

**Review article on anti-inflammatory activity of various herbal plants and various animal models**

Rajesh Asija, Prateek Choudhary, Sourabh Sharma, Ravi Kumar Pareek, Pravesh Kumar Sharma

Maharishi Arvind Institute of pharmacy, Mansarovar, Jaipur, Rajasthan, India- 302020

Accepted 10 September 2014; Published 13 September 2014

ABSTRACT

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, injured cells or irritants. It is characterized by redness, inflamed joints, joint hurt, loss of joint function its and stiffness. Inflammation is currently treated by NSAIDs. The developments of potent anti-inflammatory drugs from the natural products are now under thought. Natural products are rich source for discovery of new drugs because of their component diversity. A herbal product from medicinal plants plays a major role to cure many diseases associated with inflammation. The traditional drug available in the market to treat inflammation produces various side-effects. There is need for the search of newer drugs with less or no side-effects. This review is given on the anti-inflammatory activity of the most familiar medicinal plants of medicine. This matter may be helpful for the researchers, academicians and preclinicians about the potential herbs having anti-inflammatory activity.

Keywords: Herbaceous Plant, NO, inflammation, NSAIDs and Screening models.

INTRODUCTION:

Inflammation is a normal, protective response to tissue injury caused by noxious chemicals physical trauma or microbiological agents. There are mainly two types of inflammation which are as follows:

1. Acute inflammation:

It is associated with increased, capillary infiltration, vascular permeability and emigration of leukocytes.

2. Chronic inflammation:

It is associated with infiltration of mononuclear immune cells, fibrosis, fibroblast activation macrophages, proliferation (angiogenesis), neutrophils, and Monocytes. Inflammation is a common clinical conditions and rheumatoid arthritis is a chronic incapacitating autoimmune disorder², that affects about 1% of the population in developed countries². The classic signs of inflammation are local redness, swelling, pain, heat and loss of function³. Nitric oxide (NO) is a gaseous short lived free radical has been pinched in as a mediator of inflammation and modulation of biosynthesis or activity of NO results in amelioration of acute inflammation and experimental arthritis model^{4,5}. NO is generated via the oxidation of the terminal guanidine nitrogen atom of L-arginine by the enzyme Nitric Oxide Synthase (NOS). Increased NOS activity or NO release has been demonstrated in both acute and chronic models of inflammation. Further, administration of L-arginine a

precursor for NO synthesis increased the paw swelling in adjuvant arthritis⁶. NSAIDs are among the most commonly used drugs are prescribed for orthopaedic conditions such as soft-tissue injuries, osteoarthritis, and fractures etc⁸. NSAIDs e.g. naproxen and ibuprofen etc. are used in the above said conditions. The other class of drugs is glucocorticoids e.g. cortisone and prednisone etc. However, besides their elevated costs, severe adverse reactions and toxicity, including some risk of infections in subsets of patients being treated with biological response modifiers e.g. Tumour necrosis factor, alpha blocking agents. The side-effects with currently used drugs are G.I. ulceration and bleeding, Hypertension, Hyperglycemia Renal damage. As well as the above side-effects, the greatest disadvantage in presently available powerful synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. So, the screening and development of drugs for their anti-inflammatory activity is the need of the hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants.^{6,7} A plant contains a multiple of different molecules that act synergistically on targeted elements of the complex cellular pathway. The use of herbaceous medicines becoming popular due to toxicity and side-effects of allopathic medicines. Herbaceous plants play an important role in the development of potent therapeutic agents⁸. There are many practitioners of

traditional medicinal system using medicinal plants in defensive, promotional and healing applications^{9,10}.

Table 1: Herbal Plants having anti-inflammatory potential

Sr. No.	Plant Name	Family	Plant Part	Ref.
1.	<i>Achillea millefolium</i>	Asteraceae	Whole Plant	11
2.	<i>Aconitum heterophyllum</i>	Valeraneaceae	Root	12
3.	<i>Adhatoda vasica</i>	Acanthaceae	Leaves	13
4.	<i>Bacopa monnieri</i> Linn	Scrophulariaceae	Whole Plant	14
5.	<i>Cassia fistula</i> L.	Caesalpiniaceae	Leaves	15
6.	<i>Daphne pontica</i> Linn.	Thymelaeaceae	Roots	16
7.	<i>Emblica officinalis</i>	Euphorbiaceae	Fruit	17
8.	<i>Garcinia mangostana</i> Linn.	Guttiferae	Fruit	18
9.	<i>Lantana camara</i> Linn.	Verbenaceae	Leaves	19
10.	<i>Lycopodium clavatum</i> Linn.	Lycopodiaceae	Aerial Part	20

