Research Article

Anti-Inflammatory Activity of *Murraya exotica* Linn against Cotton Pellet induced Granuloma in Wistar Rats

M. N. L. Aishwarya[1], P. Mounika[1], J. Mounika[1], M. Shankar[*1], G. Mallikarjuna[2], M. Niranjan Babu[3], N. Nagaraju[4]
[1]Department of Pharmaceutical Chemistry, Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh, India.
[2]Department of Pharmacology, Seven Hills College of Pharmacy, Tirupati, Tirupati, Andhra Pradesh, India.
[3]Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, Tirupati, Andhra Pradesh, India.
[4]Department of Microbiology, Sri Venkateswara Arts College (UG & PG), Tirupati, Andhra Pradesh, India.
***Corresponding author's E-mail:** shankarmanichellappa@gmail.com

Received: 10-03-2018; **Revised:** 28-04-2018; **Accepted:** 12-04-2018.

ABSTRACT

Traditional medicine usage has gain importance in management of various disorders. The present study was aimed at evaluating anti-inflammatory activity of hydroalcoholic extract of Murraya exotica in cotton pellet induced granuloma in wistar rats. The cotton pellets weighing 50 mg were sterilised in an autoclave for 30min. at 1200 C under 15 lb pressures. Each pellet was implanted subcutaneously in lumbar region. The animals were treated with Aceclofenac (50mg/kg b.wt) and hydroalcoholic extract of Murraya exotica at 200mg/kg b.wt and 400mg/kg b.wt for 7 days. On 8th day the pellets were weighed for wet weight and dry weight and calculated for granuloma tissue formation and exudate inhibition. Haemotological parameters RBC & WBC count was determined. The extract at both dose levels significantly suppressed the granuloma formation and exudate inhibition as evidenced by restoration of altered haematological parameters reporting anti inflammatory activity.

Keywords: Anti-Inflammatory activity, Murraya exotica Linn, Aceclofenac, Granuloma tissue.

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction to prevent the spread of injurious agent and to remove the necrosed cells and tissues[1]. It helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli[2]. Inflammation may be caused by bacteria, viruses, fungi, parasites, antigen-antibody reaction, Mechanical trauma, organic and inorganic poisons and foreign bodies. Inflammatory responses occur in three different phases, each apparently mediated by different mechanisms: An acute transient phase characterised by local vasodilation and increased capillary permeability. A subacute phase, characterised by infilteration of leukocytes and phagocytic cells. A chronic proliferative phase, in which tissue degeneration and fibrosis occurs[1.]

A sign of inflammation includes redness, swelling, heat, pain, loss of function. There are two types of inflammation, Acute and Chronic. Acute inflammation is of short duration and represents the early body reaction, resolves quickly and is usually followed by healing. Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at same time[1]. Acute and chronic inflammatory disorders account for most of the painful medical problems encountered all over the world.

The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cycloxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation[3].

Inflammation is the response of living tissues to injury and it involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair. Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research[4]. The most important mechanism of anti-inflammatory action of NSAIDs is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins[5]. Since the currently used anti-inflammatory agents both steroidal and nonsteroidal are prone to evoking serious adverse reactions[4], there is a for the development of newer, more effective and safe anti-inflammatory drugs is necessary[6].

Traditional medicinal plants were used for the treatment of various diseases from many centuries where gained their importance in traditional knowledge periodically[7]. India is known as the Emporium of Medicinal plants due to availability of several thousands of medicinal plants in the different bioclimatic zones. Anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population[8]. Herbal drugs or their extracts are prescribed widely, even when their biologically active compounds are unknown.

Exploration of the chemical constituents of plants and pharmacological screening would provide the basis for developing new lead molecules in strategic favor of Natural product Drug Discovery. This shows the need for planned activity guided pharmacological evaluation of herbal drugs[9] [abt+int.pdf in arthritis] Plant drugs are known to play a vital role in management of inflammatory diseases. The phytoconstituents from plant origin are potential against inflammatory diseases. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with Ayurvedic treatment and extending to the European and other systems of traditional medicines[8].

