e-ISSN : 2455-5258

Research in Pharmacy and Health Sciences

Research Article

Evaluation of Antiasthmatic Activity of *Murraya Koenigii L. Leaves*

Rathore Jitendra Singh*, Gilhotra Dr. Umesh Kumar

Department of Pharmacology, G.D. Memorial College of Pharmacy, Jodhpur-342001. Rajasthan, INDIA

ABSTRACT

The present investigation was designed to evaluate the anti-asthmatic activity of ethanolic extract of *Murraya Koenigii* Leaves in experimental animals. *Murraya Koenigii* Leaves was evaluated for anti-histaminic activity using isolated Guinea pig tracheal chain preparation, histamine induced Bronchoconstriction in Guinea pig and milk induced leucocytosis in mice. Ethanolic extract of *Murraya Koenigii* Leaves oral dose 400mg/kg body weight significantly inhibited dose dependent contraction of Guinea pig tracheal chain produced by histamine and also showed significant protection by prolonging PreConvulsion Dyspnoea time (PCT) in guinea pigs. *Murraya Koenigii* Leaves was evaluated by milk induced leucocytosis in mice showed significant decreased of total leukocyte count. Thus, *Murraya Koenigii* Leaves showed anti-allergic activity against histamine hence possesses potential role in the treatment of asthma.

Received: 16-1- 2017

Revised: 29-1-2017

Accepted: 02-03-2017

***Correspondence to:**
Rathore Jitendra Singh,
Email:
jsrathore1011@gmail.com

Funding: Nil

Competing Interests: None

Keywords: Anti-asthmatic, *Murraya Koenigii*, Histamine aerosol, Chlorphenaramine Maleate

INTRODUCTION:

Asthma is a chronic inflammatory disorder of the airways that can cause repeated episodes of cough, wheezing and breathing difficulty. It has been identified as one of the five pressing global lung problems [1]. It has many causes but more specifically due to inflammation of air passage, hypersensitivity of afferent glossopharyngeal and vagal ending in larynx and afferent trigeminal endings in the nose, pulmonary edema and congestion of lungs caused by left ventricular failure (cardiac asthma). The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Approximately 300 million people worldwide currently have asthma, with estimates suggesting that asthma prevalence increases globally by 50% every decade [2]. With the projected increase in the proportion of the world‘s urban population from 45% to 59% in 2025, there is likely to be a marked increase in the number of asthmatics worldwide over the next two decades [3]. The prevalence of childhood asthma with wheezy bronchitis ranges from 9.9 to 33% child asthmatic [4]. Although the fundamental causes of asthma are not completely understood, the strongest risk factors for developing asthma are inhaled asthma triggers such as pollens and moulds, tobacco smoke and Chemical irritants in the workplace [5]. There are two types of asthma, Atopic and non-atopic. Atopic occurs in children and young adults who have atopic (type-I) hypersensitivity to foreign proteins. Again when the same antigen comes into contact the antibody/antigen reoccurs resulting in release of histamine and other factors increase mucous secretion and muscular contraction that narrows the airways. Attacks become less frequent and less severe with age. Non atopic occurs in adult life with no childhood history and is associated with chronic inflammation of upper respiratory tract. Eventually impaired lung ventilation leads to hypoxia, pulmonary hypertension and right sided heart failure. Anti-asthmatic drugs like corticosteroids, theophylline and salbutamol are widely used in the treatment of asthma but these drugs produce some adverse effects like immune suppression, cardiac problems [6]. Large numbers of drugs are used for in the treatment of asthma. However none of them seems to be an ideal drug [7]. The currently used drugs for the treatment of asthma in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use [8, 9]. Now a day the approach on herbal medicine to reduce the adverse effects has been increased and *Murraya koenigii* L. is one of the traditional herbal medicine which claims to have many therapeutically beneficial effects.

