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EVALUATION OF ANTI INFLAMMATORY AND ANALGESIC ACTIVITIES OF THE EXTRACT PREPARED FROM *ALOYSIA POLYSTACHYA* IN EXPERIMENTAL ANIM ALS

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ABSTRACT

Aloysia polystachya used as an appetite suppressant herb for millennia. It also has antioxidant, ant diabetic, and nootropic actions. It is proved that it is a natural anti obesogenic agent and is widely consumed in India. Its actions like anti-atherosclerotic is of high medicinal value. The phytochemical screening of extract shows the presence of alkaloids, phytosterols, phenolic compunds and tannins using various methods. In the present work an attempt has been made to evaluate the anti inflammatory, analgesic activities of ethanolic extract of *aloysia polystachya* (100mg/kg, 200mg/kg) and the results were found to be positive. The results were compared with the standard drug indomethacin (10mg/kg), pentazocin (10mg/kg) and aspirin (10mg/kg). Hence, *aloysia polystachya* contains anti inflammatory and analgesic activity. The present work was done to demonstrate the anti inflammatory and analgesic activity of the ethnolic extract obtained from the leaves of *aloysia polystachya* (verbenaceae). Inflammation was induced by carrageenan induced paw edema and pain was induced by eddy's hot plate and tail flick method. Thermal and radiant heat is used in hot plate and tail flick method respectively.

KEYWORDS: *Aloysia polystachya*, analgesic, anti inflammatory activity.

1. INTRODUCTION

Pain is the most common reason for physician consultation. It is a major symptom in many medical conditions. It can significantly interfere with a person's quality of life and general functioning.[1] It is a part of the body's defence system, producing are flexiveretraction from the painful stimulus, and tendencies to protect the affected body part while it heals, and avoid that harmful situation in the future.[2,3] Pain is the most common reason for using complementary and alternative medicine.[4,5] Pain is primarily managed with analgesics. Opioid analgesics are commonly used for treatment of pain. Although opioids are strong analgesics, there are other drugs used for the treatment of pain.

Inflammation is the body's immediate response to damage to its tissues and cells by pathogens, noxious stimuli such as chemicals, or physical injury.[6] It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation can be classified as either acute or chronic status depending on onset time. Acute inflammation is the primary response of the body to injurious stimuli and it involves the local vascular and immune response. On the other hand, chronic inflammation is a pathological condition characterized by progressive destruction and recovery of the injured tissue from the inflammatory response.[7]

Though a variety of chemical mediators or signalling molecules such as histamine, serotonin, leukotrienes, prostaglandins are involved in the inflammatory response the mechanism of inflammation injury is attributed to release of ROS (reactive oxygen species) from activated neutrophils and macrophages. The over production of ROS by macrophages causes oxidative damage to membrane lipids, DNA, proteins and lipoproteins.[8] In addition, ROS propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor and interferon which stimulate recruitment of additional neutrophils and macrophages. Further ROS activates Nuclear Factor ƙ-β(NF-ƙ-β) which regulates various cellular genes involved in immune and acute phase inflammatory responses and in cell survival. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation.

2. MATERIAL AND METHODS

2.1 Material

Drugs and chemicals

1. Pentazocine inj. (Ranbaxy laboratories limited).

2. Indomethacin capsules (Ranbaxy laboratories limited).
3. Aspirin (Ranbaxy laboratories limited).

All Other chemicals used for this investigation were of analytical grade from S.D Fine chemicals, Mumbai, India.

Plant sample collection
The whole plant (leaves) of *aloysia polystachya* will be collected and will be authenticated by dr k madhava chetty, department of botany, Sri Venkateswara University, tirupathy.

Extraction of Plant Material
The plant leaves are sun dried for 2 weeks then grinded in to a coarse powder with the help of a mixer (remi motor grinder-rm-200).

2.2 Hot Continuous Extraction (Soxhlet)
In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the soxhlet apparatus. The extracting solvent in flask A is heated, and its vapours condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A.

This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

Evaporation of Solvent
The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vaccum dissecator for 7 days.