1. *Achillea millefolium* Linn. (Asteraceae):

Achillea millefolium L. is used externally for treatment of, burns, swollen irritated skin and wounds. It is belonging to family Asteraceae. Whole plant is used as herbaceous. Aqueous and alcoholic extracts of *A. millefolium* are used in traditional medicine internally in treatment of gastro-intestinal and hepato-biliary disorders. The crude plant extract and two fractions enriched the flavonoids inhibit human neutrophil elastase as well as the matrix metalloproteinases, which are associated with anti-inflammatory process *in vitro* studies¹¹.

2. *Aconitum heterophyllum* (Valeraneaceae):

A. heterophyllum is used for the treatment of nervous system, fever and rheumatism digestive system. It is belong to family Valeraneaceae. The study of ethanol extract of root of *A. heterophyllum* contains alkaloids, sterols glycosides, and flavonoids. The cotton pellet-induced granuloma is widely used to determine the anti-inflammatory activity. The administration of *A. heterophyllum* extract has been observed to inhibit the weight of wet cotton pellet in a dose dependent manner and the higher dose of *A. heterophyllum* exhibited inhibition of inflammation very close to the inhibitory effect of diclofenac sodium¹².

3. *Adhatoda vasica* (Acanthaceae):

The plant *Adhatoda vasica* is use to treat, whooping cough, chronic bronchitis, asthma, sedative expectorant, cold, cough antispasmodic, anthelmintic rheumatic painful inflammatory swellings, and rheumatism. The drug is employed in different forms such as fresh juice, decoction, infusion and powder. It is also given as alcoholic extract and liquid extract or syrup. This plant contains alkaloids, tannins, flavonoids, terpenes, sugars and glycosides. The anti-inflammatory potential of ethanolic extract has been determined by using

carrageenan-induced paw edema assay, formalin-induced paw edema assay in albino rats.¹³

4. *Bacopa monnieri* Linn. (Scrophulariaceae):

The *Bacopa monnieri* is used as a brain tonic to enhance, learning concentration and memory development. The plant possesses anti-inflammatory activity on carrageenan-induced rat paw edema and it has shown 82% edema inhibition when compared to indomethacin¹³.

5. *Cassia fistula* L. (Caesalpiniaceae):

Cassia fistula tree is useful in the treatment of rheumatism, anorexia skin diseases, jaundice and inflammatory diseases. The bark extracts of *Cassia fistula* possess significant anti-inflammatory effect in the acute and chronic anti-inflammatory model of inflammation in rats. The main constituents responsible for anti-inflammatory activity of *Cassia fistula* are flavonoids and bio-flavonoids¹⁵.

6. *Daphne pontica* Linn. (Thymelaeaceae):

Daphne pontica is used for the treatment of inflammatory ailment and rheumatic pains. the roots of *Daphne pontica* which is reported to have antitumour activity. Several *Daphne* species have been used against inflammatory disorders. The root extracts inhibits the production of PGE₂.

7. *Emblica officinalis* (Euphorbiaceae):

Emblica officinalis is used for antipyretic activities and anti-inflammatory. The water fraction of methanol extract inhibited migration of human PMNs in relatively low concentrations¹⁷.

8. *Garcinia mangostana* Linn. (Guttiferae):

Garcinia mangostana is used as the treatment of trauma and skin infections. The xanthones, γ -mangostins, α - and are major bioactive compounds found in the fruit hulls of mangosteen. The xanthones exhibits their biological

effects by blocking inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). It was reported that two mangostins decrease prostaglandins (PGE₂) levels through inhibition of COX-2 activity and NO production. It is reported that α -mangostin shows a more potent inhibition of PGE₂ release than either histamine or serotonin¹⁸.

9. *Lantana camara* Linn. (Verbenaceae):

Lantana camara Linn. is widely used in treatment of cancer and tumours. The additional uses of plant has antimalarial, anti-bacterial and anti-diarrhoeal activities. It has been reported that aqueous extract of *Lantana camara* leaves has promising analgesic, anti-inflammatory and anti-hemorrhoidal activities¹⁹.