Murraya koenigii commonly known as Curry tree,

belongs to the family Rutaceae. *Murraya koenigii* is rightly called the "Botanical garden of the World". The *Murraya Koenigii* contain compounds Carbohydrates, Protein, Phytosterols, Tannin, Flavonoids, Volatile oil, Anthroquinone, Glycoside, and Alkaloids [10]. *Murraya Koenigii* Leaves contain anti-inflammatory activity and antioxidant activity [11, 12]. *Murraya koenigii* is a rich source of biologically active carbazole alkaloids [13]. Mahanimbine, murrayanol and mahanine are three carbazole alkaloids isolated from fresh leaves of Murraya koenigii. Of these three, murrayanol showed anti-inflammatory activity, while mahanimbine displayed antioxidant activity [14]**.** Mahanimbine and koenigine, two carbazole alkaloids isolated from the leaves of *Murraya koenigii* showed antioxidant activity [15]. It is traditionally used as a whole or in parts as antiemetics, antidiarrheal, febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain and vomiting [16].

Traditionally, the Curry Leaves used as antiemetic, antidiarrheal, febrifuge and blood purifier.

Curry Leaves is found to be effective as antioxidant, antidiabetic, antibacterial, antihypertensive, cytotoxic and also in the treatment of bronchial respiratory difficulties [17]. Therefore, by considering the traditional claim reported pharmacological activity and chemical constituents present in plant, the present study was to evaluate the Antiasthmatic Activity of *Murraya Koenigii L*. Leaves in laboratory animals.

MATERIALS AND METHODS

Plant collection and Authentication

The collection of the plant material of *Murraya koenigii* Leaves were done in month in the March from Jodhpur region, Rajasthan. Since the plants will be enriched with phytoconstituents during that time. The *Murraya koenigii* Plant was authenticated by Dr.Vinod.Maina, Scientist 'D' & H.O.O. Botanical Survey of India, Jodhpur (Raj) (voucher specimen no: No.: BSI/AZRC/TECH./2015-16 (PI. Id)/832) and submitted at G.D. Memorial College of Pharmacy, Jodhpur, (Raj.)

Preparation of plant extract

Fresh Leaves was separated and washed thoroughly under running tap water followed by rinsing with distilled water, were shade dried at room temperature. The shade dried leaves passed through a 10-mesh sieve. After proper sieving, the coarsely powdered materials (500 g) weight of the powder was obtained. These powders were used for extraction.

coarsely powder (10 size mesh) were used for the soxhlet extraction petroleum ether (60-80^{0c}c) and ethanol. Plant material 20 g was extracted with 250 ml of petroleum ether in soxhlet apparatus for 40hrs at temp.60-80^{0}C to defatted the powder and then mark was extracted with 250 ml ethanol for 72 hrs. at temp.75-80^{0}C. The extracts were collected and evaporated to dryness to give dry crude extract. The yield of ethanolic extract was 4.80% w/w. The obtained extracts were subjected to phytochemical investigation.

Experimental animals

Dunkin-Hartley Guinea pig weighing 350-400 gm of both sex and Swiss Albino mice 20-30 gm either sex were procured from animal house of G. D. Memorial College of Pharmacy, Jodhpur (Raj.). They were housed in well ventilated cage under controlled condition of light (12 hrs. light-dark) and temp (20-22^{0}C). The animals were allowed standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. no. (1491/PO/a/11/CPCSEA) & (Ref. No.: GDMCP/2016-17/132(A).

Phytochemical Screening (**Table 1**)

Ethanolic extract of *Murraya koenigii Leaves* was screened for presence such as Carbohydrate, Alkaloids, Glycosides, Flavonoids, Tannins. The screening was done using standard protocol described in Khandelwal and Kokate [18, 19].

Acute Toxicity Study

Acute oral toxicity was performed as per OECD-423 guidelines. Acute oral acute toxicity study in albino mice were carried out with ethanol extract of *Murraya Koenigii* in accordance with OECD guidelines no.423. The animal were kept fasting for overnight providing only water. Then the extracts (Leaf) were administered orally at the dose of 400 mg/kg by intragastric tube and Extract was found to be safe and 400 mg/kg of *Murraya Koenigii* was chosen and administered i.p. for this study based on acute toxicity testing [20].

Antiasthmatic activity

Isolated Guinea pig tracheal chain preparation

The guinea pigs tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb's solution (Composition NaCl-5.9, KCl-0.35, $CaCl_2$-0.28, $MgSO_4$-0.11, $NaHCO_3$-2.1, KH_2PO_4-0.16 and Glucose-2.0 gm/liter). Guinea pigs trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution and maintained at 37

Fresh Leaves was separated and washed

±1ºC, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 400 mg. A dose response curve for histamine was recorded at variant molar concentrations by maintaining 15 mint time cycle.
After obtaining dose response curve of histamine (30ug/ml) on trachea, the ethanolic extract of *Murraya Koenigii* Leaves (100 µg/ml) was added to reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative log of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine.
In absence and in presence of ethanolic extract of *Murraya Koenigii* Leaves and standard drug Chlorpheniramine maleate (1 µg/ml) [21].