2.3 ACUTE TOXICITY STUDIES:
The acute oral toxicity test of the extract was determined prior to the experimentation on animals according to the OECD (Organisation for Economic Co-operation and Development) guidelines no. 423. Albino mice (25-30g) were taken for the study and dosed once with 2000 mg/kg. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing LD50 dose as 2000 mg/kg. Therefore therapeutic dose was calculated as $1/10^{th}$ and $1/20^{th}$ie; 100 mg/kg and 200 mg/kg of the dose were chosen for subsequent experimentation.

2.4 PHYTOCHEMICAL SCREENING
The extract where subjected to preliminary phytochemical screening for possible presence of bioactive anti inflammatory and analgesic compounds.

Table1: Phytochemical screening method.

Test	EEAP
Alkaloids	+
Carbohydrates and Glycosides	-
phytosterols	+
Fixed oils and fats	-
Phenolic componds and tannins	+
Proteins and amino acids	-

2.5 Experimental Animals and Housing of Animals
Albino mice weighing 25-30 g of either sex were used for the study in different models. The animals were procured from National institute of Nutrition (Hyderabad) at least 2 weeks prior to the study, so that animals could acclimatize to the new environment.

Animals were kept in well-maintained room under standard hygienic conditions. Commercial pellet diet and water were made available *ad libitum*. They were housed in propylene cages (32 x 24 x 16 cm) with stainless steel grill top, bedded with rice husk.

2.6 Screenings of anti-inflammatory activity
Carageenan induced hind paw edema in mice: Albino mice weighing between 25-30gms were divided into 5 groups of 6 mice each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

Grouping
Group 1: Animals were administered 0.1ml saline p.o.
Group 2: Animals served as disease control (carrageenan induced).
Group 3: Animals were administered standard (Indomethacin 10 mg/kg) p.o.
Group 4: Animals were administered *Aloysia polystachya*(100 mg/kg) p.o.
Group 5: Animals were administered *Aloysia polystachya*(100 mg/kg dose) p.o.

Procedure: All mice of II, III, IV & V (except I group) groups were injected with 0.1ml of carageenan (1%) in normal saline into sub planter area of right hind paw. All the drugs were given orally 1hr prior to carageenan injection.

Paw volume was measured by mercury plethysmograph at 0, 1, 2, 3, 6, hrs after the carageenan injection.

2.7 Screening of analgesic activity

A. Eddy's hot plate method

Albino mice weighing between 25-30gms were divided into 4 groups of 6 mice each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

Grouping

Group 1: Animals were administered 0.1ml saline p.o.

Group 2: Animals were administered standard reference Pentazocine (10 mg/kg)i.p.

Group 3: Animals were administered *Aloysia polystachya*(100 mg/kg) p.o.

Group 4: Animals were administered *Aloysia polystachya*(200 mg/kg) p.o.

Procedure: The time for licking paws or jumping in hot plate was recorded as response, prior and 0, 30, 60, 90,120 min after administration of respective drugs.

B.Tail flick method

Grouping

Group 1: Animals were administered 0.1ml saline p.o.

Group 2: Animals were administered standard reference aspirin (10 mg/kg)i.p.

Group 3: Animals were administered *Aloysia polystachya*(100 mg/kg) p.o.

Group 4: Animals were administered *Aloysia polystachya*(200 mg/kg) p.o.

Procedure

The tail flick latency was assessed by analgesiometer (INCO, INDIA).The strength of the current passing through the naked nichrome wire was kept constant at 6 amperes. The distance between the heat source and tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5c.m. measured from the root of tail. The cutoff reaction time was fixed at 10 seconds to avoid tissue damage.

3. RESULTS AND DISCUSSION

3.1 STATISTICAL ANALYSIS

Results were expressed as mean±S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant ($P < 0.05$).

3.2 Carageenan induced paw edema in mice

In carageenan induced paw edema *aloysia polystachya* significantly inhibited the edema in a dose dependent manner as shown in Table.3. The paw volume in normal control group rats on 2ndhr was found to be 0.2148 ± 0.0122ml. The paw volume in mice pretreated with lower dose of *aloysia polystachya* (100 mg/kg/day), higher dose of *aloysia polystachya* (200 mg/kg/day) and indomethacin (10 mg/kg/day) at 2ndhr were found to be 0.191 ± 0.0061 ml, 0.158 ± 0.0042** ml and 0.1369 ± 0.0054** ml.

