INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

ANTI-INFLAMMATORY EVALUATION OF METHANOL EXTRACT AND AQUEOUS FRACTION OF THE BARK OF BAUHINIA VARIEGATA (LEGUMINOSAE)

SM. Bairagi[1]*, AA. Aher[1], N. Nema[2] and PK.Nimase[1]

[1]MES College of Pharmacy, Sonai, Ahmednagar, Maharashtra, India.

[2]Department of Pharmaceutical Sciences, Dr. H.S. Gaur Central University. Sagar, Madhyapradesh, India.

ABSTRACT

The main objective of the study is that the methanolic and aqueous fraction of the bark of *Bauhinia variegata* was investigated for its acute inflammation potential in animals. Albino rats were used for the experiment respectively. Anti-inflammatory activity determined by carrageenan induced paw edema and dextran induced paw edema. The extract of the bark administered orally at a dose 200 mg/kg and 250 mg/kg. The anti-inflammatory activity of methanolic and aqueous fraction of *Bauhinia Variegata* was carried out. The anti-inflammatory activity determined by carrageenan induced paw edema were not too significantly different ($P>0.05$) from the control at 200 mg/kg and 250 mg/kg. The aqueous fraction of the methanol extract significantly inhibited inhibited ($P<0.05$) carrageenan induced paw edema in rat at 250 mg/kg. Significant activity against dextran induced paw edema in rats was exhibited by both methanol extract ($P<0.01$) and aqueous extract ($P<0.05$) when administered orally at 200 mg/kg and 250 mg/kg. The bark powder of *Bauhinia Variegata* contains flavone glycosides', flavonoids. It is revealed from the screening models used that the methanol and aqueous fraction of this plant shows the acute anti-inflammatory activity. The activity was attributed to the presence of phytoconstitutents in the tested extract.

Keywords: Anti-inflammatory activities, *Bauhinia variegata*, Dextran induced edema.

INTRODUCTION

Bauhinia variegata (Leguminosae) is a medium sized deciduous tree, sparingly grown in India. This plant is used traditionally in scrofula, diarrhea, anticancer, pain, rheumatism, delirium, and depressant[1]. The steam of the plant is used as an astringent in the treatment of diarrhea. Its decoctions are recommended for ulcers as a useful wash solution[2]. The plant are reported to contain flavone glycosides, flavonoids (2*S*)-5,7-dimethoxy-3′,4′-methylenedioxyflavanone, serine proteinase inhibitor 6-butyl-3-hydroxy flavanone and a new dihydrodibenzoxepin, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz [b,f] oxepin[3-8]. These active constituents have been attributed the therapeutic activity of the plant. Therefore, the present study was undertaken to evaluate their anti-inflammatory activities.

MATERIALS AND METHODS

Plant collection and identification

The fresh bark of *Bauhinia Variegata* were collected from local area of MES College of pharmacy, sonai, Maharashtra, India in Apr 2010 and identified and authenticated from Botanical survey of India, pune the bark was air dried and powdered with a mechanical grinder, filtered and fine powder was stored in a non-toxic polyethylene bag.

Preparation of the extract
Air-dried fine bark powdered (500 g) material of *Bauhinia Variegata* was macerated in 2.5 liters methanol for 48 hours, filtered concentrated in a rotary evaporator, weighed (73 g) and stored. Thirty five gram of the concentrate was dissolved in methanol successively and exhaustively partitioned into various fraction using n-hexane, chloroform and water. The aqueous of methanol extract were used.

Phytochemical screening[9-11]
The extract was subjected to phytochemical screening and the preliminary chemical examination of ethanol extract revealed the presence of steroids, flavonoids, tannins, coumarins, carbohydrates and reducing sugars. Flavonoids exhibit varied biological activities that include analgesic,anti-inflammatory, antioxidant, hepatoprotective and antiulcer activities. Tannins are protectants. Based on this, it was contemplated to carry out the screening of ethanolic extract for analgesic, anti-inflammatory activities. Petroleum ether extract and chloroform extract revealed the presence of steroids and hence these were not tested. The results are compiled in Table 5

Drugs and chemicals
Methanol (Research-lab fine chem. Industries, Mumbai, India), Chloroform (Research-lab fine chem.Mumbai, Industries), Diclofenac sodium (Fortstar-Cadila, India)

Pharmacological evaluation
Animals: Albino rats (150-200 g) were obtained from the animal house colony of the MES College of Pharmacy sonai, India. All animals were allowed free access to water and were kept on a constant standard diet. All procedures involving animals were carried out in accordance with the guide for the care and use of laboratory animals and were approved by the Animals Ethical Committee.