Histamine induced Bronchoconstriction in Guinea pig

Overnight fasted guinea pigs were randomly divided into three groups (n=6). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The Preconvulsive dyspnoea time (PCT) was noted for each animal. The Preconvulsive dyspnoea time is the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnoea (PCD) commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnoea for 24hours. This time for preconvulsive dyspnoea was recorded as basal value. After 24 hours, animals belonging to group I served as control and were administered with phosphate buffer (1ml/kg, p.o.); Animals belonging to group II were administered with Chlorpheniramine maleate (2 mg/kg, i.p.) while group III was received respective doses of ethanolic extract of *Murraya Koenigii* Leaves. These animals were again subjected to histamine aerosol later at an interval of 1 hr., 4 hrs. and 24 hrs. to determine Preconvulsive dyspnoea time (PCT) [22]. The protection offered by the treatment was calculated by using the following formula:

% protection = (1 – T1/T2) X 100

T1 =the mean of PCT before administration of test drugs.
T2 = the mean of PCT after administration of test drugs at 1 hr., 4 hrs. and 24 hrs.

Milk induced Leucocytosis in mice

Mice were divided into three groups, six animal in each group. Animal belonging to group-I served as control and received only boiled and cooled milk (4 ml/kg, s.c.). Animal belonging to group II received boiled and cooled milk injection in dose of 4ml/kg (s.c.). Animals belonging to group II served as standard and was received Dexamethasone (50 mg/kg i.p.) while animals belonging to group III served as test group and was received respective doses of ethanolic extract leaves of *Murraya koenigii* and 1 hr. later boiled and cooled milk (4 ml/kg, s.c.) were administered to the same animals. After 24 hrs. Blood samples were collected from all animals from their retro orbital plexus, under light ether anesthesia. Total leukocytes count was done in each group 24 hrs. after milk injection [23].

Statistical Analysis

Statistical Analysis The analysis was performed using Graph Pad Prism software. The results of various studies were expressed as mean ± SEM (n=6). Data analyzed using one-way ANOVA followed by Student's test in Histamine induced Bronchoconstriction in Guinea pig & Dunnett's test for individual comparison of group with control P value were measured moderate significant at $p<0.05$.

RESULT:

Phytochemical Screening : (Table 1)

Effect of ethanolic extracts of Leaves of *Murraya Koenigii* on histamine induced contraction of isolated guinea pigs tracheal chain preparation :(Table 2)

In the present study, histamine (30µg/ml) produced dose dependent contraction of guinea pigs tracheal chain preparation maximum percentage of contractile response versus negative log molar concentration of histamine. The modified physiological salt solution containing Chlorpheniramine maleate (1 µg/ml) significantly inhibited ($p<0.05$) the contractile effect of histamine. The modified physiological salt solution containing ethanolic extract of *Leaves of Murraya Koenigii* (100 µg/ml) significantly inhibited ($p<0.05$) the contractile effect of histamine. Hence Chlorpheniramine maleate and ethanolic extract of *Leaves of Murraya Koenigii* (100 µg/ml) shifted the DRC of Histamine towards the right side indicating that there was competitive antagonism between histamine and both the drugs for histaminergic receptors. (Fig 2).

Effect of ethanolic extract of Leaves of *Murraya Koenigii* on histamine induced
Bronchoconstriction in guinea pigs (Table 3)

The guinea pigs when exposed to 0.2%w/v histamine aerosol showed signs of progressive Dyspnoea leading to convulsions. Chlorpheniramine maleate

(2mg/kg, i.p.) significantly prolonged ($p<0.05$) the PreConvulsive Dyspnoea in 1 hr., 4 hrs. and 24 hrs. as compared to control and the percent (%) protection observed was respectively. The ethanolic extract of Leaves of *Murraya Koenigii* at doses of 400 mg/kg ($p<0.05$) significantly prolonged the PreConvulsive dyspnoea later at 1 hr., 4 hrs. and 24 hrs. as compared to control. Thus showed more protection against PreConvulsive Dyspnoea as compared to control, following exposure to histamine aerosol.

