International Standard Serial Number: 0976-1381

Deccan J. Natural Products 1(2): April-June 2010

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

Potentiation Of Anti Inflammatory Activity Of Rasna By "Trikatu"

Authors: Ghodake Pradnya B[1] *., Dhavan Kailas[2], Shaikh Gazi[1], Patil S.M[1].,

1. ASPM's K.T.Patil college of Pharmacy , Osmanabad, Maharashtra (India)

2. Rajgadh Dnyanpeeth,s college of Pharmacy, Bhor, Pune, Maharashtra (India)

For correspondance: pradnyaghodake20@gmail.com

1. ABSTRACT:

Inflammation is a response of vascularised living tissue to the local injury.It is the body defence mechanism, which is closely interwined with the process of repair.Although the anti-inflammatory activity of Rasna was carried out but the literature survey revealed that no work has been carried out on potential effect of Trikatu preparation for enhancing anti-inflammatory activity of Rasna . In the present study Trikatu a herbal formulation was investigated for enhancing anti-inflammatory activity of Rasna(Alpinia officinarum). 'Trikatu', a compound Ayurvedic preparation containing Piper longum (Piperaceae) as one of the major ingredients, and others are Piper nigrum, Zingiber officinale. When Trikatu was given in the form of suspension in 1% CMC with Rasna in different proportions like 'A', 'B', 'C' in dose 1ml each.The methanolic extract of dried fruits of Trikatu potentiate the anti-inflammatory activity of Rasna and shown maximum potentiation done by preparation 'B'.The anti-inflammatory activity of these extracts was evaluated by carrageenan-induced rat paw oedema method, using Aspirin(150 mg/kg p.o.) suspension as a reference standard drug. The result was found significant with preparation 'B', with p value<0.01.

2. INTRODUCTION

Inflammatory diseases are very common throughout the world. It serves to destroy or dilute the injurious agents and also reconstitute the damaged tissue by regeneration. The severe side effects of steroidal and non-steroidal anti-inflammatory drug evoked us to search for the new anti-inflammatory drugs from the natural botanical source.Several indigenous drugs have been described in Ayurveda for management of inflammatory diseases. Thus, the present investigation was carried out to enhance anti-inflammatory activity of Alpinia offcinarum by herbal extracts of Trikatu in carrageenan-induced rat paw edema method as an experimental animal model.Trikatu is a herbal formulation containing Piper nigrum(Piperaceae),Piper longum(Piperaceae), Zingiber officinale(Zingiberaceae) which are known for their anti-inflammatory activity in rats.

2.1 Inflammation and the mechanism of action of anti-inflammatory drugs

Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, and, more recently, platelet- activating factor (PAF) and interleukin-1. Evidence for their involvement comes from studies with competitive antagonists for their receptors and inhibitors of their synthesis. H1 histamine antagonists are effective for hay fever and some skin allergies such as urticaria, which indicates the importance of histamine in these conditions. Symptoms of rheumatoid arthritis are alleviated by the aspirinlike anti- inflammatory drugs, which inhibit the cyclo-oxygenase enzyme and reduce synthesis of prostanoids. Corticosteroids prevent the formation of both PGs and LTs by causing the release of lipocortin, which by inhibition of phospholipase A2 reduces arachidonic acid release. They suppress the inflammation of rheumatoid arthritis and asthma. Currently, high doses of nonsedating H1 antihistamines and PAF antagonists are being tested for the treatment of allergic asthama.

2.2 Causes

- Burns
- Chemical irritants
- Frostbite
- Toxins
- Infection by pathogens
- Physical injury, blunt or penetrating
- Immune reactions due to hypersensitivity
- Ionizing radiation

2.3 Types

Comparison between acute and chronic inflammation:

	Acute	Chronic
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Resolution, abscess formation, chronic inflammation	Tissue destruction, fibrosis

Acute inflammation is a short-term process, usually appearing in a few minutes or hours and ceasing once the injurious stimulus has been removed.[4]. It is characterized by five cardinal signs:[5]

rubor (redness), calor (increased heat), tumor (swelling), dolor (pain), and functio laesa (loss of function). The first four (classical signs) were described by Celsus (ca 30 BCE–38 CE), while loss of function was added later by Virchow in 1870.[5][4]

