Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(3): 477-481

ISSN: 0975-4873

Research Article

Evaluation of *In vitro* and *In vivo* Anti-Inflammatory Activity of Aqueous Extract of *Gliricidia sepium* Flowers in Rats

*Kola Phani Kumar[1], Vadite Siva Naik, V.Bhuvan Chandra, R. Lavanya, K. Narendra Kumar, V.Bhagyasree, B.Soumya , Lakshmi Sudeepthi N[2]

[1]*Department of pharmacology, University College of Pharmaceutical sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur , Andhra Pradesh-522 510, India.*
[2] *KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Krishna, Andhra Pradesh-520010, India.*

Available Online: 1st September 2014

ABSTRACT
The present study was aimed for scientific evaluation of the anti-inflammatory activity of aqueous extract of *Gliricidia sepium* linn. (fabaceae) flowers *by in-vitro and in vivo models.*The anti-inflammtory activity of aqueous extract obtained by decoction was evaluated by in vitro HRBC membrane stabilization assay and in vivo carrageenan induced paw edema model in albino wistar rats. Aqueous extract showed dose dependant anti-inflammatory activity in human red blood cell membarane stabilization method at different concentrations (100-500 µg/kg) with a percentage protection of 7.15, 11.25, 22.71, 24.83 and 26.95 compared to standard diclofenac 32.09% at 10 µg/kg. Diclofenac sodium at 10 mg/kg, aqueous extract administered at a dose of 250 and 500 mg/kg p.o. at 1, 3, 6 and 8 hours significantly ($p<0.05$) decreased and increased the volume of paw edema & % protection compared to carrageenan group and dicclofenac, respectively. The aqueous extract has shown a significant ($p<0.05$, $p<0.01$, $p<0.001$) percentage inhibition of paw edema 69.81±2.93 and 78.07±3.19 on 8th hour at 250 and 500mg/kg, respectively.These results provide a scientific basis for the use of the flowers of *Gliricidia sepium* as an anti-inflammatory agent.

Keywords: *Gliricidia sepium*, Carrageenan Anti-inflammatory, Diclofenac.

INTRODUCTION

Inflammation is a fundamental pathological process of an immune system towards tissue damage, tissue malfunction and infection[1].This complex reaction results from the release of local hormones like Prostaglandins, histamine, serotonin and cytokines. These biochemical molecules regulate both homeostatic and pathological reactions such as inflammation, fever, pain [2]. Inflammation is associated with various diseases. To treat this many of anti-inflammatory agents like corticosteroids and NSAIDS are available but these synthetic agents produces adverse effects such as damage to GIT, gastric erosions and in extreme case severe hemorrhage and death [3, 4]. Consequently there is a need for development of new anti-inflammatory agents with minimum side effects. In this context the value of Herbal medicinal plants, herbs and spices is priceless as the treatment is 100% natural with no side effects.

Gliricidia sepium is a medium size, semi deciduous trees, native to central America which grows about 10m [33ft] height and belonging to the family fabaceae [5]. It is a fast-growing, nitrogen-fixing tree used throughout the tropics for the many environmental services. Gliricidia is widely used to provide crop shade for cacao, coffee, and other shade loving crops [6,7]. The tree is also an important source of green manure, fodder, and fuel wood. Several phytochemicals like flavonoids [8], triterpenoid saponins, stigmastanol glucoside, rhamnogalactoside of kaempferol, coumarin, coumaric acid and melilotic acid and 12a-hydroxy retenoids were reported in various parts of the plant and bark, respectively [9]. Furthermore, different parts of the plants are reported to have viz. antimicrobial [10], nematicidal [11], larvicidal [12] antioxidant [4] and it's a folk remedy for rheumatism, headache, fever, wounds, fractures and urticaria etc [13]. *Gliricidia sepium* is a folk remedy for some of inflammation related diseases fractures, gangrene, head-ache, itch, prickly heat, rheumatism, urticaria, and wounds [14], till today there is no scientific report on this plant. Hence, the present study was aimed to investigate the anti-inflammatory activity of *Gliricidia sepium* aqueous extract of flowers.

MATERIALS AND METHODS

Plant collection and identification: Flowers of *Gliricidia sepium* was collected from Acharya Nagarjuna University campus, Guntur district of Andhra Pradesh. The plant was identified, confirmed and authenticated by comparing with voucher specimen available at survey of Medicinal plants and collection unit, Department of Botany.