The percent protection observed for Leaves of *Murraya Koenigii* at the dose of 400 mg/kg was 53.8, 58.9, and 4.45% in 1 hr., 4 hrs. and 24 hrs. respectively. The percent protection observed for at the dose of Chlorpheniramine maleate (2mg/kg, i.p.) was 81.1, 84.5, and 73.8% in 1 hr., 4 hrs. and 24 hrs. respectively. (Fig-3).

Effect of ethanolic extracts of Leaves of *Murraya Koenigii* on milk induced Leucocytosis in mice (Table 4)

Subcutaneous administration of boiled and cooled milk (4ml/kg) into the Swiss Albino Mice as antigen and produced allergic response in mice**.** The total leucocytes count was increased 24 hours after milk injection.

The total leucocytes count in control group was significantly higher as compared to ethanolic extract of Leaves of *Murraya Koenigii* group & Dexamethasone group. (Fig-4)

Treatment with ethanolic extract of *Murraya Koenigii* dose of 400mg/kg, p.o. showed significant inhibition of milk induce leukocytosis as compare to control. And Treatment with Dexamethasone at the dose of 50 mg/kg, i.p. has significantly ($p<0.05$) inhibited milk-induced leucocytosis as compared to control.

DISCUSSION

Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli, in which many cells play a role. Different agonists like acetylcholine, histamine, 5-HT and other mediators are responsible for contractile responses. Ultimately the mediators promote vascular permeability, smooth-muscle contraction and mucus production, which cause symptoms of asthma including airway constriction, coughing, shortness of breath and wheezing. The stimulation of H1 receptor causes contraction of bronchial smooth muscle.

In the present study with screening of ethanolic extract of Leaves of *Murraya Koenigii* has antagonized the histamine induced contractions on guinea pigs tracheal chain preparation which have shown a significant relaxation indicated by right shift of DRC of histamine. In isolated guinea pig tracheal chain preparation, a right side shift of dose response curve of histamine was observed in the presence of the extract indicating antihistaminic action (Table 2) Histamine is the major inflammatory mediator in asthma, causing hyper responsiveness and bronchial airway inflammation [24]. Total leukocyte count was carried out for doses 400 mg/kg p.o. body weight. The result show significant decrease in total leukocyte count, indicating possible usefulness of *Murraya Koenigii* extract in antiasthmatic treatment. (Table 4) Thus the possible mechanism of its antiasthmatic activity may be due to inhibition of various inflammatory mediators of asthma, but still exact mechanism is yet to be carried out and separation of active chemical constituents is yet to be done to determine exactly which constituent is responsible for activity.

CONCLUSION

The drug may be further explored for its phytochemical profile to identify the active constituents. It can be concluded that ethanolic extracts of *Leaves of Murraya Koenigii* may possesses antihistaminic activity which may be due to H_1-receptor blocking or anti-allergic activity, Bronchodilating activity, Anti-inflammatory activity and Anti-oxidant activity. Thus *Leaves of Murraya Koenigii* may be used in the management of asthma.

ACKNOWLEDGEMENT

I thankful to Mr. S.N. Kuchhawaha Sir, Chairman, G. D. Memorial College of Pharmacy, Jodhpur, for providing all necessary facilities for carry out research work. Special thanks to my respected guide Dr. Umesh Kumar Gilhotra Sir, Professor and Principal, G. D. Memorial College of Pharmacy, Jodhpur (Raj.) For her excellent guidance and dedicated efforts.

REFERENCES

1. Chitra V, Raju D, Sri Hari DK, Silambu JP. Evaluation of Anti-asthmatic Activity of Aqueous Extract of *Achillea mellifolium* Linn Flowers. Scholar Research Library Archives of Applied Science Research 2009;1(2):2 87-293.

2. Barnes PJ, Drazen JM, Rennard SI, Thomson NC. Pathophysiology of Asthma: Basic Mechanisms and Clinical Management. Edn 2, Science Direct e-book, 2009, 401-423.