Redness and heat are due to increased blood flow at body core temperature to the inflamed site; swelling is caused by accumulation of fluid; pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes.[5]

These five signs appear when acute inflammation occurs on the body's surface, whereas acute inflammation of internal organs may not result in the full set. Pain only happens where the appropriate sensory nerve endings exist in the inflamed area — e.g., acute inflammation of the lung (pneumonia) does not cause pain unless the inflammation involves the parietal pleura, which does have pain-sensitive nerve endings.[5]

2.4 Process of acute inflammation

The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages (e.g. dendritic cells, histiocytes, Kuppfer cells and mastocytes, depending on the tissue). Once activated by an injurious agent (infection, burn, etc.), they undergo activation and release inflammatory mediators responsible for the signs of inflammation. Vasodilation and its resulting increased blood flow causes the redness (rubor) and increased heat (calor). Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (Edema), manifesting as swelling (tumor). Some of the released mediators, e.g. bradykinin, increase the sensitivity to pain (hyperalgesia, dolor). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils, outside of the blood vessels (extravasation) into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. [4] The loss of function (functio laesa) is probably the result of a neurological reflex in response to pain.

In addition to cell-derived mediators, several acellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria, and the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma. [4]

The acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators have short half lives and are quickly degraded in the tissue. Hence, inflammation ceases once the stimulus has been removed.[4]

Exudative component

The exudative component involves the movement of plasma fluid, containing important proteins such as fibrin and immunoglobulins (antibodies), into inflamed tissue. This movement is achieved via the chemically-induced dilation and increased permeability of blood vessels, which results in a net loss of blood plasma. The increased collection of fluid into the tissue causes it to swell (edema).

Vascular changes

Acute inflammation is characterised by marked vascular changes, including vasodilation, increased permeability, and the slowing of blood flow, which are induced by the actions of various inflammatory mediators. Vasodilation occurs first at the arteriole level, progressing to the capillary level, and brings about a net increase in the amount of blood present, causing the redness and heat of inflammation. Increased permeability of the vessels results in the movement of plasma into the tissues, with resultant stasis due to the increase in the concentration of the cells within blood - a condition characterised by enlarged vessels packed with cells. Stasis allows leukocytes to marginate along the endothelium, a process critical to their recruitment into the tissues. Normal flowing blood prevents this, as the shearing force along the periphery of the vessels moves cells in the blood into the middle of the vessel.

Plasma cascade systems

The complement system, when activated, results in the increased removal of pathogens via opsonisation and phagocytosis.

The kinin system generates proteins capable of sustaining vasodilation and other physical inflammatory effects.

The coagulation system or clotting cascade which forms a protective protein mesh over sites of injury.

The fibrinolysis system, which acts in opposition to the coagulation system, to counterbalance clotting and generate several other inflammatory mediators.

Plasma derived mediators

Non-exhaustive list

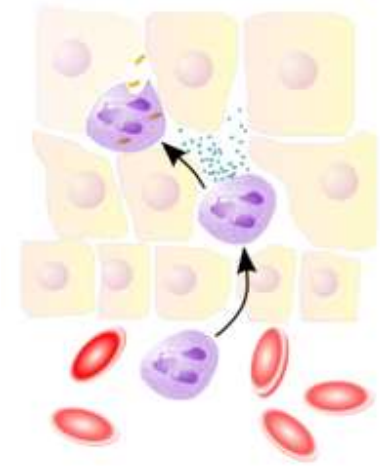
Name	Produced by	Description
Bradykinin	Kinin system	A vasoactive protein which is able to induce vasodilation, increase vascular permeability, cause smooth muscle contraction, and induce pain.
C3	Complement system	Cleaves to produce C3a and C3b. C3a stimulates histamine release by mast cells, thereby producing vasodilation. C3b is able to bind to bacterial cell walls and act as an opsonin, which marks the invader as a target for phagocytosis.
C5a	Complement system	Stimulates histamine release by mast cells, thereby producing vasodilation. It is also able to act as a chemoattractant to direct cells via chemotaxis to the site of inflammation.
Factor XII (Hageman Factor)	Liver	A protein which circulates inactively, until activated by collagen, platelets, or exposed basement membranes via conformational change. When activated, it in turn is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.
Membrane attack complex	Complement system	A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the membrane attack complex, which is able to insert into bacterial cell walls and causes cell lysis with ensuing death.
Plasmin	Fibrinolysis	Able to break down fibrin clots, cleave complement protein C3, and