10. *Lycopodium clavatum* Linn. (Lycopodiaceae):

The study carried out by four extracts prepared with methanol, petroleum ether, ethyl acetate and chloroform as well as the alkaloidal fraction from the aerial parts of *Lycopodium clavatum* using acetic acid-induced increase in capillary permeability assessment in mice revealed that only the chloroform extract and the alkaloid fraction displayed marked anti-inflammatory effect as compared to Indomethacin²⁰.

In vivo animal models:^{21,22}

The inflammatory response is accompanied by clinical signs of hyperalgesia, erythema and edema. Inflammatory responses occur in three different phases, each apparently mediated by different mechanisms:

1. An acute transient phase characterised by local vasodilation and increased capillary permeability.
2. A subacute phase, characterised by infiltration of leukocytes and phagocytic cells.
3. A chronic proliferative phase

Testing acute and sub acute inflammation are:

1. UV-erythema in guinea pigs
2. Vascular permeability
3. Oxazolone-induced ear edema in mice
4. Croton-oil ear edema in rats and mice
5. Paw edema in rats (various modifications and various irritants)
6. Pleurisy tests
7. Granuloma pouch technique (various modifications and various irritants)

The proliferative phase is measured by following methods:

1. Cotton wool granuloma
2. Glass rod granuloma

Methods for testing sub acute and acute inflammation:

1. UV-erythema in guinea pigs:

Prostaglandin E (PGE) levels in the skin have been shown to be elevated during the 24 h period following exposure

of guinea pig skin to ultraviolet radiation from 280-320 nm. The development of augmented PGE levels paralleled the development of the overdue phase of erythema. Hindrance the development of ultraviolet erythema on albino guinea pig skin by general pretreatment with clinically equivalent doses of phenylbutazone and other nonsteroidal anti-inflammatory agents. Erythema (redness) is the original sign of inflammation, not yet accompanied by plasma exudation and edema. This model depicts the hindrance in development of UV erythema on albino guinea pig skin by general pretreatment with clinically equivalent doses of phenylbutazone and other NSAIDs.

Procedure: Albino guinea pigs of both sexes with an average weight of 350g are used. Four animals are used each for treatment and control group. 18 hr prior testing, the animals are shaved on both the flanks and on the stern and then they are chemically depilated by a commercial depilation product or by a suspension of barium sulphide. 20 min later, the depilation paste and the hair are rinsed off in running warm water. On the next day the test compound is dissolved in the vehicle and half of the test compound is administered by gavage (at 10 ml/kg) 30 min before UV exposure. Control animals are treated with the vehicle alone. The guinea pigs are placed in a leather cuff with a hole of 1.5 \times 2.5 cm size punched in it, allowing the UV radiation to reach only this area. Then animals are rendered to UV radiation. After 2 min of rendering the remaining half of the test compound is administered. The erythema is scored 2 and 4 hr after exposure²³.

2. Vascular permeability:

Procedure: Albino Wistar are used each group containing 4 rats. Control group will receive distilled water 1%w/v 1ml/100g by oral route and other group will receive test compound by oral route and standard group will receive diclofenac 10ml/kg by intraperitoneal route. After 1h of these administration rats are injected with 0.25ml of 0.6% v/v solution of acetic acid intraperitoneally. Immediately, 10 ml/kg of 10%w/v Evans blue is injected intravenously via tail vein. After 30 min, the animals are anesthetized with ether anaesthesia and sacrificed. The abdomen is cut open and exposed viscera. The animals are held by a flap of abdominal wall over a Petri dish. The peritoneal fluid (exudates) collected, filtered and made up the volume to 10 ml using normal saline solution and centrifuged at 3000 rpm for 15 min. The absorbance (A) of the supernatant is measured at 590 nm using spectrophotometer²⁴.