Determination of Anti-inflammatory activity
The inhibitory activity of the studied compounds on Carrageenan-induced rat's paw edema was determined according to the method of Winter et al[12, 13]. The rats were divided in groups of 5 each. Acute inflammation was induced by intraplantar administration of 0.1 ml 1% freshly prepared Carrageenan or Dextran solution in to the right hind paw of each rat. The rats were treated with normal saline, crude extract or aqueous fraction (200 mg/kg or 250 mg/kg P.O) 1 hour before administration of inflammatory agent. Paw volume of rats were measured prior to administration of inflammatory agent and then at predetermined intervals. For Carrageenan the interval was 1 hour for 6 hours, while Dextran was 30 minute for 4 hours, change in paw volume was measured using Vernier caliper and anti-inflammatory activity calculated.

Statistical analysis
Results were expressed as mean $\pm$ standard error of mean. Statistical analysis of the data was done using one–way analysis of variance (ANOVA) followed by Dunnett's test and significance determined using P-values <0.05.

RESULTS
The methanol extract of *Bauhinia variegata* bark inhibited (P>0.05) from the control (table 1and graph 1). The aqueous fraction of the methanol extract significantly inhibited (P<0.05) Carrageenan induced paw edema, at the third hour of the activity of the aqueous fraction is higher than that elicited by Diclofenac in a dose-dependent manner. At 250 mg/kg the methanol extract significantly inhibited dextran induced edema (P<0.01) after 60 min (table 3 and graph 3). The aqueous fraction also inhibited dextran induced paw edema significantly (P<0.05) both at 200 mg/kg and 250 mg/kg after 60 minutes (table 4 and graph 4).
The methanol extract showed a dose dependent activity but was less than that produced by Diclofenac (table 2 and graph 2), the aqueous fraction showed an improved activity as compared to the methanol extract with a maximal effect produced by Diclofenac after 60 minutes (table 4 and graph 4).

DISCUSSION
Inflammation is the response of living tissue to injury. Which involve activation of various enzyme, mediators release, cell migration, tissue breakdown and repair[14]. The present shows the anti-inflammatory activity of the methanol extract of *Bauhinia variegata* and the aqueous extract in a number of experimental models. Carrageenan induced paw edema is suitable experimental animal model for evaluation anti- edematous effect of natural product[15].
And this is involve three phases, in first phase (1 hr after Carrageenan induce) involves the release of serotonin and histamine from mast cells, in second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cycloxygenase product and lipoxygenase products[16]. From the result the methanol extract of *Bauhinia variegata*

bark inhibited (P>0.05) from the control (table 1and graph 1). The aqueous fraction of the methanol extract significantly inhibited (P<0.05) Carrageenan induced paw edema, at the third hour of the activity of the aqueous fraction is higher than that elicited by Diclofenac in a dose-dependent manner. Diclofenac sodium is cycloxygenase inhibiter. It inhibits prostaglandin synthesis and somewhat cycloxygenase-2 selective. The methanol extract has activity which is comparable to Diclofenac can be said to inhibit the cycloxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carragennan paw edema[17].

Dextran induced rat paw edema in which the edema is a consequence of liberation of histamine and serotonin from mast cells[18]. At 250 mg/kg the methanol extract significantly inhibited dextran induced edema (P<0.01) after 60 min (table 3 and graph 3). The aqueous fraction also inhibited dextran induced paw edema significantly (P<0.05) both at 200 mg/kg and 250 mg/kg after 60 minutes (table 4 and graph 4).

The methanol extract showed a dose dependent activity but was less than that produced by Diclofenac (table 2 and graph 2), the aqueous fraction showed an improved activity as compared to the methanol extract with a maximal effect produced by Diclofenac after 60 minutes (table 4 and graph 4). Preliminary phytochemical screening shows that the extract contain steroid,tannins, flavonoids carbohydrates, reducing sugars and Free anthraquinones.