Murraya is one of the 150 genera from the family Rutaceae. Of the 14 global species belonging to this genus, *Murraya koenigii*, and *Murraya exotica* have been found in India[10]. *Murraya exotica* Linn. Mantiss. (Family: Rutaceae), synonym *Murraya paniculata* (Linn.) Jack locally known as Kamini; Chinese Box in English, is a small evergreen tree with smooth and slender barks having glossy green foliage and large clusters of fragrant flowers. This plant has been used in ethnomedicine. The plants have stimulant and astringent, abortive properties and are used to treat diarrhoea, dysentery, cuts, body aches, venereal diseases. Infusion of the leaves and flowers of M. exotica is tonic and stomachic. It is said to be aromatic, refrigerant, digestive, and beneficial in coughs, giddiness, hysteria, thirst, and burning of the skin[11].

Figure 1: *Murraya exotica* plant

Figure 2: *M.exotica leaves* after collection

MATERIALS AND METHODS

Collection and identification of plant material

The plant Murraya exotica was collected from Tirupati, Seshachalam Hills, Andhra Pradesh. The samples of the plant were identified and authenticated (voucher No.: NN253) by Dr.N.Nagaraju, Asst. Professor in Botany & HEAD, Dept. of Microbiology, Sri Venkateswara Arts College (UG & PG), Tirupati, Andhra Pradesh.

Preparation of Plant Extract

The collected leaves of the plant were separated from undesirable materials. They were dried in open air for 4 weeks as shown in figure 3. The shade dried leaves were ground into a coarse powder with the help of a pulverizer. The powdered drug was subjected to percolation with 70% ethanol using soxhlet apparatus in 1:4 ratio. The extraction was carried out until the solvent becomes colourless. The extract was evaporated on a hot plate at 40^{o}C. The marc of crude drug obtained was set for complete drying in a dessicator using calcium chloride until dried powder of the crude drug was obtained. The powdered form of crude drug was suspended in 1% CMC (Micro Crystalline Cellulose), to produce a concentration of 200mg and 400 mg/kg, b.wt.as low dose & high dose.

Figure 3: *M.exotica* leaves set for shade drying

Figure 4: Marc of crude drug set for drying in desicator

Animal husbandry

Healthy, Adult, Male, Wistar rats weighing 130-150 gms were maintained under standardized conditions with 12-hour light/dark cycle, 24°C and 35 to 60% humidity,

provided free access to pellet diet and purified drinking water *ad libtium*[11]. The experimental protocol was approved by Institutional Animal Ethical Committee (Approval no. 1995/PO/Re/S/17/CPCSEA).