3. Oxazolone-induced ear edema in mice:

Procedure: Using 12 mice in each group, the same skin site of the right ear is sensitized by a single application of 10 μ l (each 5 μ l for inner and outer of ear) of 0.5% oxazolone in acetone 7 days before the first challenge (day 0), and 10 μ l of 0.5% oxazolone in acetone was repeatedly applied to the sensitized right ear 3 times per week. In the nonsensitized animals, acetone alone was applied to the right ear. The mice are challenged 8 days later again under anesthesia by applying 0.01 ml 2% oxazolone solution to the inside of the right ear in control group and 0.01 ml of oxazolone solution in which the test compound and the standard is solved. Groups of 10 to 15 animals are treated with the irritant alone or with the solution of the test compound. The left ear is remains untreated. The maximum of inflammation is occurs 24 h later. This time the animals are sacrificed under anesthesia and a disc of 8 mm diameter is punched on both sides. The discs are immediately weighed on a balance. The weight difference is an indicator of the inflammatory edema.²⁵

4. Croton-oil ear edema in rats and mice:

Procedure: A total of 15 μ l of an acetonetic solution containing 75 μ g of croton oil is applied to the inner surface of right ear of each mouse. Left ear remains untreated. Control animals receives only the irritant while indomethacin (100 μ g/ear) serves as reference. Varying dose levels of test drug are applied to the inner surface of right ear of each mouse by dissolving them in inflammation inducing solution. Animals are sacrificed by cervical dislocation 6 hr later and a plug (6 mm in diameter) is removed from both the untreated and treated ear. The difference in weight between the two plugs is taken as measure of edematous response. Since tetradecanoyl phorbol acetate (TPA) is the chief ingredient of croton oil, purified TPA has also been used to induce ear edema in mice. Evaluation: The antiphlogistic effect can be determined by expressing the increase in weight of the treated ear as percentage of the weight of the contralateral control ear. The difference between both ears or excised discs is calculated as the average values for treated and control groups and the effect is evaluated by statistical methods.²⁶

5. Paw edema in rats:

Procedure: Male Sprague-Dawley rats with a body weight between 150 g and 100 g are used. The animals are fasting overnight and provide drink water. The rats be given 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same amount. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into

the plantar side of the left hindpaw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to mark. The paw volume is measured immediately after injection, again 3 and 6 h, and in the long run 24 h after challenge. Various devices have been developed for plethysmography of the paw, like mercury for immersion of the paw, more sophisticated apparatus based on the principle of transforming the volume being increased by immersion of the paw into a proportional voltage using a pressure transducer, sensitive method of measuring mouse paw volume by interfacing a Mettler Delta Range top-loading balance with a microcomputer, commercially available plethysmometer.²⁸

6. Pleurisy tests:

Procedure: The mouse pleurisy is induced by a single intrapleural injection of 0.1 ml of carrageenan (1%). After 4 h the animals are Euthanasia with an overdose of ether, the thorax was opened and the pleural cavity was washed with 1.0 ml of sterile PBS, containing heparin. The Samples of the pleural lavage are collecting for determination of exudation, myeloperoxidase, adenosine-deaminase activities, and nitric oxide levels, as well as for determination of total and differential leukocyte counts. Total leukocyte counts are performing in a Neubauer chamber. The cytospin preparations of pleural wash are stained with May-Grunwald Giemsa for the differential count which was performed under an oil immersion objective. The serum level of the C-reactive protein was also analysed. In another set of experiment animals were treated 30 min before carrageenan with a solution of Evans blue dye (25 mg/kg, i.v.) in order to evaluate the degree of exudation in the pleural space. A sample of the fluid leakage collected from the pleural cavity is store in a freezer (20° C) to further determine the concentration of Evans blue dye and on the day of experiments, a group of samples is flux at room temperature and the amount of dye was estimated by colorimetry using an Elisa plate reader at 600 nm, by interpolation from a standard curve of Evans blue dye in the range of 0.01 to 50 μ g/ml.²⁹

7. Granuloma pouch technique:

Procedure: Male Sprague-Dawley rat with a body weight between 150g and 200 g are used. Ten animals are taken for test and controls for groups. The asternside of the animals is shaved and disinfected. a very thin needle a pneumoderma is made in the middle of the dorsal skin by injection of 20 ml of air under anesthesia and the resulting oval airpouch 0.5 ml of a 1% solution of Croton oil in sesame oil is injected avoiding any leak of air, 48 hours later the air is withdrawn from the pouch and 72 h later any resulting adhesions are split. Instead of croton

oil 1 ml of a 20% suspension of carrageenan in sesame oil can be used as irritation and starting with the formation of the granuloma, the animals are treated every day either subcutaneously or orally with the standard or test compound and testing local activity, the test compound is injected directly into the air sac at the same time as the irritation. and the 5th day the animals are sacrificed under anesthesia. The pouch is opened and the exudate is collected in glass cylinders. The pouches are washed with 1 ml of saline, exudates are immediately cooled on ice and the volume is recorded. Total no. of leukocytes migrated into the pouch are evaluated after staining with Erythrosine B and remaining exudates is centrifuged at 3000 rpm for 10 min at 4 degrees and supernatant stored at -20 degrees until use.³⁰