	system	activate Factor XII.
Thrombin	Coagulation system	Cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin, which aggregates to form a blood clot. Thrombin can also bind to cells via the PAR1 receptor to trigger several other inflammatory responses, such as production of chemokines and nitric oxide.

Cellular component

The cellular component involves leukocytes, which normally reside in blood and must move into the inflamed tissue via extravasation to aid in inflammation. Some act as phagocytes, ingesting bacteria, viruses, and cellular debris. Others release enzymatic granules which damage pathogenic invaders. Leukocytes also release inflammatory mediators which develop and maintain the inflammatory response. Generally speaking, acute inflammation is mediated by granulocytes, while chronic inflammation is mediated by mononuclear cells such as monocytes and lymphocytes.

Leukocyte extravasation



Neutrophils migrate from blood vessels to the inflamed tissue via chemotaxis, where they remove pathogens through phagocytosis and degranulation

Main article: Leukocyte extravasation

Various leukocytes are critically involved in the initiation and maintenance of inflammation. These cells must be able to get to the site of injury from their usual location in the blood, therefore mechanisms exist to recruit and direct leukocytes to the appropriate place. The process of leukocyte movement from the blood to the tissues through the blood vessels is known as extravasation, and can be divided up into a number of broad steps:

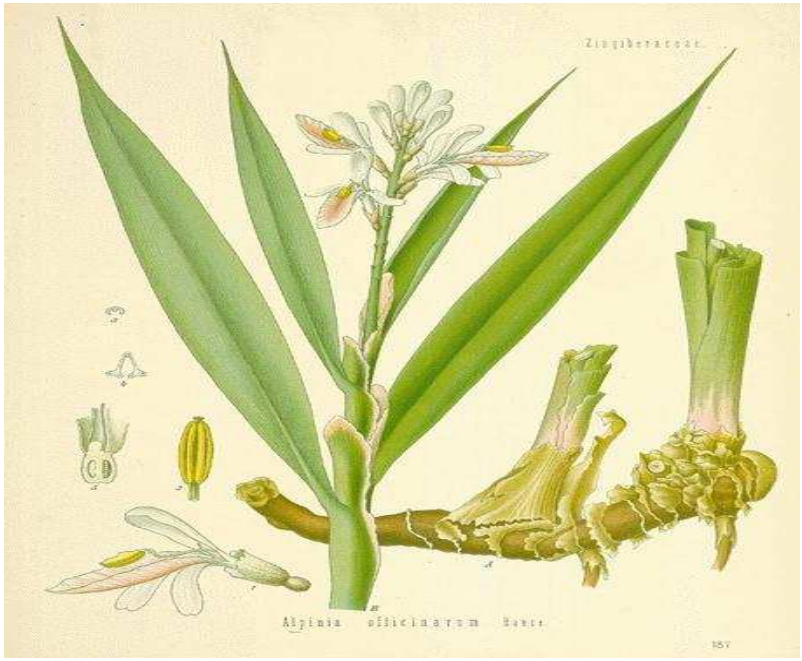
Leukocyte localisation and recruitment to the endothelium local to the site of inflammation – involving margination and adhesion to the endothelial cells: Recruitment of leukocytes is receptor-mediated. The products of inflammation, such as histamine, promote the immediate expression of P-selectin on endothelial cell surfaces. This receptor binds weakly to carbohydrate ligands on leukocyte surfaces and causes them to "roll" .