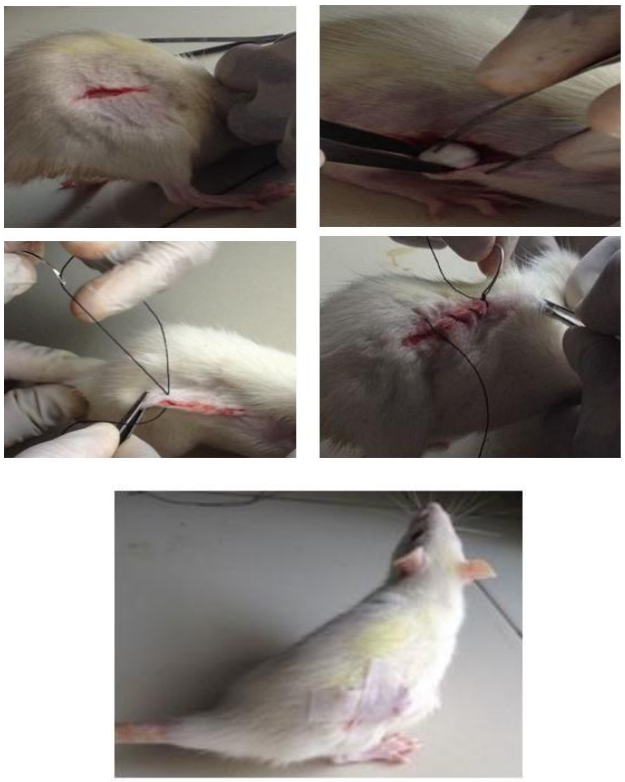


Figure 5-9: Represents implantation of cotton-pellet into lumbar region of rats

Procedure

The cotton pellet-induced granuloma in rats was evaluated as specified by winter and Porter[12]. The cotton pellets weighing 50mg were sterilized in an autoclave for 30 min at 120^{0}C under 15 Ib pressure. One pellet of cotton was implanted subcutaneously (s.c.), on lumbar region of each rat under light ether anaesthesia (Lagishetty and Naik, 2008)[13] and sterile technique after shaving the area and disinfecting it with 70% ethanol[5] as shown in figure 5-9. The animals were divided into four groups each containing 6 animals. Group-I animals received 0.5 ml of Aceclofenac (100 mg/ml p.o.) intraperitoneally (i.p.) served as standard, group- II & III received HAME (200 and 400 mg/ kg, b.wt, p.o.) and group-IV served as vehicle control received 0.5 ml of normal saline p.o. The drugs were given once daily for 7 days. On the 8th day the animals were anaesthetized and the pellets were carefully removed and made free from extraneous tissues.

The pellets were weighed for wet weight and then dried in an incubator at 60^{o}C until a constant dry weight was obtained (all the exudates were dried), after that the dried pellets were weighed again[14]. The weight of exudate in mg was calculated by subtracting constant dry weight of pellet from the immediate wet weight of pellet. Granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet (50 mg) from the constant dry weight of pellet and taken as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined using the following formulae [14].

$$\text{Exudate Inhibition (\%)} = \left(1 - \frac{\text{Weight of exudate in mg of treated group of rats}}{\text{Weight of exudate in mg of control group of rats}}\right) \text{x } 100$$

$$\text{Granuloma Inhibition (\%)} = \left(1 - \frac{\text{Weight of granuloma in mg of treated group of rats}}{\text{Weight of granuloma in mg of control group of rats}}\right) \text{x } 100$$

RESULTS

Table 1: Results of phytochemical screening of HAME

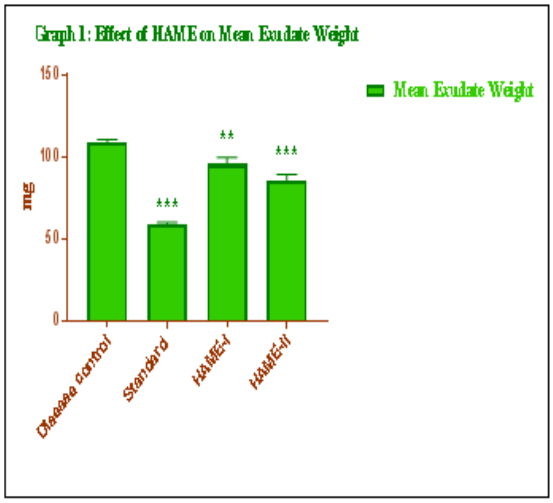
Test for phytochemical group	Reagent	Results of HAME
Carbohydrates	Fehling's Test	+
Alkaloids	Dragendroff's Test	+
Tannin	Ferric chloride test	+
Saponin	Frothing Test	+
Amino Acids	Ninhydrin Test	-
Glycosides	Legal's Test	+
Cardiac Glycosides	Raymonds Test	-
Flavonoids	Shinoda test	+

+: Positive result; −: Negative result

Table 2: Effect of HAME on Exudation in Cotton Pellet induced granuloma in rats.

Groups	Mean Exudate Weight(mg)	Exudate inhibition(%)
Disease control	109.2±1.302	-
Standard [Aceclofenac -50 mg/kg, b.wt]	58.50±1.544***	46.41
HAME-I [200 mg/kg, b.wt]	95.50±1.023**	12.52
HAME-II [400 mg/kg, b.wt]	85.83±3.628***	21.38

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001. as compared with arthritic control (One-way ANOVA followed by Dunnett's test).