3. Braman SS. The Global Burden of Asthma. Chest 2006,4S-12S.

4. Nichols D, Longsworth FG. Prevalence of exercise induced asthma in schoolchildren in Kingston, Jamaica. West Indian Med J 1955;44:16-9.

5. Gurudeeban S, Satyavani K, Ramanathan T. Bitter Apple (*cittrus colocynthis*): An over view of Chemical Composition and Biomedical Potential. Asian Journal of Plant Science 2010;9(7):394-401.

6. Sutherland DC, Beaglehole R, Fenwick J, Jackson RT, Mullins P, Rea HH et al. Medicinal Journal of Chemistry 1984;97:845-848.

7. Verstraelen S, Bloemen K, Nelissen I et al. Cell types involved in allergic asthma and their use in vitro models to assess respiratory sensitization. Toxicology in Vitro 2008; 22:1419-1431.

8. Borhade P, Deshmukh T, Patil V, Khandelwal K. Review on *Citrullus colocynthis*. Int J Pharm chem. 2013;3(1):46-53.

9. Verstraelen S, Bloemen K, Nelissen I et al. Cell types involved in allergic asthma and their use in vitro models to assess respiratory sensitization. Toxicology in Vitro 2008;22:1419-1431.

10. Handra HK, Jha PK, Shruthi SD. Pharmacognostic and Phytochemical Studies on The Leaves of *Murraya Koenigii* (L) Spreng. Pharmacophore 2010;1(3):231-238.

11. Patel S, Rathod H, Kabra S, Vaishnav M. Evaluation of Anti Inflammatory Activity of Hydroalcoholic Leaves Extracts of Polyherbal Combination of *Vitex Negundo* and *Murraya Koenigii* against Carrageenan Induced Paw Edema in Rats. J Mol Pharm Org Process Res 2014, 2(3).

12. Jat BL, Joshi R, Chaudhary K, Choudhary CR, Maheshwari RK. Determination of antioxidant activities of different extracts of in vitro plantlets of *Murraya koenigii* L. Spreng. 2014;2(2):2321-4902.

13. Banerji J, Nayak A. Review on chemistry and pharmacology of *Murraya koenigii* Spreng (Rutaceae). J Chem Pharm Res 2010,2(2):286-299.

14. Kureel SP, Kapil RS, Popli SP. Chemistry and Industry. 1970:958.

15. Rao LJM, Ramalakshmi K, BB Borse, B Raghavan. Food Chemistry. 2006;100:742-747.

16. Handra HK, Jha PK, Shruthi. SD. Pharmacognostic And Phytochemical Studies On The Leaves Of *Murraya Koenigii* (L) Spreng. Pharmacophore. 2010;1(3):231-238.

17. Ajay S, Rahul S, Mishra A, Paras M. Comprehensive review: *Murraya Koenigii* Linn. Asian J Pharm Life Sci. 2011;1(4):417-422

18. Khandelwal KR. Practical Pharmacognosy techniques and experiments. Edn 8, Nirali Prakashan, Pune, India, 2000, 149-155.

19. Kokate CK. Practical Pharmacognosy. Edn 4, Vallabh Prakashan, Delhi, 1996, 107-111.

20. Organization for Economic Co-operation and Development (OECD) Guideline for The Testing of Chemicals: Acute oral toxicity. 2001:423.

21. Saxena P. In-Vivo or In-Vitro Evaluation of Anti-asthmatic activity of Rhizome Extract of *Acorus Calamus* Linn. in Guinea pig. Res J Pharm Sci 2014;3(5):1-6.

22. Sharma P, Hullatti KK, Kuppasth IJ and Sharma S. Study on Anti-asthmatic property of *Cyamopsis Tetrogonoloba* L. Leaves Extracts. J Natural Reme 2010;10(1):81-86

23. Patil SS, Panchal CV, Poul B, Langde MM, Burande MD. Pharmacological Evaluation of Polyherbal Formulation for Anti-Asthmatic Study. WIJPPS 2015;9(4):570-591.

24. Gosh MN. Fundamental of Experimental Pharmacology. Edn 5, Calcutta, Scientific book agency, 1984.

TABLE-1: RESULT OF PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *MURRAYA KOENIGII* LEAVES.