Table 2: Anti-inflammatory effect of *Aloysia polystachya* on carageenan induced paw edema in rats.

Treatment	Paw volume in ml at different Hrs (Mean + S.E.M.)				
	0	1	2	3	6
Normal Control	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058
Inflammatory control	0.1225 ± 0.0079^{+++}	0.1876 ± 0.007^{+++}	0.2148 ± 0.0122^{+++}	0.2083 ± 0.0094^{+++}	0.165 ± 0.0076^{+++}
Indomethacin 10mg/kg, p.o.	0.1249 ± 0.0061	0.1427 ± 0.0071**	0.1369 ± 0.0054**	0.1442 ± 0.007**	0.1449 ± 0.0060
Aloysia polystachya (100mg/kg)	0.1210 ± 0.0186	0.152 ± 0.008	0.191 ± 0.0061	0.196 ± 0.006	0.159 ± 0.009*
Aloysia polystachya (200mg/kg)	0.1016 ± 0.0070	0.132± 0.0057**	0.158 ± 0.0042**	0.1542 ± 0.0071**	0.1542 ± 0.0136

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test.

+++ P<0.001 Vs Normal control &** P< 0.01 Vs Inflammatory Control.

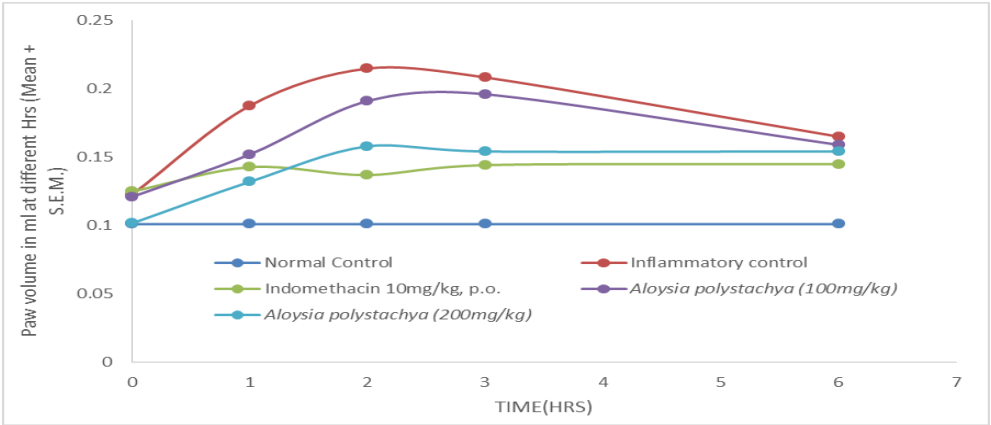

Figure 1: Anti-inflammatory effect of *Aloysiapolystachya* on carrageenan induced paw edema in mice.

3.3 Analgesic activity

Eddy's hot plate

Aloysia polystachya showed maximum analgesic activity at 60, 90 min for 100 and 200mg/kg dose. The reaction time in normal control group at 60, 90 min was found to be 3.52±0.002, 4.08±0.161 sec. The reaction time (paw licking / jumping response) in mice pretreated with lower dose of *Aloysia polystachya* (100mg/kg), higher dose of *Aloysia polystachya* (200mg/kg/day) and Pentazocine(10 mg/kg) at 60, 90 min were found to be 9.26±0.851, 7.16 ± 0.193, 9.82 ± 0.894 and 8.60 ± 0.992, 9.12 ±0.372, 14.12±3.182 respectively when compared to control group mice.

The duration of analgesic effect was more in 200 mg/kg compared to 100 mg/kg and reference drug pentazocine at 10 mg/kg dose significantly increased the reaction time at 90 minutes as shown in Table 2.

Table 3: Effect of *Aloysia polystachya* on reaction time (sec) in Eddy's hot plate.