Table 1: Effect of methanol extract of *Bauhinia Variegata* on Carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg)	Mean paw edema (paw circumference) (mm)					
		1 hr	2 hr	3hr	4hr	5hr	6hr
Normal saline	5 ml/kg	42.2±4.1	59.3±3.3	63.1±2.3	62.1±3.8	58.8±1.8	61.5±5.3
Methanol Extract	200	28.3 ± 2.4	38.2±2.4	50.2±1.2	47.8±1.8	50.3±1.4	42.3±3.1
Methanol Extract	250	35.1±5.8	37.2±3.6	53.1±0.8	51.4±1.2	39.5±1.5	34.5+2.1
Diclofenac sodium	10	27.3±6.1	37.2±9.8	42.3±7.1	38.9±7.1	38.2±7.1	33.7±8.7

Value are expressed as Mean ± SEM P<0.05 when compared with control group (n=5)

Table 2: Effect of aqueous fraction of the methanol extract of *Bauhinia Variegata* on carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg)	Mean paw edema (paw circumference) (mm)					
		1 hr	2 hr	3hr	4hr	5hr	6hr
Normal saline	5 ml/kg	42.2+4.1	59.3±3.3	63.1±2.3	62.1±3.8	58.8±1.8	61.5±5.3
Aqueous fraction	200	43.7 ± 1.8	51.7±5.1	45.7±5.2	46.8±7.8	47.3±7.7	44.7±7.5
Aqueous fraction	250	28.9+9.3	31.2+7.3	*24.2+8.3	*22.6+6.8	*22.6+6.8	*22.9+6.5
Diclofenac sodium	10	27.3±6.1	37.2±9.8	42.3±7.1	38.9±7.1	38.2±7.1	33.7±8.7

Values are expressed as Mean ± SEM **P<0.01, *P< 0.05 when compared with control group (n=5)

Table 3: Effect of methanol extract of *Bauhinia variegata* on dextran-induced paw edema in rats

Treatment	Dose (mg/kg)	Mean paw edema (paw circumference) (mm)							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Normal saline	5 ml/kg	57.7±7.4	66.5±7.8	56.8±6.3	43.1±7.3	40.8±5.3	32.9±5.7	32.1±4.0	21.8±6.1
Methanol Extract	200	69.8± 2.9	63.8±9.4	56.3±7.2	47.6±7.2	37.8±5.6	37.8±5.2	25.3±4.5	18.0±4.6
Methanol Extract	250	54.2±6.4	*50.7±6.5	**40.6±5.8	*33.2±5.9	22.2±5.1	22.2+3.7	16.4+4.1	11.9+4.1
Diclofenac sodium	10	48.8±4.5	*40.1±4.7	**29.2±1.8	26.0±2.9	20.0±5.3	20.0±5.3	8.8±4.5	4.1±2.6

Values are expressed as Mean ± SEM **P<0.01, *P<0.05 when compared control group. (n=5)

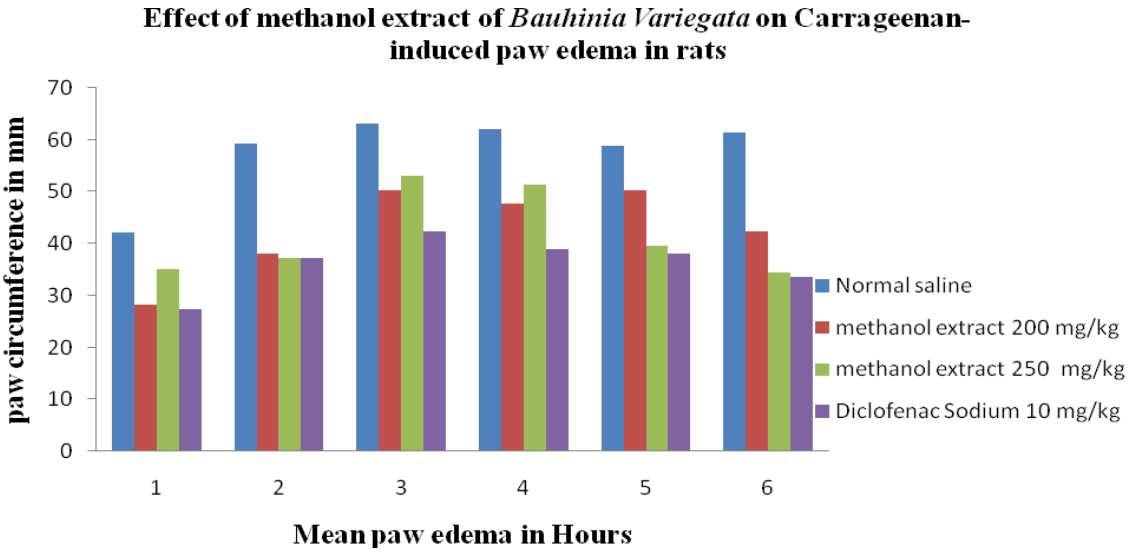
Table 4: Effect of aqueous fraction of the methanol extract of Bauhinia variegata on Dextran -induced paw edema in rats