8. Cotton wool granuloma:

Procedure: Male rats weighing 180.200 g were used. Test drugs were administered orally on a once daily dosage regimen for 7 days, and the control group received vehicle. Two sterilized pellets of cotton wool were implanted subcutaneously, one on each side of abdomen of the animal, under the light ether anesthesia and sterile technique. The rats were sacrificed on the eighth day. The implanted pellets were dissected out and recorded for wet weight. Thymuses were also dissected out. Both pellet and thymus were dried at 60 °C for 18 h and the dry weight was recorded.³¹

9. Glass rod granuloma:

Procedure: A Glass rod with a diameter of 6 mm is cut to a length of 40 mm and the ends rounded off by flame melting. Male Sprague-Dawley rats with an initial weight of 120 g are anaesthetized with the back skin shaved and disinfected. From an incision in the coccyx region a subcutaneous subway is formed in cranial direction with a closed blunted forceps. A glass rod is introduced into this tunnel finally lying on the back of the animal and incision wound is closed by sutures. The animals are set to the left in cages. The rods remain for 20 or 40 days and then cure with drugs is either during the complete period or only during the last 10 or 3 days and end the animals are sacrificed below CO₂ anesthesia. The glass rods are prepared together with the surrounding connective tissue which forms a tube around the glass rod and incision at one end the glass rod is extracted and the granuloma sac inverted forming a plain piece of pure connective tissue. Wet heft of the granuloma tissue is recorded and finally the granuloma tissue is dried and the dry weight is recorded²².

REFERENCES:

1. Kumar S, Bajwa, Singh K and Kalia Anti-inflammatory activity of herbal plants: A review international journal of advances in pharmacy, biology and chemistryijapbc apr-jun, 2013 – vol. 2-2.
2. Cardinali PD and Esquifino IA. Circadian disorganization in experimental arthritis. *Neuro Signals*. 2003;12:267-282.
3. Pervical M. Understanding the natural management of pain and inflammation, *Clinical Nutrition insights*. 1999;4:1-5.
4. Daniel SF. Therapeutic Administration of a selective inhibitor of nitric oxide synthase Does not ameliorate the chronic inflammation and tissue damage associated with adjuvant-induced arthritis in rats, *J Pharmacol Expt Ther*.1998;32:714-721.
5. Zumora RA and Billar TR. Inducible nitric oxide synthase and inflammatory disease. *Mol Med* 2000;6:347-356.
6. Corbett JA. Interleukin-1 β -induced formation of EPR-detectable iron-nitrosyl complexes in islets of Langerhans. *J Biol Chem*.1991;266:21351-21354.
7. Mederos M,. Effect of chronic nitric oxide synthesis inhibition on the inflammatory response induced by carrageenan in rats, *Eur J Pharmacol*.1995; 285:109.
8. Malizos KN. Do steroids, conventional non-steroidal anti-inflammatory drugs and selective Cox-2 inhibitors adversely effect fracture healing. *J Musculoskeletal Neuronal Interact*. 2009;9:44-52.
9. Barnes PM. Complementary and alternative medicine use among adults, United states. *Adv Data*. 2002;343:1-19.
10. Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandan SK and Ravi Prakash V. Evaluation of anti-inflammatory activity of *Pongamia pinnata* in rats, *J Ethnopharmacol*. 2011;78:151-157.
11. Benedek B, Kopp B and Melzig MF. *Achillea millefolium* L.- Is antiinflammatory activity mediated by protease inhibition. *J Ethnopharmacol*. 2007;2:312-317.
12. Santosh V, Shreesh O and Mohammad R. Anti-inflammatory activity of *Aconitum heterophyllum* on cotton pellet-induced granuloma in rats, *J Medicinal Plants Research*. 2010;4:1566- 1569.
13. Wahid a M, Suyog D, Suraj B J, Ajinkya M P, Mukhtar S K and Madhukar R V. Evaluation of anti-inflammatory and analgesic activities of ethanolic extract of roots *Adhatoda vasica* Linn. *International journal of PharmTech Research*. 2010;2:1364-1368.