Treatment	Reaction time in seconds				
	0	30	60	90	120
Control	3.51 ± 0.277	3.80 ± 0.343	3.52 ± 0.455	4.08 ± 0.161	3.93± 0.067
Pentazocine (10mg/kg)	4.11 ± 0.238	6.64 ± 0.430**	9.82 ± 0.894**	14.12 ± 3.182**	9.41± 0.650**
Aloysia polystachya (100mg/kg)	4.02 ± 0.194	5.01 ± 0.332	9.26 ± 0.851**	8.60 ± 0.992	6.30± 0.259**
Aloysia polystachya (200mg/kg)	3.81 ± 0.230	7.09 ± 0.523**	7.16 ± 0.193**	9.12 ± 0.372	8.21± 0.671**

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test.
** P< 0.001 Vs control, * P< 0.05 Vs control.

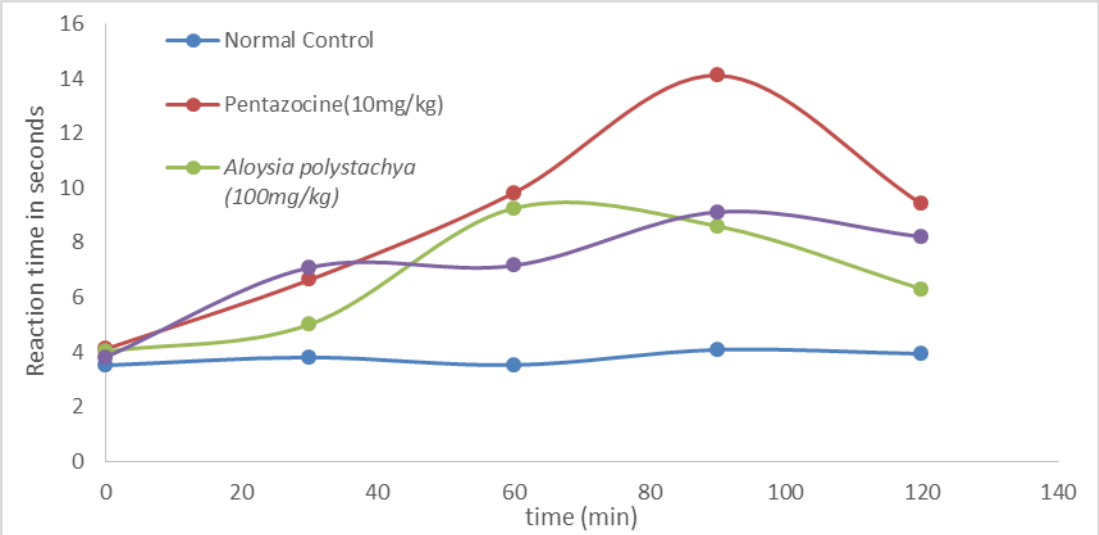

Figure 2: Effect of*Aloysia polystachya* on reaction time (sec) in Eddy's hot plate method.

Tail flick method

Table 3: Mean TFL of Tail flick method at Various Time Interval.

Treatment	DOSE Mg/kg	MEANBASAL TIME (Seconds)	MEAN RESPONSE OF TAIL FLICK METHOD AT VARIOUS TIME INTERVAL(in minutes)					
			30	60	90	120	180	240
Distilled water	0.5ml	4.19±0.029	3.76±0.041	4.68±0.046	4.77±0.041	4.77±0.035	4.23±0.047	4.07±0.037
Asprin	10	4.29±0.045	7.88±0.042	8.41±0.042	9.47±0.046	9.47±0.036	10.68±0.037	9.94±0.041
Aloysia polystachya (100mg/kg)	100	4.52±0.057	4.41±0.010	4.52±0.034	5.32±0.013	5.77±0.020	5.52±0.022	5.38±0.041
Aloysia polystachya (200mg/kg)	200	4.21±0.016	4.79±0.016	4.89±0.005	5.48±0.025	6.82±0.012	6.16±0.010	0.07±0.035