Treatment	Dose (mg/kg)	Mean paw edema (paw circumference) (mm)							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Normal saline	5 ml/kg	55.7±5.6	63.5±4.8	59.8±6.3	43.1±7.3	40.8±5.3	32.9±5.7	32.1±4.0	21.8±6.1
Aqueous Fraction	200	48.8± 6.1	*38.8±8.2	40.3±9.7	37.6±9.2	36.8±8.6	34.3±8.3	25.3±7.5	22.3±7.5
Aqueous Fraction	250	41.5±6.6	*36.2±5.5	*34.6±7.2	26.1±4.7	24.7±5.8	23.2+6.1	14.4+4.3	10.9+4.1
Diclofenac sodium	10	48.8±4.5	*40.1±4.7	**29.2±1.8	26.0±2.9	20.0±5.3	13.0±5.3	8.8±4.5	4.1±2.6

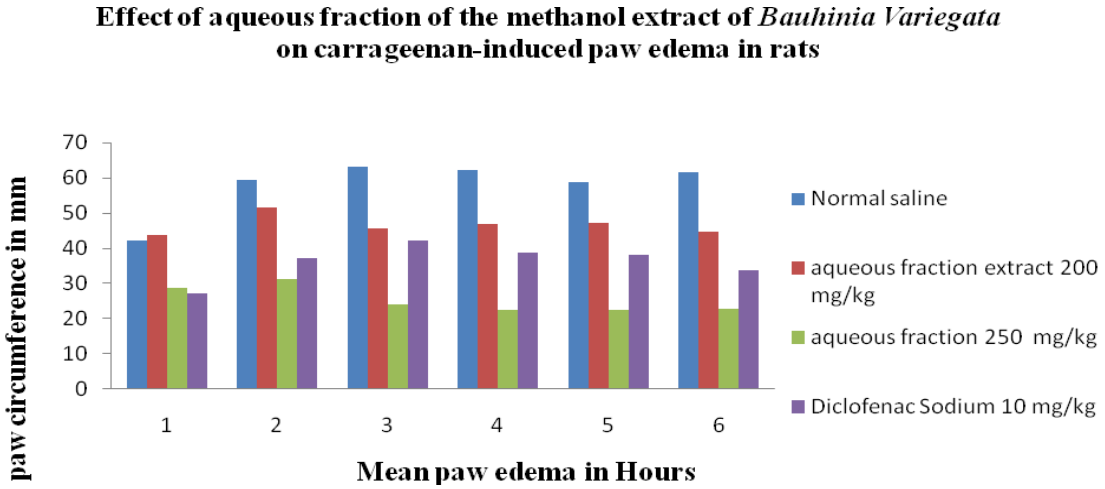
Values are expressed as Mean±SEM **P<0.01 *P<0.05 when compared with control group (n=5)

Table 5: Phytochemical investigation[9-11] of various extracts of *Bauhinia Variegata*