3. OBJECTIVE

The study reported that Trikatu' increased their bio-availability either by promoting rapid absorption from the gastrointestinal tract or by protecting the drug from being metabolised during its first passage through the liver after being absorbed, or by combination of both mechanisms. By adjusting constituent of Trikatu, anti-inflammatory activity of Rasna enhanced. So this work was undertaken to estimate capability of Trikatu preparation to enhance anti-inflammatory activity of Rasna.

4. MATERIAL AND METHODS

4.1 Alpinia officinarum



Synonyms:

Galanga. China Root. India Root. East India Catarrh Root. Lesser Galangal. Rhizoma Galangae. Gargaut. Colic Root. Kaempferia Galanga.

Biological source:

Therhizome of *Alpinia officinarum* Hance. *Family* : Zingiberaceae

Description---The genus *Alpinia* was named by Plumier after Prospero Alpino, a famous Italian botanist of the early seventeenth century. The name Galangal is derived from theArabic *Khalanjan*, perhaps a perversion of a Chinese word meaning 'mild ginger.'

The drug has been known in Europe for seven centuries longer than its botanical origin, for it was only recognized in 1870, when specimens were examined that had been found near Tung-sai, in the extreme south of China, and later, on the island of Hainan, just opposite. The name of *Alpinia officinarum* was given to the herb, as the source of Lesser Galangal. The Greater Galangal is a native of Java (*A. galanga* or *Maranta galanga*), and is much larger, of an orange-brown colour, with a feebler taste and odour. It is occasionally seen at London drug sales, but is scarcely ever used. There is also a resemblance to *A. calcarata*.The herb grows to a height of about 5 feet, the leaves being long, rather narrow blades, and the flowers, of curious formation, growing in a simple, terminal spike, the petals white, with deep-red veining distinguishing the lippetal.

The branched pieces of rhizome are from 1 1/2 to 3 inches in length, and seldom more than 3/4 inch thick. They are cut while fresh, and the pieces are usually cylindrical, marked at short intervals by narrow, whitish, somewhat raised rings, which are the scars left by former leaves. They are dark reddish-brown externally, and the section shows a dark centre surrounded by a wider, paler layer which becomes darker in drying. Their odour is aromatic, and their taste pungent and spicy. They are tough and difficult to break, the fracture being granular, with small, ligneous fibres interspersed throughout one side. The drug is exported, chiefly from Shanghai, in bales made of split cane, plaited, and bound round with cane. The root has been used in Europe as a spice for over a thousand years, having probably been introduced by Arabian or Greek physicians, but it has now largely gone out of use except in Russia and India. Closely resembling ginger, it is used in Russia for flavouring vinegar and the liqueur 'nastoika': it is a favourite spice and medicine in Lithuania and Esthonia. Tartars prepare a kind of tea that contains it, and it is used by brewers. The reddishbrown powder is used as snuff, and in India the oil is valued in perfumery.

Constituents---
The root contains a volatile oil, resin, galangol, kaempferid, galangin and alpinin, starch, etc. The active principles are the volatile oil and acrid resin. Galangin is dioxyflavanol, and has been obtained synthetically. Alcohol freely extracts all the properties, and for the fluid extract there should be no admixture of water or glycerin.

Medicinal Action and Uses---
Stimulant and carminative. It is especially useful in flatulence, dyspepsia, vomiting and sickness at stomach, being recommended as a remedy for sea-sickness. It tones up the tissues and is sometimes prescribed in fever. Homoeopaths use it as a stimulant. Galangal is used in cattle medicine, and the Arabs use it to make their horses fiery. It is included in several compound preparations, but is not now often employed alone.

4.2 Zingiber officinale

.

- Botanical Name : Zingiber Officinalis
- Family Name : Zingiberaceae
- Common Name : ginger , Calamus, Sweet Ginger, Ginger Root, Sonth (dried) cooking ginger, Canton ginger, Chinese ginger
- Part Used : Fresh And Dried Rhizomes.
- Habitat : South east asia and through out india.
- Product offered : Rhizomes, Oil

- Biological source : it consist of rhizomes of *Zingiber officinale* .