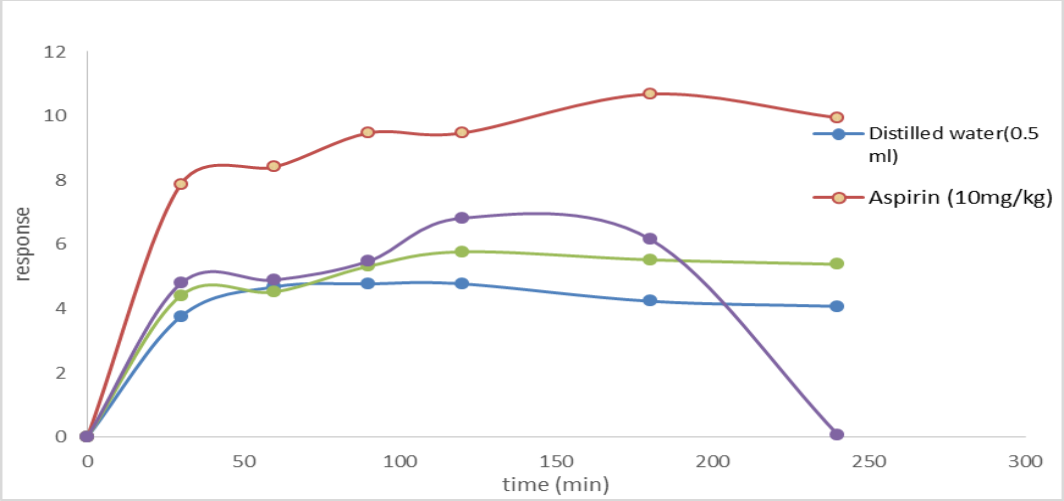

Figure 3: Mean TFL of Tail flick method at Various Time Interval.

3.4 DISCUSSIONS

The present study was done about anti inflammatory and analgesic activity of ethanolic extract of *aloysia polystachya*. Inflammation was induced by carragenan induced paw edema and pain was induced by eddy's hot plate and tail flick method.

The physical appearance of the ethanolic extract of *aloysia polystachya* was greenish black with semisolid and non sticky in nature.

The phytochemical investigation revealed the presence of significant phytoconstituents in the extract such asalkaloids, phytosterols, phenolic compunds and tannins for ethanolic extract of *aloysia polystachya*.

Carragenan paw edema method was used to determine anti inflammatory activity of the extract by using 0.1 ml of 1 % w/v carrageenan was injected into the sub plantar tissue of right hind paw of each mice.

Carragenan inuced paw edema is a commonly used primary test for the screening of new anti inflammatory agents and is believed to be biphasic. The first phase (1-2h) is due to the release of histamine or serotonin,bradykinin, and to a less extent prostaglandins produced by cyclooxygenase enzymes (COX) and the second phase of edema is due to the release of prostaglandins.[9] Release of the neutrophil-derived free radicals, nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), and interleukin-1 β (IL-1 β) also involved in the delayed phase of carrageenan-induced acute inflammation.[10]

The results of this study indicate that the extract of *aloysia polystachya* (100mg/kg,200mg/kg) significantly reduces carragenan induced paw edema in mice. Therefore, the mechanism of action by inhibition of histamine, serotonin or prostaglandins synthesis.The results were compared with the standard drug indomethacin (10mg/kg).

This study also involves the investigation of the anti nociceptive property of the extract of *aloysia polystachya* by thermal models of nociception- eddys hot plate method and tail flick method. These models are based on polysynaptic reflexes intiated at the spinal level and modulated from supraspinal centers. Pro nociceptive mediators activate primary afferent neurons directly or indirectly to enchance nociceptive signal transmission to the CNS excitation of primary afferent by peripherally originating mediators so called "peripheral sensitization".

Peripheral sensitization is the synthesis of ATP, glutamate, kinins, cytokinins and tropic factors. These reflexes are generated due to the application of heat, cold, mechanical and electrical stimulus.

Thermal and radiant heat are used in hot plate and tail flick method respectively. In eddy hot plate the duration of analgesic effect was more in 200 mg/kg compared to 100 mg/kg and reference drug pentazocine at 10 mg/kg dose significantly increased the reaction time at 90 minute and in tail flick method analgesic effect was observed at 100mg/kg when compared with the standard drug asprin (10mg/kg).