Extract	Glycoside	Carbohydrate	Amino acid	Flavonoid	Alkaloid	Tannin	Fats&Oils
Ethanolic Extract of *M.K.*Leaves	+	+	-	+	+	+	-

(+): Persent; (-): Absent

TABLE -2: EFFECT OF ETHANOLIC EXTRACTS OF LEAVES OF *MURRAYA KOENIGII* ON HISTAMINE INDUCED CONTRACTION OF ISOLATED GUINEA PIG TRACHEAL CHAIN PRPERATION

Sr. No.	Dose of Histamine (30µg /ml) (ml)	- ve Log molar concentration of Histamine	% Maximum Contraction		
			Control	Test	Standard
1	0.1	6.61	22.43±1.60	20.02±2.26**	9.89±1.32**
2	0.2	6.31	44.61±1.56	34.80±3.72**	24.03±1.56**
3	0.4	6.01	60.45±2.10	39.02±3.24**	29.56±2.02**
4	0.8	5.71	75.12±1.56	44.20±2.12**	37.12±1.03**
5	1.6	5.40	87.32±2.46	58.05±2.53**	46.08±1.89**
6	3.2	5.10	99.23±1.46	61.02±1.03**	52.06±1.27**

Data are expressed as Mean± S.E.M. Where, n= 6,
Statistical analysis done by using Student's-test & ANOVA by followed by Dunnett's test Where, level of significance chosen was $p<0.05$ when compare to control group.

Control = D.R.C of histamine (30µg /ml) in absence of test drugs of *Murraya Koenigii.*

Test = D.R.C histamine (30µg /ml) in presence of Murraya *Koenigii.*

Standard = D.R.C of histamine (30µg /ml) in presence of Chlorpheniramine maleate.

TABLE 3: EFFECT OF ETHANOLIC EXTRACTS OF LEAVES OF MURRAYA KOENIGII ON PERCENT PROTECTION IN HISTAMINE INDUCED BRONCHOCONSTRICTION IN GUINEA PIGS;

Data are expressed as Mean± S.E.M. Where, n= 6,
Statistical analysis done by ANOVA followed by Dunnett's test, where level of significance chosen was $p<0.05$ compared to control group ; ns= not significant
Group- I (Control) = Aerosolized Histamine (0.2 % w/v) + Phosphate Buffer saline (1ml/kg, p.o.)
Group-II (Standard) = Aerosolized Histamine (0.2 % w/v) + Chlorpheniramine maleate (2 mg/kg, i.p.)
Group-III (Test. *MK*-400) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of Leaves of *Murraya Koenigii (*400mg/kg, p.o.)

TABLE 4: EFFECT OF ETHANOLIC EXTRACTS OF *LEAVES OF MURRAYA KOENIGII* ON MILK INDUCED

Group (n=6)	Preconvulsive dyspnea (in min.) (Mean±SEM)at				(%) Percent protection		
	Before treatment	After treatment					
		1 hr.	4 hrs.	24 hrs.	1 hr	4hrs.	24hrs.
I	2.24±0.47	-	-	-	_	_	_
II	7.41±0.23	11.85±0.43**	14.45±0.49**	08.52±0.39^{ns}	81.1	84.5	73.8
III	4.64±0.59	04.84±0.29 ^{ns}	05.45±0.50 ^{ns}	04.10±0.37**	53.8	58.9	45.4

LEUCOCYTOSIS IN MICE

Group (n=6)	**Difference in number of Leucocytes (per cu mm)) (Mean ± SEM)**
I	6243.5±5.53
II	4602.2±5.30**
III	5381.3±6.18**

Data are expressed as Mean± S.E.M. Where, n= 6,
Statistical analysis done by ANOVA followed by Dunnett's test, level of significance chosen was $p<0.05$. All treated groups were compared with control group.

Group-I (Control) = Distilled water (10 ml/kg, p.o.) + Milk (4 ml/kg, s.c.)

Group-II (Standard) = Dexamethasone (50 mg/kg, p.o.) + Milk (4 ml/kg, s.c.)

Group-III (Test,EE*MK*-100)=Ethanolic extracts *of Murraya Koenigii* (400mg/kg, p.o.) + Milk (4ml/kg., s.c.)

FIGURE-1

FIGURE-2: HISTAMINE INDUCED CONTRACTION OF ISOLATED GUINEA PIGS TRACHEAL CHAIN PREPARATION.