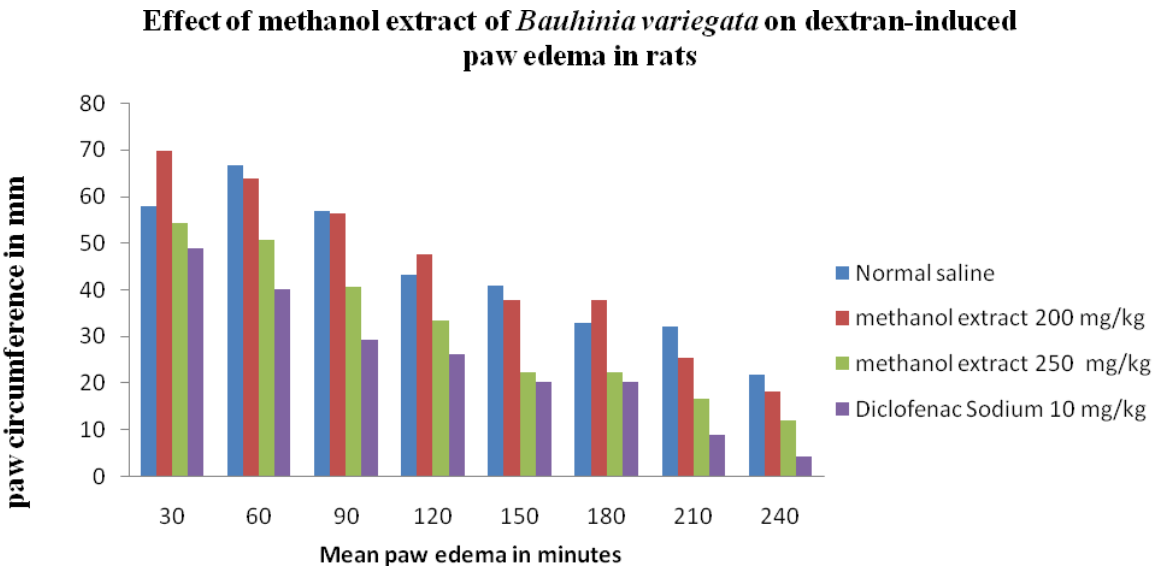
Constituents investigated	Powdered bark
Steroids	+
Free anthraquinones	+
Flavonoids, coumarins, carbohydrates and	+
Carbohydrates and reducing sugars	+
Alkaloid	-
Tannins,	+
Reducing sugars	+



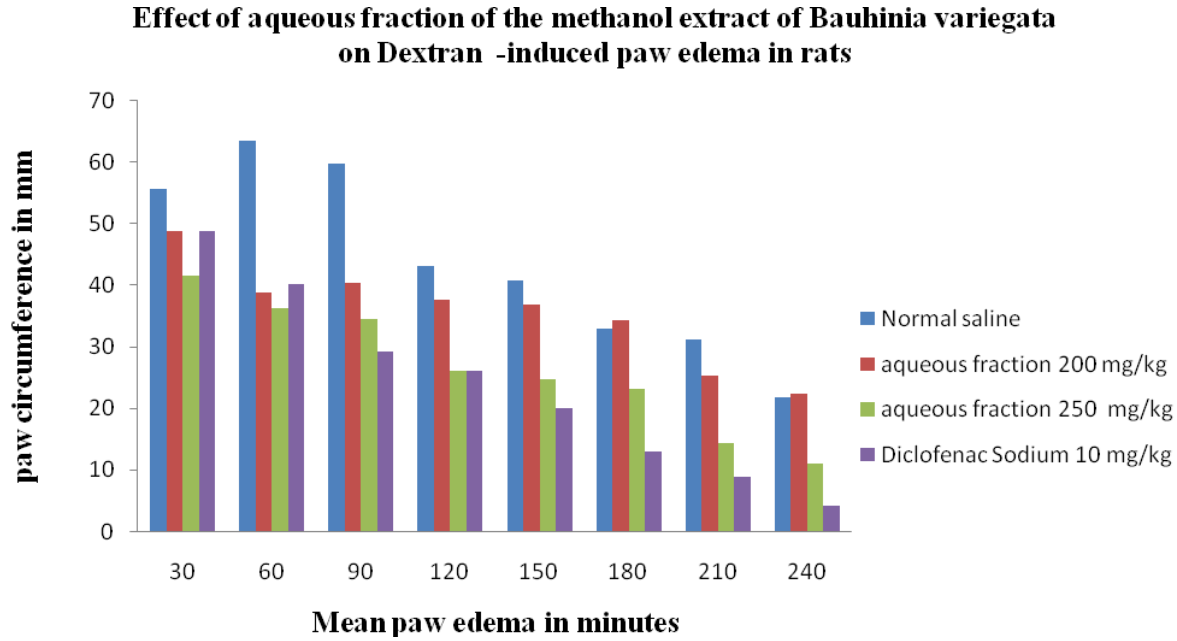
Graph 1: Effect of methanol extract of *Bauhinia Variegata* on Carrageenan-induced paw edema in rats