Preparation of Aqueous Plant Extract: The flowers were cut into small pieces, powdered and the crude drug is boiled directly with distilled water at 60º-70^0 c for 3hourrs. Then it is cooled and filtered. The extract was concentrated under reduced pressure and stored in vacuum desiccators,

*Author for correspondence: Email: phanisurya163@gmail.com

Table 1: *In vitro* anti-inflammatory activity of aqueous extract of *Gliricidia sepium*

Groups	% Hemolysis Mean ± SEM	% protection
Diclofenac 10µg/ml	67.9±0.085	32.09
GSAE 100 µg/ml	92.8±0.0236***	7.15
GSAE 200µg/ml	88.7±0.0731***	11.25
GSAE 300 µg/ml	77.3±0.0853***	22.71
GSAE 400 µg/ml	75.2±0.0873***	24.83
GSAE 500 µg/ml	73.0±0.101***	26.95

*Results are MEAN±SEM, ***P<0.001 [Dunnett's post hoc test] significant when compared with standard Diclofenac.*

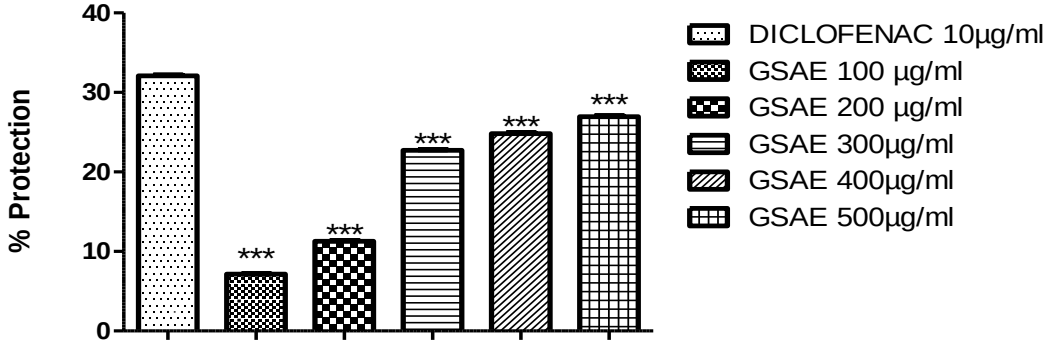

Fig. 1: in vitro anti-inflammatory activity of aqueous extract of Gliricidia sepium and diclofenac.

Table 2: Effect of *Gliricidia sepium* aqueous flower extract on carrageenan induced rat paw oedema volume

S.No.	1h	3h	4h	8h
Control [Saline]	0.48±0.021	0.56±0.017	0.60±0.035	0.71±0.029
Diclofenac 10 mg/kg	0.31±0.017	0.27±0.019	0.22±0.014	0.11±0.01
Aq.extract 250 mg/kg	0.45±0.023***	0.36±0.015*	0.29±0.026ns	0.21±0.014**
Aq.extract 5oo mg/kg	0.37±0.03*	0.31±0.028ns	0.25±0.032ns	0.16±0.023ns

*Results are MEAN±SEM, [n=6] *P < 0.05, **P<0.01 and ***P<0.001 [Dunnett's post hoc test] significant when compared with standard Diclofenac.*

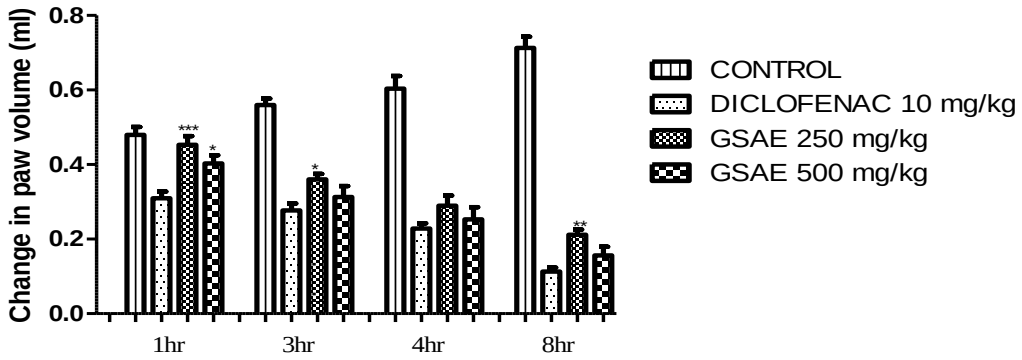

Fig. 2: Effect of Gliricidia sepium aqueous flower extract on carrageenan induced rat paw volume
*Results are MEAN±SEM, [n=6] *P < 0.05, **P<0.01 and ***P<0.001 [Dunnett's post hoc test] significant when compared with standard Diclofenac*

which was used for *in-vitro* and *in-vivo* anti-inflammatory investigations.