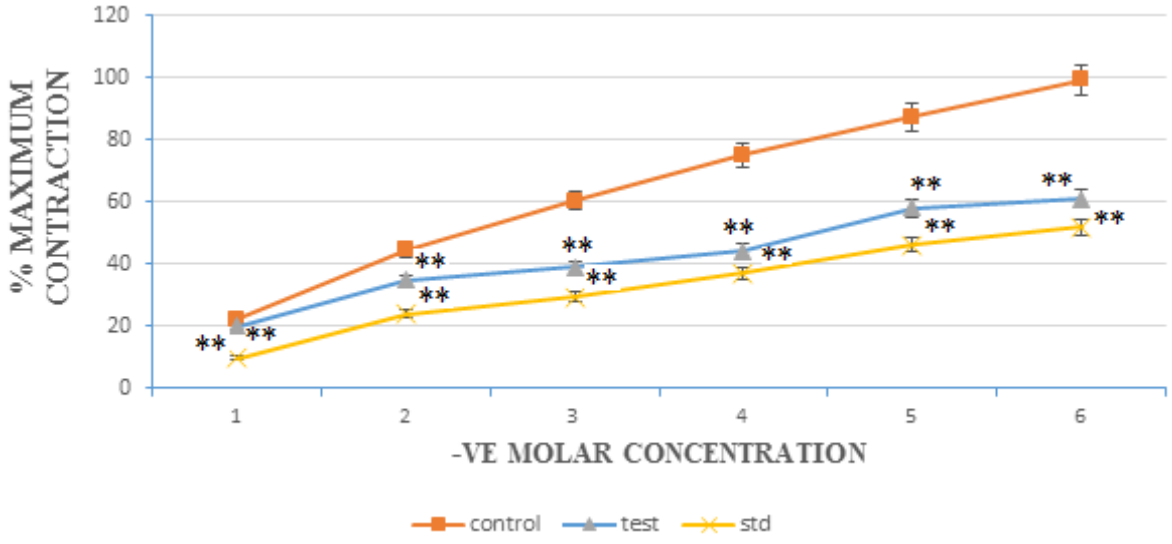

FIGURE-3: HISTAMINE INDUCED BRONCHOCONSTRICTION IN GUINEA PIGS

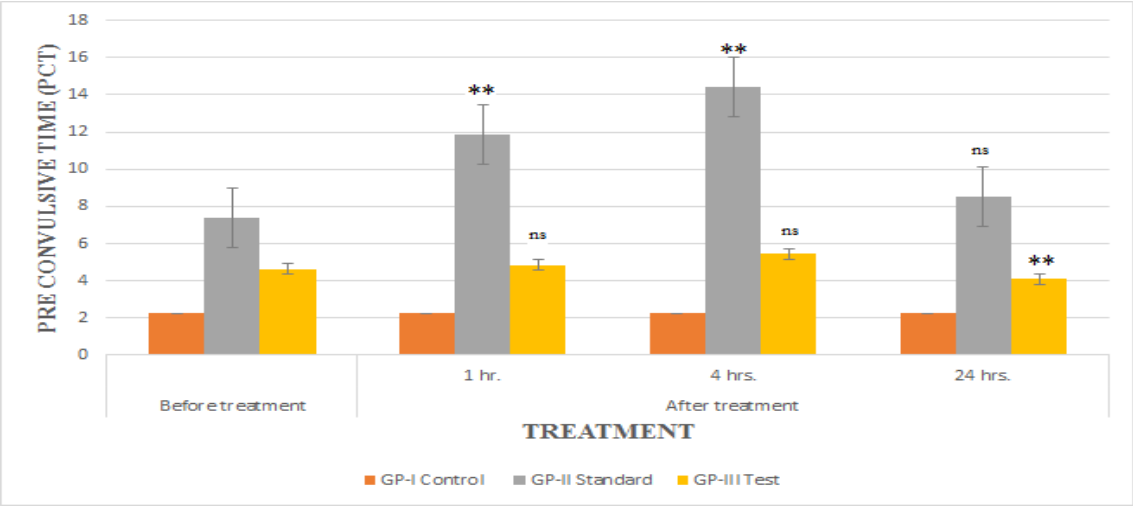

FIGURE-4: MILK INDUCED LEUCOCYTOSIS IN MICE