14. Viji V and Helen A. Inhibition of Proinflammatory mediators: role of Bacopa monniera (L.) Wettst, Inflammo Pharmacology. 2010; 210-215.
15. Ilavarasan R, Mallika M and Venkataraman S. Anti-inflammatory and Antioxidant activities of Cassia fistula Linn bark extracts. Afr J Trad CAM. 2005;1:70-85.
16. Kupeli E, Tosun A and Yesilada E, Assesment of anti-inflammatory and antinociceptive activities of Daphne pontica. (Thymelaeaceae). J Ethnopharmacol. 2007;113:332-337.
17. Asmawi MZ, Kankaanranta H, Moilanen E and Vapaatalo H. Anti-inflammatory activities of Emblica officinalis Gaertn leaf extracts. J Pharm Pharmacol. 1993;45:581-584.
18. Chen L, Yang L and Wang C. Antiinflammatory activity of mangostins from Garcinia mangostana. Food Chem Toxicol. 2008;46:688-693.
19. Gidwani BK, Bhargava S, Rao SP, Majoomdar A, Pawar DP and Alaspure RN. Analgesic, Anti-inflammatory and Anti-Hemorrhoidal activity of aqueous extract of Lantana camara Linn, Research J Pharm and Tech. 2009;2:378-381.
20. Orhan I, Kupeli E, Sener B and Yesilada E. Appraisal of anti-inflammatory potential of the clubmoss, Lycopodium clavatum L. J Ethnopharmacol. 2007; 109:146.
21. Patel M, Murugananthan, Gowda K, *In Vivo* Animal Models in Preclinical Evaluation of Anti- Inflammatory Activity *International Journal of Pharmaceutical Research & Allied Sciences* Volume 1, issue2 (2012), 01-05
22. Gerhard vogel H, Wolfganga HV, Bernward AS, Jurgen S, Gunter M, Wolfganga FV. Drug discovery and evaluation pharmacological assays. 2nd ed , Berlin, Germany : Springer ;2002. p. 725-71.
23. Snyder. DS. Effect of topical indomethacin on uvr-induced redness and prostaglandin e levels in sunburned guinea pig skin. Prostaglandins. 1976;11(4):631-43.
24. Yashraj.Y, Mohanty.P.K, Kasture.S.B. Antiinflammatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. flower in rats. International Journal of Pharmacy & Life sciences. 2011;2(8):977-81.
25. Tadafumi.T, Toru.A, Kenji.O, Haruhiko.M. The effects of olopatadine hydrochloride on the number of scratching induced by repeated application of oxazolone in mice. Eur J Pharmacol. 2005;524:149-54.
26. Rogerio.A.S, Mariana.K.A, Romagna.C.Oliveira, Kleber.DP, Gerlania.O.L, Marta.R.K Topical anti-inflammatory effect of *Caryocar coriaceum* Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. J Ethnopharmacol. 2011;136:504- 10.
27. Gupta.SK. Drug screening methods. 2 ed. New Delhi: Jaypee; 2009; 2.
28. Penna.S.C, Medeiros.M.V, Aimbire.F.S.C, Faria-Neto.H.C.C, Sertie.J.A.A, Lopes- Martins.R.A.B. Anti-inflammatory effect of the hydralcoholic extract of *Zingiber officinale* rhizomes on rat paw and skin edema. Phytomedicine. 2003;10:381-5.
29. Vargas.A.J, Geremias.D.S, Provensi.G, Fornari.P.E, Reginatto.F.H, Gosmann.G, et al. *Passiflora alata* and *Passiflora edulis* spraydried aqueous extracts inhibit inflammation in mouse model of pleurisy. Fitoterapia. 2007;78:112-9.
30. Maier.P, Manser.P, Zbinden.G. Granuloma pouch assay i. induction of ouabain resistance in vivo. Mutation Research. 1978;54:159--65.
31. Martin.S.W, Stevens.A.J, Brennan.B.S, Davies.D, Rowland.M, Houston.J.B. The sixday-old rat air pouch model of Inflammation: Characterization of the inflammatory response to carrageenan. JPM. 1994;32 (3):139-47.