Graph 2: Effect of aqueous fraction of the methanol extract of *Bauhinia Variegata* on carrageenan-induced paw edema in rats



Graph 3: Effect of methanol extract of *Bauhinia variegata* on dextran-induced paw edema in rats



Graph 4: Effect of aqueous fraction of the methanol extract of Bauhinia variegata on Dextran -induced paw edema in rats

CONCLUSION

It can be concluded that both the menthol extract and aqueous fraction of the methanol extract of *Bauhinia variegata* has anti-inflammatory activity against Carrageenan and dextran induced paw edema in rats. These activities may be due to their content of tannins, steroids, flavonoids and carbohydrates.

The aqueous fraction of the methanol showed better activity profile compared to the methanol extract. This study demonstrates the efficacy of *Bauhinia variegata* as an anti-inflammatory agent and also scientifically justifies the use of this plant as an anti-edematous agent. However further study is required to determine the constituents responsible for the anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors are grateful to the principal of MES College of Pharmacy, sonai for their valuable support for the completion of the work.

REFERENCES

1. Manandhar NP. Plants and People of Nepal Timber Press. Oregon. 2002;192:527-6.
2. Kirthikar KR and Basu DB. Indian Medicinal Plants. Vol. 4. 2nd ed. Dehradun: Oriental Enterprises, 2000:1255-7.
3. Pinto, Luciano S, Andrade Neto, Manoel, Bacarin, Marco A et al. Chemical and biochemical characterization of Bauhinia variegata L. seeds. Rev bras eng agríc ambient. 2005;9(3):385-390.
4. Salantino A and Blatt TT. Foliar Flavanoids of nine species of Bauhinia. Revista Brsileira de Botanica. 1999;22:17-20.
5. Panda S and Kar A. Withania Somnifera and Bauhinia variegata in the regulation of circulating thyroid hormone concentrations in female mice. J Ethnopharmacol. 1999;67:233-9.
6. Vijayakumari KP and Siddhuraru. Chemical composition, amino acid content and quality of protein in the legume of Bauhinia variegata. J Sci Food Agr. 1997;73:279-86.
7. Ramachandran, Reena and Joshi BC. Chemical examination of Bauhinia variegata ß owers. Curr Sci. 1967;36:574-5,.
8. Pettit GR, Numata A, Iwamoto C, Usami Y, Yamada T, Ohishi H et al. Isolation and structures of bauhiniastatins 1-4 from Bauhinia variegata. J Nat Prod. 2006;69:323-7.
9. Wagner H, Baldt S and Zgainski EM. Plant drug analysis. Springer Verlag. Berlin/New York: 1984:126-69,291-305.
10. Harborne JB. Phytochemical methods. London: Chapman and Hall Ltd.; 1973:52-105.
11. Stahl E. Thin layer chromatography: A laboratory hand book, Springer International. New York: 1969:206-58.
12. Weithmann KU, Jeske S and Schlotte V. Effect of leflunomide on constitutive and inducible pathways of cellular eicosanoid generation. Agent Actions. 1994;41:164–170.
13. Vinegar R, Trauz JF and Selph JL. Concurrent evaluation and resolution in an acute inß ammatory model of rat carrageenan-induced pleurisy. Proc Soc Exp Biol Med. 1973;143:711-4.
14. Katzung BG. Basic and Clinical pharmacology, 9th Ed adrenocorticosteroid and adrenocortical antagonists. McGraw Hill, London. 2004:641-646.
15. Turner RA. Screening methods in pharmacology. New York: Academic Press. 1965:158.
16. Vinegar R, Schriber W and Hugo R. Biphasic development of Carrageenan induced in rats. Journal of pharmacology and experimental therapeutics. 1969;166:96-103.
17. Essentials of medical pharmacology by KD Tipathi, Jaypee Brothers, 6th Ed. 2009:192-193.
18. Chawla AS, Singh M, Murthy M S, Gupta MP and Singh H. Anti-inflammatory action of ferulic acid and its ester in Carrageenan induced rat paw edema model. Indian journal of experimental biology. 1987;25:187-189.