Phytochemical Screening: Preliminary qualitative phytochemical screening of *Gliricidia sepium* flower extract shows a positive test for carbohydrates [Benedict's test and Barfoed's test], proteins [Millons test], flavonoids [Shinoda test], saponins [froth formation test] and Tannins [color reaction with ferrous chloride].

Acute Toxicity [LD50] Study: Acute oral toxicity of was studied as per OECD guidelines 425 using albino Swiss mice. The extract was found to be safe up to 2000 mg/kg body weight.

Pharmacological Evaluation-

In vitro Anti-inflammatory activity-

HRBC membrane stabilization assay [15, 16]: Fresh human blood was collected, centrifuged and prepared 10% v/v

Table 3: % protection of aqueous extract of *Gliricidia sepium* flowers against carrageenan induced paw oedems

S.No.	1h	3h	4h	8h
Diclofenac10 mg/kg	33.66±4.37	50.21±4.06	61.73±2.75	84.04±1.54
Aq.extract 250 mg/kg	5.66±1.21***	35.12±4.ns	51.06±5.90ns	69.81±2.93**
Aq.extract 5oo mg/kg	14.81±3.3**	43.15±5.51ns	57.62±6.07ns	78.07±3.19ns

*Results are MEAN±SEM, [n=6], *P < 0.05, **P<0.01, ***P<0.001 [Dunnett's post hoc test] significant when compared with standard Diclofenac.*

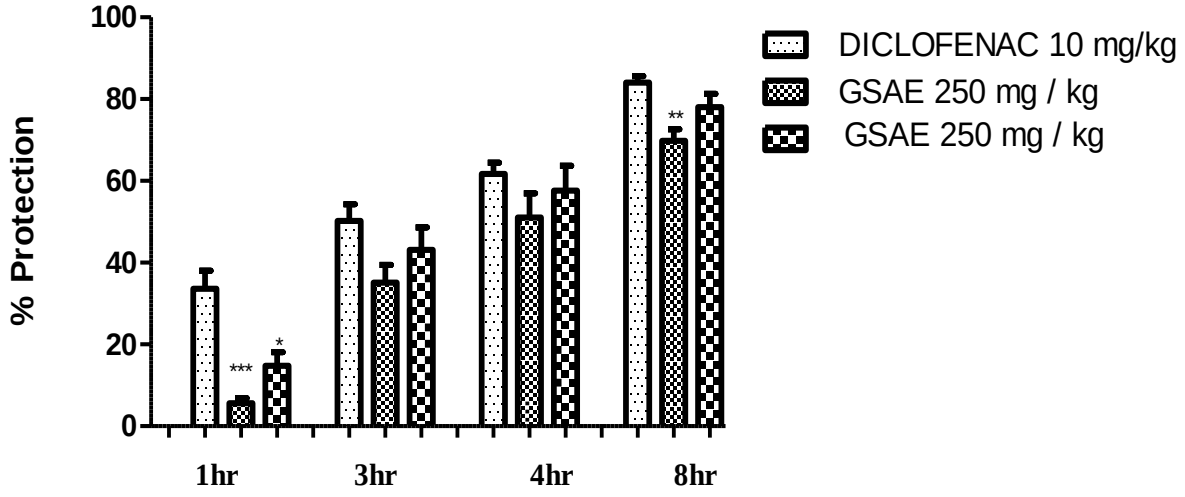

Fig. 3: % protection of Gliricidia sepium extract on carrageenan induced paw oedema
*Results are MEAN±SEM, [n=6], *P < 0.05, **P<0.01, ***P<0.001 [Dunnett's post hoc test] significant when compared with standard Diclofenac.*

suspension with normal saline. The reaction mixture consists of total volume 4.5ml i.e. 1ml of different concentrations of extract [100, 200, 300, 400, 500 µg/ml], 2ml of 0.25% NaCl, 1ml of 0.15M phosphate buffer [pH 7.4] and 0.5ml 10% HRBC 10% v/v was added. Diclofenac used as standard. The mixtures were incubated at 56^0C for 30 minutes and centrifuged at 3000rpm for 20 minutes. The supernatant solution was collected and subjected to spectrophotometric analysis at 560 nm. Percentage membrane stabilization was calculated.