- Chemical constistuent :zinger oil consist of Monoterpen hydrocarbon Sesquiterpen Hydrocarbo,zingiberene,volatile oil(1-4%),starch(40-60%) ,fat(10%)
- Uses : Clinically proved as prophylactic of nausea and vomiting associated with motion, sickness, sea sickness and pregnancy. Known for its gastrointestinal benefits and as an anti inflamatory and carminative. It is useful in bronchitis, colds, congestion, diarrhea, flu, headache, nausea, rheumatism, sore throat. It is also used as an adjunct to many tonic and stimulating remedies. As an aromatic ,a carminative, stimulant, flavouring agent, stomachic.

4.3 *Piper nigrum*

Botanical Name : *Piper nigrum*

- Family Name : Piperaceae
- Common Name : Black Pepper, Peppercorns
- Part Used : Dried Unripe Fruits, Usually Known As Peppercorns
- Habitat : Extensively cultivated in hotter and moist part of india.
- Product offered : Whole plant, Fruits

- Biological source: pepper is the dried unripe fruit of Perenial Climbing Vine *Piper nigrum* Linn
- Family: Piperiaceae

- Chemical constistuent: pepper contain an Alkaloid piperine(5-9%),volatile oil(1-2.5%),pungent resin(6%),piperidine and starch about 30%

- Uses : Black pepper oil can be used to help in the treatment of pain relief, rheumatism, chills, flu, colds, increase circulation, exhaustion, muscular aches, physical and emotional coldness, nerve tonic and fevers. It furthermore increases the flow of saliva, stimulates appetite, encourages peristalsis, tones the colon muscles and is a general digestive tonic. Sometimes it is used in place of cubebs for gonorrhoea. As a gargle it is valued for relaxed uvula, paralysis of the tongue. On account of its stimulant action it aids digestion and is especially useful in atonic

dyspepsia and turbid condition of the stomach. It will correct flatulence and nausea. It has also been used in vertigo, paralytic and arthritic disorders. It has also been advised in diarrhoea, cholera, scarlatina and in solution for a wash for tinea capititis. Externally it is used for its rubefacient properties and as a local application for relaxed sore throat and some skin diseases. Its oleoresin has bacteriostatic and fungistatic properties.

4.4 *Piper longum*

Botanical Name : *Piper longum*

- Family Name : Piperaceae
- Common Name : Long Papper, Pipli
- Part Used : Fruit, Root, Stem
- Habitat : Most deciduus to evergreen forests
- Product offered : Seeds, Roots, Fruit, Stem
- Biological source: The consistof fruits of pipper longum.

- Major chemical constituents

- Alkaloids and amides
- The fruit contains a large number of alkaloids and related compounds, the most abundant of which is piperine, together with methyl piperine, pipernonaline, piperettine, asarinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, retrofractamide A, pergumidiene, brachystamide-B, a dimer of desmethoxypiplartine, N -isobutyldecadienamide, brachyamide- A, brachystine, pipercide, piperderidine, longamide, dehydropipernonaline piperidine and tetra hydro piperine. Piperine, piperlongumine, tetrahydropiperlongumine, trimethoxy cinnamyol-piperidine and piperlonguminine have been found in the root.

- Lignans
- Sesamin, pulviatilol, fargesin and others have been isolated from the fruits.

- Esters
- The fruits contain tridecyl-dihydro-pcoumarate, eicosanyl-(E)-p-coumarate and Z-12-octadecenoic-glycerol-monoester.

- Volatile oil
- The essential oil of the fruit is a complex mixture, the three major components of which are (excluding the volatile piperine) caryophyllene and pentadecane (both about 17.8%) and bisaboline (11 %). Others include thujine, terpinoline, zingiberine, p-cymene, p-methoxyacetophenone and dihydrocarveol.
- Uses : Aromatic, stimulant, carminative ,good for constipation, for gonorrhea, paralysis of the tongue ,advised in diarrhea , cholera, scarlatina ,Chronic Malaria, Viral hepatitis.Piper Longum is most commonly used to treat respiratory infections such as stomachache, bronchitis, diseases of the spleen, cough, tumors, and asthma. When applied topically, it soothes and relieves muscular pains and inflammation. In Ayurvedic medicine, it is said to be a good rejuvenator. Piper Longum helps stimulate the appetite and it dispels gas from the intestines. An infusion of Piper Longum root is used after birth to induce the expulsion of the placenta. It is used as sedative in insomnia and epilepsy. Also as cholagogue in obstruction of bile duct and gall bladder.