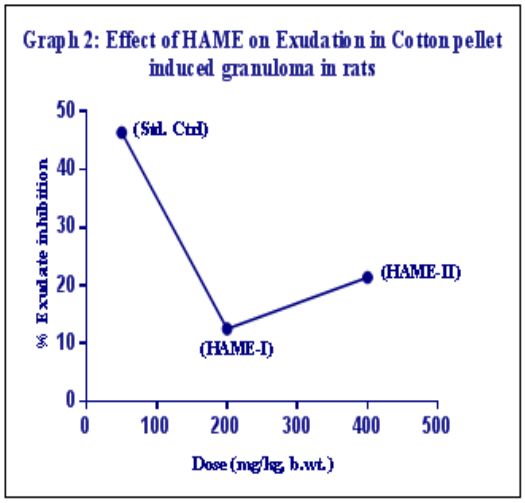
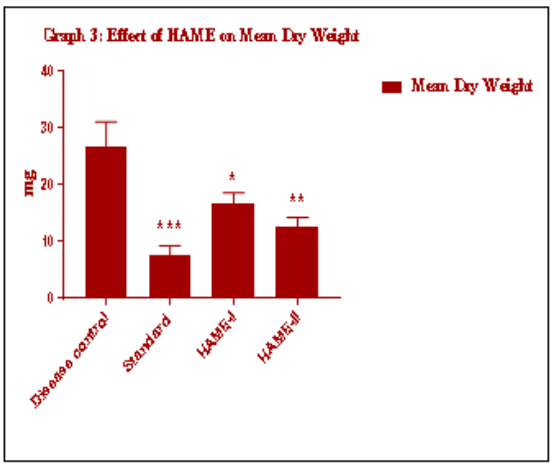


Table 3: Effect of HAME on Granular tissue formation in Cotton Pellet induced granuloma in rats

Groups	Mean Dry Weight (mg)	Granuloma inhibition (%)
Disease control	26.67±4.462	-
Standard [Aceclofenac -50 mg/kg, b.wt]	7.66±1.58***	69
HAME-I [200 mg/kg, b.wt]	16.66±1.925*	26.98
HAME-II [400 mg/kg, b.wt]	12.48±1.79**	49.07

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001. as compared with arthritic control (One-way ANOVA followed by Dunnett's test).



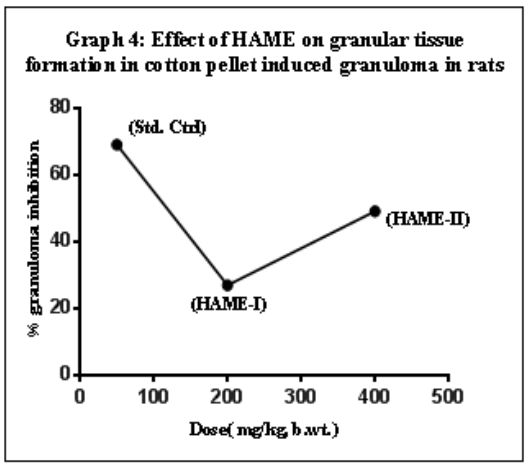
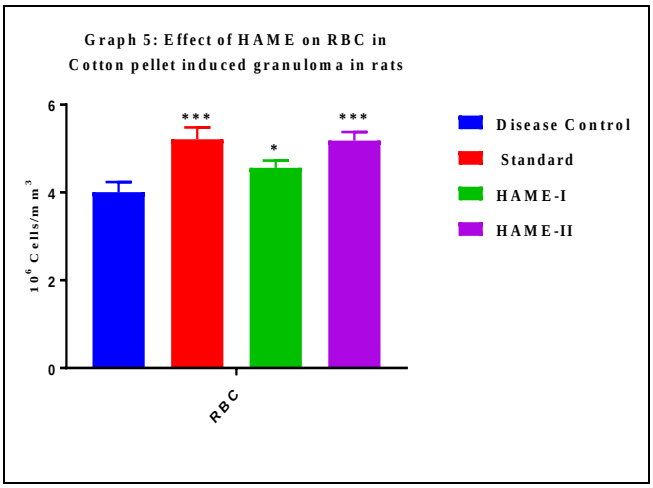
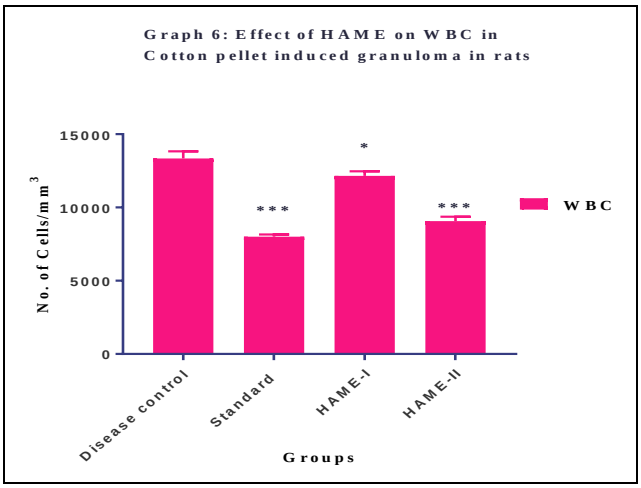