% Hemolysis = [Absorbance of Test sample / absorbance of Control] X 100

% Membrane Stabilisation = 100 – [[Absorbance of Test sample / Absorbance of Control] X100]

In vivo anti-inflammatory activity

Experimental Animals: Albino wistar rats weighing 150-250g was procured from Biogen, Bangalore.. Animals were maintained under controlled condition of temperature at 27º ± 2º C and 12-h light-dark cycles. They were housed in polypropylene cages and had a free access to standard pellets [Amruth] and water *ad libitum*. All the animal studies were conducted according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals [Reg No: 1725/GO/a/13/CPCSEA], Govt. of India.

In-vivo anti-inflammatory activity [17,18, 19]: Animals were divided into 4 groups of 6 animals each. Before 1h of carrageenan injection into rat paw Group I received saline, Group II received Diclofenac 10 mg/kg, Group III received GSAE 250 mg/kg and group IV received GSAE 500 mg/kg [Group IV].

Before carrageenan injection, the paw volumes for each rat were measured separately by water plethysmometer (INCO INDIA PVT LTD). Edema caused by carrageenan was measured at 0, 1, 3, 4 and 8 hours. The anti-inflammatory potency of the extract was determined by comparing its effect with standard diclofenac sodium. Then percent inhibition of edema was calculated for each group with respect to the control group as follows.

Percentage of inhibition of paw edema =

[Vc – Vt / Vc] × 100

Where Vc and Vt represent average paw volume of control and drug treated animals respectively.

Statistical Analysis: Results were analyzed by using one-way ANOVA followed by post hoc Dunnett's test using Graph pad Prism-5 v software. The results were expressed as Mean ± SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001 was considered as significant.

RESULTS

The aqueous extract of the *Gliricidia sepium* was studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. The plant extract showed dose dependant anti-inflammatory activity and % protection of HRBC in hypotonic solution. Results were compared with standard diclofenac [Table &Figure 1].

Aqueous extract of flowers 250 & 500 mg/kg and standard drug were tested for anti inflammatory activity at different hours in carrageenan induced paw edema model using water plethysmometer. Diclofenac sodium at 10 mg/kg, Aqueous extract administered at a dose of 250 and 500 mg/kg p.o. at 1, 3, 6 and 8 hours significantly[**P*<0.05,

**P<0.01, ***P<0.001] decreased and increased the volume of paw edema & % protection compared to carrageenan group and diclofenac respectively. Aqueous extract at 250 p.o. at 1, 3, 4 and 8 hours prevented the carrageenan induced paw edema with a percentage inhibition of 5.66±1.21, 35.12±4.36, 51.06±5.90, 69.81±2.93 at 1, 3, 4, 8 hour, respectively., while 14.81±3.37, 43.15±5.51, 57.62±6.07, 78.07±3.19 at dose of 500 mg/kg p.o. at 1, 3, 4, 8 hour, respectively. Diclofenac sodium decreased the carrageenan induced paw edema with a percentage inhibition of 33.66±4.37, 50.21±4.06, 61.73±2.75, 84.04±1.54 at 1, 3, 4, 8 hour, respectively [Table 1,2 & Figure 1,2].

DISCUSSION

Inflammation is the common problem in all the age groups irrespective of gender and the use of NSAIDS to treat inflammation produces vascular and gastrointestinal complications.Anti-inflammatory drugs available in the market produces symptomatic relief with adverse effects. Now a day's medicinal plants and their formulations are used for various disorders in ethno medical practices as well in the traditional system of medicine in India. Furthermore, these natural remedies can be used to cure problems rather than just mask symptoms [20]. Herbal medicines importance is increased in treatment of chronic and acute diseases with free of adverse effects. *Solanum trilobatum* [21], *Plumeria acuminate* [22], *Thesium chinense*, Mexican medicinal plant extracts [23] and Crude Saponin Extracts [24] also showed the anti-inflammatory effect in carrageenan induced paw edema.

In the present study the plant extract exhibited dose dependant membrane stabilization effect by inhibiting hypotonicity induced lyses of RBC membrane in *in vitro* assay. The RBC membrane is analogous to the lysosomal membrane and its stabilization indicates lysosomal membrane stabilization. It plays a vital role in reduction of pathological mechanisms invovlved in inflammation by augmenting the release of activated neutrophil, bactericidal enzymes and proteases, which produce further tissue inflammation and damage [25].

