IN MILK INDUCED LEUCOCYTOSIS IN MICE

RESULTS AND DISCUSSION: Leukocyte recruited during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein which promote ongoing inflammation.⁹ The eosinophil are the most characteristic inflammatory cells in bronchial biopsies taken from asthma patients and may be seen in the submucosal and epithelial layers¹⁰.

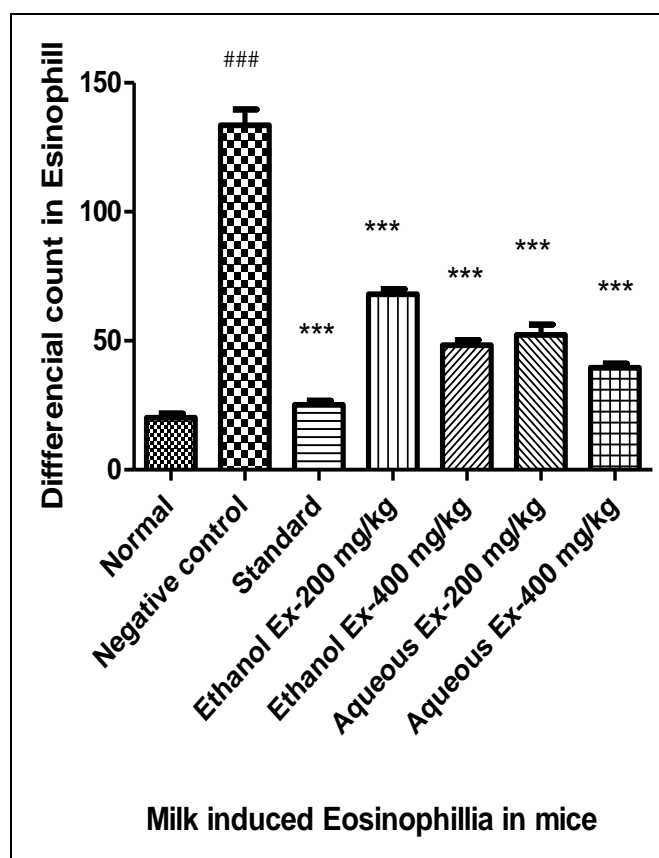


FIGURE 2: EFFECT OF ETHANOL & AQUEOUS EXTRACT OF *PARTHENIUM HYSTEROPHORUS* ON EOSINOPHILIA LEVEL IN MILK INDUCED EOSINOPHILIA IN MICE

An abnormal increase in peripheral eosinophil count to more than 4% of total leukocyte is termed as eosinophilia¹¹. In asthmatic patients, there is increase in eosinophil count. The involvement of eosinophil in bronchial mucosa, in which allergic inflammation occurs, is a critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion.

In the late phase, especially in the development of allergic asthma, eosinophil plays role as inflammatory cell¹². Eosinophil secretes mediators such as eosinophil cationic protein, tumor necrosis factor, eosinophil-derived neurotoxin, and prostaglandin, which results in epithelial shedding, bronchoconstriction, and Promotion of inflammation in respiratory tract often allergic¹³.

It has been demonstrated that parental administration of milk produces a marked and significant increase in leukocyte and eosinophil count after 24 hours of administration. The milk-induced leukocytosis and eosinophilia in mice model helps to evaluate the stress-induced asthma¹⁴.

The results of present study revealed that aqueous and ethanol extracts of leaves *Parthenium hysterophorus* of caused reduction in the count of these inflammatory cells. Among both these extracts, aqueous extract has shown significant activity as compared with ethanol extract in a dose-dependent manner. However, we are screening Both extracts for other animal models of asthma to evaluate their efficacy in the management of asthma and also working on phytochemical investigation of these extracts to pin point the chemical constituent responsible for the activity.

CONCLUSION: In conclusion, our data suggest that the ethanolic and aqueous extract of the leaves of *Parthenium hysterophorus* have a significant in antieosinophilic activity and have beneficial effect in asthma.

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How to cite this article:

Rajasekaran S: Evaluation of effects of *Parthenium hysterophorus* L., leaves extracts against milk-induced eosinophilia in mice. *Int J Pharm Sci Res* 2014; 5(4): 1303-06. doi: 10.13040/IJPSR.0975-8232.5(4).1303-06

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