Table 4: Effect of HAME on Hematological Parameters

Groups	RBC(millions of cells / mm^3)	WBC(Cells / mm^3)
Disease control	4.01±0.229	13340±487.4
Standard [Aceclofenac -50 mg/kg, b.wt]	5.21±0.273***	8011±151.4***
HAME-I [200 mg/kg, b.wt]	4.562±0.167*	12153±312.5*
HAME-II [400 mg/kg, b.wt]	5.183±0.1926***	9069±294.9***

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001. as compared with arthritic control (One-way ANOVA followed by Dunnett's test).





DISCUSSION

Due to increase in the frequency of NSAID's usage with reported adverse effects, there is needed to focus on the scientific exploration of herbal drugs with minimum or no side effects. So there is a continuous out search for indigenous drugs providing relief to the inflammation. Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue[5].

The dried leaves of Murraya *exotica* was pulverized to fine powder and subjected to percolation by soxhlet using 70% ethanol in 1:4 ratio. The HAME obtained was phyto pharmacologically evaluated for various phyto chemical constituents and anti-inflammatory activity. The percentage yield of HAME was 23%. The phyto chemical analysis of HAME revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, saponins and glycosides which was shown in Table-1. Flavonoids and Tannins possess different mechanisms in various pharmacological activities[15].

Based on the previous data of acute toxicity studies conducted as per OECD - 423, high dose and low doses were selected as 10% and 20% of administered maximum dose of 2000 mg/kg, b.wt.[16]. The choice of the animal strain has been found to be very important for the performance of this test. Wistar albino rats have been proven to be very suitable in contrast to other substrains. This was mainly due to the structural homology of rat TNF and similarity in CYP 450 enzyme system[17].

The current research was aimed at provoking foreign body granulomas in rats by subcutaneous implantation of pellets of compressed cotton. After specified duration of research, histologically giant cells and undifferentiated connective tissue was observed besides the fluid infiltration. The amount of newly formed connective tissue was measured by weighing the dried pellets after removal[1].

In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the test treatment, which might be due to inhibition of collagen, fibronectin and glycosaminoglycan synthesis[3,6] compared to standard treatment.

In cotton pellet induced granuloma, there is increase in the WBC count due to expression of adhesion molecules on endothelial cells, activation of neutrophils, proliferation of lymphocytes and monocytes by increase in the binding and transmigration of leukocytes[18]. Upon treatment with test and standard significantly ameliorated a WBC count owing to its anti-inflammatory action. There is decrease in RBC count in cotton pellet induced granuloma rats indicating anaemia of varying degree with chronic inflammation[19]. Upon treatment with standard and test, the RBC levels were significantly restored and this was shown in Table-4, Graph -5 & 6.

CONCLUSION

Current study as investigated the scientific evidence for folkloric use of *Murraya exotica* in the management of inflammation. From the above results, it was concluded that the HAME at both dose levels (200 mg/kg b.wt & 400 mg/kg b.wt) revealed the presence of anti-inflammatory activity. It may be due to the presence of various phytochemical constituents such as flavanoids and tannins having crucial role in suppressing the release of inflammatory mediators. Further investigation is required to identify the active principle responsible for anti-inflammatory activity.

REFERENCES

1. Mitul P, Murugananthan, Shivalinge GKP, In Vivo Animal Models in Preclinical Evaluation of Anti- Inflammatory Activity- A Review, Ijpras , 1(2), 2012, 01-05.