Carrageenan induced inflammation is the most sensitive and reliable model for evaluating acute phase and orally active anti-inflammatory agents. The time course of increase in paw edema is represented as biphasic event. Development of carrageenan induced inflammation during 1 hour is due to the release of histamine and serotonin [26-28] along with trauma of injection, where as the second phase and third phase is attributed to the release of kinin like substances and prostaglandins, respectively [29-31] [Cyclooxygenase - 2], protease and lysosome. Mediators like histamine, serotonin and COX-2 released prostaglandins increases the vasodilatation, hyperemia, pain and edema. Table 4 & 5 represent the *in vivo* anti-inflammatory activity of extract. Significant [*P<0.05, P**<0.01,***P<0.001] anti-inflammatory activity was observed at 4th and 8th hours compared to standard reference diclofenac. Aqueous extract showed dose dependant anti-inflammatory activity at third phase of carrageenan induced paw edema, with maximum anti-inflammatory activity at 500 mg/kg.

In conclusion, extract showed anti-inflammatory activity in later phases in dose dependent manner and the inhibitory effect of aqueous extract may be due to the inhibition of cyclooxygenase induced prostaglandin synthesis and neutrophil mobilization. This anti-inflammatory effect of the extract observed might be due to the presence of flavonoids and saponins in the plant. The present investigation has also opened avenues for further research especially with reference to the isolation and development of potent phytomedicines from this extract for treatment of inflammation.

REFERENCES

1. Ashley NT, Weil ZM, and Nelson RJ. Inflamation: mechanisms, Costs, and Natural Variation. Annu.Rev. Ecol. Evol.Syst. 2012; 43: 385-406.
2. Miller MJ, Ahmed S, Bobrowski P, Haqqi TM. The chondroprotective actions of a natural product are associated with the activation of IGF-1 production by human chondrocytes despite the presence of IL-1b. BMC Complement Altern Med. 2006; 6:13.
3. Almekinders LC. Anti-inflammatory treatment of muscular injuries in sport. An update of recent studies. Sports Med. 1999; 28:383–388.
4. Akharaiyi FC, Boboye B and Adetuyi FC. Antibacterial, Phytochemical and Antioxidant Activities of the Leaf Extracts of *Gliricidia sepium* and Spathodea campanulata. World Applied Sciences Journal. 2012; 16 [4]: 523-530.
5. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008; 454:428-435.
6. Chadhokar PA. *Gliricidia maculate*, Apronising legume forage plant. World Animal Review. 2010; 44: 36-43.
7. Csurhes S. And Edwards. Potential environmental weeds in australia; candidate species for preventative control. Queens land department of natural resources. 1998; 164-168.
8. Rastrelli L, Berger I, Kubelka W, Caceres A, Tommasi DN, Simone DF. New 12a-Hydroxyrotenoids from *Gliricidia sepium* bark. J Nat prod.1999; 62 [1]:188-190
9. Beena Jose, And Joji Reddy L. Evaluation Of Antibacterial Activity of The Leaf And Flower Essential Oils of *Gliricidia sepium* From South India. International Journal of Applied Pharmaceutics. 2010; 2[3]:177-179.
10. Nazli R, Sohail T, Nawab B And Yaqeen Z. Antimicrobial property of *Gliricidia sepium* plant extract.Pakisthan J. agric. Res. 2011;24 : 1-4.
11. Nazli R, Akhter M, Ambreen S, Solangi AH and Sultana N. Insecticidal, Nematicidal and antibacterial activities of *Gliricidia sepium*, Pak. J. Bot. 2008; 40[6]: 2626-2629.
12. Kaliyamoorthy Krishnappaa, Shanmugam Dhanasekarana, Kuppusamy Elumala. Larvicidal, ovicidal and pupicidal activities of *Gliricidia sepium* [Jacq.] [Leguminosae] against the malarial vector,