- Medicinal and pharmacological activities

- Immunomodulatory activity
- Stimulant effects: Isolated piperine showed a central stimulant action in frogs, mice, rats and dogs and increased the hypnotic response in mice. It antagonised respiratory depression induced by morphine or pentobarbitone in anaesthetised dogs anda petroleum ether extract of the fruits antagonised morphine-induced respiratory depression in mice, A comparative study conducted with piperine and nalorphine,for effects against morphine-induced respiratory depression and

analgesia, found that both reversed respiratory depression but, unlike nalorphine, piperine did not antagonise morphine-induced analgesia in rats.

- Bio-availability enhancement: Piperine has been shown to enhance the bio-availability of structurally and therapeutically diverse drugs, possibly by modulating membrane dynamics, due to its easy partitioning and increasing permeability.The effect of Trikatu', a compound Ayurvedic preparation containing Piper longum as one of the major ingredients, was tested in combination with other drugs. The study reported that Trikatu' increased their bio-availability either by promoting rapid absorption from the gastrointestinal tract or by protecting the drug from being metabolised during its first passage through the liver after being absorbed, or by combination of both mechanisms.

5. METHOD:

All the herbal drugs were obtained from local market.The maceration process was applied to Trikatu by using 90% methanol for 24hrs.Simultaneously,the extraction of Rasna was carried out by 50% methanol using soxhlet apparatus. For experimental purpose Trikatu was prepared by following ways, 'A'(30gm Piper nigrum, 10gm Piper longum,20gm Zingiber officinale), 'B'(20gm Piper nigrum,30gm Piper longum, 10gm Zingiber officinale), 'C' (10gm Piper nigrum,20gm Piper longum, 30gm Zingiber officinale).The suspention of A,B,C was made in 1%CMC, suspension of standard drug Aspirin was made in the same.All these preparations were evaluated for enhancing anti- inflammatory activity of Rasna by the carrageenan induced rat paw edema method.

Thirty two adult wistar rats(200-220gm) were divided into four groups of eight each. First group received normal saline, second group received aspirin, third group received Rasna (500mg/kg p.o.) and remaining group received (700 mg/kg p.o.) body weight of each extract. Food was withdrawn overnight, but adequate supply of water was given to the rats before the experiment. The drugs were given orally with the help of an oral catheter. After one hour a sub plantar injection of 0.1 ml of 1% freshly prepared carrageenan was given to the left hind paw to all the animals. The paw volume was measured with help of Plethysmometer (UGO BASILE) immediately after injection. The paw volume was measured after 1,2,3 hrs. The average third hour paw volume of the A, B, C extract treated rats was compared with the control group and the standard drug (aspirin) group.