2. Mathew G, Lincy J and Naina S S, Evaluation of antipyretic and anti-inflammatory activity of seed extract of *Trigonella foenum graecum,* Journal of Chemical and Pharmaceutical Research, 8(1), 2016, 132-135.

3. Balasubramanian A, Ramalingam K, Sureshkumar K and Christina AJM, Anti-inflammatory Activity of Morus indica Linn., Iranian Journal of Pharmacology & Therapeutics, 4, 2005, 13-15.

4. Agbaje EO, Fageyinbo MS, Evaluating Anti-Inflammatory activity of aqueous root extracts of *Strophanthus hispidus* DC. (Apocynaceae), International Journal of Applied Research in Natural Products, Vol. 4 (4), 2012, 7-14.

5. Ghanshyam D, Trilochan S, Amit R, Animal Models for Inflammation: A Review, Asian J. Pharm. Res., Vol. 3: Issue 4, 2013, 207-212.

6. Raghdan Zeki Al-Saad, Saad Abdulrahman H and Intesar T N, Dose-response Relationship of the Anti-inflammatory Activity of Pentoxifylline in Experimental Models of Chronic Inflammation, Pharmacologia, 3(2), 2012, 39-45.

7. Raveesha P, Chandrasekhar K B, Natural Remedies – A cure for arthritis? Journal of Comprehensive Pharmacy, 2(4), 2015, 115-119.

8. Kore KJ, Shete RV, Desai NV, Anti-Arthritic activity of Hydroalcoholic extract of Lawsonia Innermis, International Journal of Drug Development & Research, 3(4), 2011.

9. Nishat F, Syeda JF, Pharmacological Screening for Anti-arthritic activity of Moringaoleifera, Asian Journal of Pharmaceutical and Clinical Research, 9(3), 2016, 106-111.

10. Saloni S, Saroj A, Pharmaceutical activities of Phytochemicals in Murraya spp. - a review, Journal of Pharmacy Research, 9(4), 2015, 217-236.

11. Mallikarjuna G, Kambagiri SC, Prabhakaran V, Niranjan Babu M, Anti-Arthritic Activity of *Ocimum gratissimum* L. on Complete Freund's Adjuvant Induced Arthritic Rat Model, Journal of International Pharmaceutical Sciences, 3(1), 2016, 25-35.

12. Winter C A and Porter C C, Effect of alteration in side chain upon anti inflammatory and liver glycogen activities in hydrocortisone ester, J. Am. Pharma. Association, 46, 1957, 515-519.

13. Lagishetty C V and Naik SR, Polyamines: Potential anti-inflammatory agents and their possible mechanism of action, Indian J. Pharmacol., 40, 2008, 121-125.

14. Khouzami L , Mroueh M, and Daher FC, The role of methanolic extract of *Quercus Infectoria* bark in lipemia, glycemia, gastric ulcer and bacterial growth, J. Med. Plants Res., 2, 2009, 224-230.

15. Longhuo WU, Pei LI, Xiao W, Zhenjing Z, Farzin F, and Ruian XU, Evaluation of anti-inflammatory and antinociceptive activities of *Murraya exotica*, Pharmaceutical Biology 48(12), 2010, 1344–1353.

16. Gautam M K , Singh A, Rao C V and Goel R K. Toxicological Evaluation of *Murraya Paniculata* (L.) Leaves Extract on Rodents**,** American Journal of Pharmacology and Toxicology, 7 (2), 2012, 62-67.

17. Burke MD, Thompson S, Weaver RJ, Wolf CR, Mayer RT, Cytochrome P450 specificities in alkoxyresorufin o-dealkylation in human and rat liver, BiochemPharmacol, 48(5), 1994, 923-936.

18. Sztrymf B, Rabiller A, Nunes H, Savale L and Lebrec D et al., Prevention of hepatopulmonary syndrome and hyperdynamic state by pentoxifylline in cirrhotic rats, Eur. Respir.J., 23, 2004, 752-758.

19. Harsh M., Text book of Pathophysiology, 7thEd, Jaypee publication, New Delhi, 2005, 135.

Source of Support: Nil, **Conflict of Interest:** None.