Anopheles stephensi Liston [Culicidae: Diptera] *Phytochemistry*: Asian Pacific Journal of Tropical Medicine. 2012; 5[8]:598–604.
13. Manners GD, Jurd L. Additional Flavonoids in *Gliricidia sepium*. Phytochemistry, 1979;18(6):1037-1042.
14. Abulude, F.O, and Adebote V.T. Antibacterial Investigation Of Crude Extracts Of The Root Bark Of *Gliricidia Sepium*. Continental J. Microbiology. 2009; 3: 23 – 26.
15. Chatterjee S, Das SN. Anti-arthritic and Anti-inflammatory effect of a poly-herbal drug [EASE@]: its mechanisam of action. Indian.J.Pharmacology. 1996; 28:166-119.
16. Rajurkar R, Jain R, Matake N, Aswar P, Khadbadi SS. Ati-inflammatory Action of *Abutilon indicum*[L] Sweet Leaves by HRBC Membrane Stabilization. Res J. Pharm. Tech. 2009; 2[2]:415-416
17. Mohamed F.Zayed, Memy H. Hassan. Syntesis and biological evaluation studies of novel quinazoline derivatives as anti-bacterial and anti inflammatory agents. Saudi Phar J 2014; 22:157-162.
18. Alam K, Pathak D, Ansari SH. Evaluation of anti - inflammatory activity of Adhatoda Zeylanica [MEDIC.] leaves extract. International Journal of Pharma and Bio Sciences. 2011; 2[1]:157-162.
19. Igbe I, Ching FP, Eromon A. Anti-inflammatory activity of aqueous fruit pulp extract of Hunteria umbellata K. Schum in acute and chronic inflammation. Acta Pol Pharm. 2010; 67[1]:81-85.
20. Mukherjee PK, Venkatesh P and Ponnusankar S. Ethnopharmacology and integrative medicine – Let the history tell the future. J Ayurveda Integr Med. 2010; 1[2]: 100–109.
21. Ravi V, Saleem TSM, Maiti PP, Gauthaman K and Ramamurthy J. Phytochemical and pharmacological evaluation of Solanum nigrum Linn. *African Journal of Pharmacy and Pharmacology*. 2009; 3[9]: 454-457.
22. Gupta M, Mazumder UK, Gomathi P, Thamilselvan V. Antiinflammatory evaluation of leaves of Plumeria acuminate. BMC Complementary and alternative medicine [Internet]. 2006; 36:1472-6882.
23. Meckes M, David-Rivera AD, Nava-Aguilar V, Jimenez A. Activity of some Mexican medicinal plant extracts on carrageenan-induced rat paw ; edema. *Phytomedicine*. 2004; 11[5]:446-51.
24. Halimatu S.H, Muhammad I.S, Muhammad A.M,Andrew A.E, Hajara I, Ali S.H, and Abdullahi H.Y. Analgesic and anti-inflammatory activities of the saponins extract of Carissa edulis root in rodents. Int. J. Biol. 2014; 4[4]: 1310-1317.
25. Govindappa M, Nagasravya S, Poojashri MN, Sadananda TS, Chandrappa CP, Gustavo S, Sharanappa P and Anil Kumar NV. Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of water extract of Wedelia trilobata[L.] Hitchc. Journal of medicinal plants research. 2011; 5[24]:5718-5729.
26. Sarika A, Purabi R, Vinod S, Avnish K, Rambir S, and Poonam S. Anti-Inflammatory Activity of Lactobacillus on Carrageenan-Induced Paw Edema inMaleWistar Rats International Journal of Inflammation, Volume [2012], Article ID 752015, 6 pages, doi:10.1155/2012/752015.
27. Anuja GI, Latha PG, Shine VJ, Suja SR, Shikha P, Satheesh Kumar K, and Rajasekharan S. Antiedematous and Analgesic Properties of Fertile Fronds of Drynaria quercifolia. Hindawi Publishing Corporation ISRN Inflammation. [2014]Article ID 302089.
28. Anuradha M, Kushwha P, Murthy N.Evaluation of diclofenac potassium microsphere for anti-inflammatory Activity. Asian journal of pharmaceutical and clinical research. 2012; 5[2]:19-22.
29. Nayak BS, Patel KN. Anti-Inflammatory Screening Of Jatropha Curcas Root, Stem And Leaf In Albino Rats. Rom. J. Biol. – plant biol.2010; 55: 9-13.
30. Rajendran V and Lakshmi KS. In vitro and in vivo anti-inflammatory activity of leaves of Symplocos cochinchnensis [Lour] Moore ssp laurina. Bangladesh Journal of Pharmacology. 2008; 3:121-124.
31. Valiollah H, Hossein S, Mohsen M. Effect of Fluvoxamine on Carrageenan-Induced Paw Edema in Rats Evaluation of the Action Sites. Services. Iranian Journal of Pharmaceutical Research 2011; 10 [3]: 611-618.