CONCLUSION

The findings in this study suggest that the *aloysia polystachya* possess anti- inflammatory and analgesic activity. The results have been obtained in carefully controlled experiments with laboratory animals where psychological factors can presumably be ruled out. In all the tests the responses have been assessed by actual measurement and not by subjective comparisons which may be influenced by the observer. Therefore the statistical validity of the findings has been proved and they provide a scientific foundation for the use of the biologically active ingredients of *aloysia polystachya* in inflammatory and pain conditions and explain the clinical effectiveness of the *aloysia polystachya*.

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REFERENCES

1. maríainésaguadoa*, néstorhugodudika, cristinamariselpérezzamoraa,b, carolaanalíatorresa,b, maríabeatriznuñeza, antioxidant and antibacterial activities of hydroalcoholic extracts from aloysiapolystachyagrisebmoldenke and lippiaturbinatagriseb (verbenaceae), international journal of pharmacy and pharmaceutical sciences, 2016; 8(3): 393-395.
2. Edieidia Souza Pina1, Juliana da Silva Coppede1, Adilson Sartoratto2, Ana Lucia Fachin1, Bianca Waleria Bertoni1, Suzelei de Castro França1 and Ana Maria Soares Pereira, Antimicrobial activity and chemical composition of essential oils from Aloysiapolystachya (Griseb.) Moldenke grown in Brazil, https://academicjournals.org/journal/JMPR/article-abstract/A52891921735.
3. FláviaSchürmann da Silva, Ana & de Andrade, Jean & R M Bevilaqua, Lia& Souza, Marcia &Izquierdo, Ivan &Henriques, Amélia&Zuanazzi, José. (2006). Anxiolytic-, antidepressant- and anticonvulsant-like effects of the alkaloid montanine isolated from Hippeastrumvittatum. Pharmacology, biochemistry, and behaviour, 2006; 85: 148-54. 10.1016/j.pbb.2006.07.027.
4. Kamau JK, Nthiga PM, Mwonjoria JK, Ngeranwa JJN, Ngugi MP. Anti-Inflammatory Activity of Methanolic Leaf Extract of Kigelia Africana (Lam.) Benth and Stem Bark Extract of Acacia Hockii De Wild in Mice. J Dev Drugs, 2016; 5: 156.
5. Anand Kale, Yasmeen A. Maniyar, Evaluation of peripheral and central analgesic activity of ethanolic extract of Clerodendruminfortunatum Linn. in experimental animals, http://www.ijbcp.com/index.php/ijbcp/article/view/205
6. 1L. D. IOR, 1S. O. Otimenyin, 2M. Umar, Anti-Inflammatory and Analgesic Activities of the Ethanolic Extract of the Leaf of SyzygiumGuineense in Rats and Mice, IOSR Journal of Pharmacy ISSN: 2250-3013, www.iosrphr.org, July-August 2012; 2(4): 33-36.
7. Manjit Singh, Vijender Kumar, Ishpinder Singh,1Vinod Gauttam,2 and AjudhiaNath Kalia2, Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. Leaves, Pharmacognosy Res, 2010 Nov-Dec; 2(6): 364–367.
8. AnnamalaiPandurangana*, RatanLalKhosaa and Siva Hemalathab, Evaluation of Anti-inflammatory and Analgesic Activity of Root Extract of SolanumTrilobatum Linn, Iranian Journal of Pharmaceutical Research, 2008; 7(3): 217-221.
9. Gilligan JP, Lovato SJ, Erion MD, Jeng AY. Modulation of carrageenan-induced hind paw edema by substance P. Inflammation, 1994; 18: 285–92. [PubMed] [Google Scholar]
10. Halici Z, Dengiz GO, Odabasoglu F, Suleyman H, Cadirci E, Halici M. Amiodarone has anti-inflammatory and anti-oxidative properties: An experimental study in rats with carrageenan-induced paw edema. Eur J Pharmacol, 2007; 566: 215–21. [PubMed] [GoogleScholar]