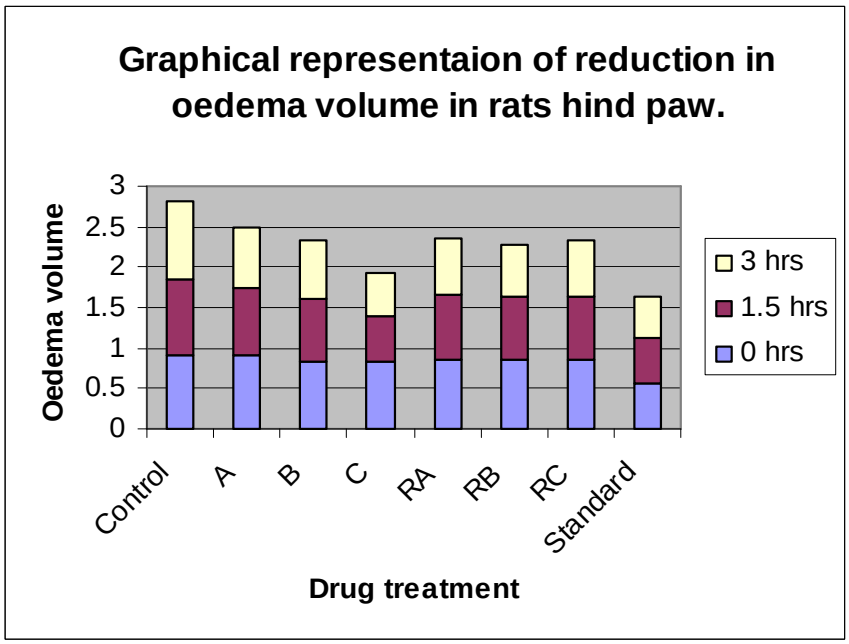
6. RESULT AND DISCUSSION

Carragenan induced oedema is a biphasic event, with early hyperemia due to the release of histamine and serotonin and delayed oedema due to the release of bradykinin and prostaglandin. The administration of Trikatu with Rasna in the proportion like A,B,C significantly inhibited paw oedema during measurement. Among them preparation 'B' had shown maximum reduction in paw volume as compared with 'A' and 'C'. The decreased paw oedema volume was measured at 0,1,2, and 3hrs after carragenan injection.It is found that anti-inflammatory activity of Rasna was potentiated by Trikatu preparation 'B' and results were found significant with p values<0.01.

TABLE: Effect of 'Trikatu' on carrageenan-induced oedema in rat's hind paw.

Group	Oedema volume Mean±SEM(ml)			't' value	'p' value
	0 hrs	1.5 hrs	3 hrs		
Control	0.92±0.090	0.94±0.102	0.96±0.101	—	—
A	0.90±0.086	0.85±0.094	0.75±0.078	t = 2.340	*p = 0.079
B	0.82±0.085	0.80±0.098	0.70±0.080	t = 4.287	*p = 0.013
C	0.82±0.075	0.58±0.078	0.54±0.082	t = 3.326	*p = 0.029
RA	0.85±0.080	0.80±0.075	0.72±0.090	t = 3.790	*p = 0.019
RB	0.86±0.082	0.78±0.102	0.63±0.085	t = 2.534	*p = 0.064
RC	0.85±0.102	0.79±0.085	0.70±0.095	t = 3.548	*p = 0.024
Standard	0.57±0.065	0.55±0.075	0.52±0.079	—	—

*p<0.05as compared to control.



Carragenan- induced paw oedema is a suitable model for screening new anti-inflammatory drugs as it is convenient & less time consuming & as it detects activity in all the clinically useful drugs.

Prostaglandins (PGs) plays significant role in diff. phase of inflammatory reactions. They elicit pain by direct stimulation of sensory nerve ending & also sensitize sensory nerve endings to other pain provoking stimuli. Moreover , PGs especially PGE_1 was reported to act on cell membrane during inflammatory conditions leadings to changes in lipoprotein structure of the cell membrane, thus change in lipoprotein structure causes destabilization of the cell membrane leadings to degenerative cellular changes. Histamine, 5-hydroxytryptamine & kinin like sub., which are relesed from the mast cell degradation during the first hour are also involved in the inflammation process.

The present study revealed that TRIKATU effective against Carragenan- induced paw oedema

7. CONCLUSION

Trikatu effectively suppress the inflammation when combined with Rasna.It is concluded that the constituents of Trikatu potentiate activity either by enhancing bioavailability or reducing metabolism of Rasna.

STATISTICAL ANALYSIS:

Results were expressed as mean ± SEM and evaluated by Dunnett multiple comparison test. Values of $P<0.01$ were considered statistically significant.

8. BIBLIOGRAPHY

1) Venkataranganna M.V. et al;Anti-inflammatory activity of JCB,a polyherbal formulation;Indian Drugs;vol-37,issue11, Nov 2000;pg.no-543.
2) Kasture S.B., Handbook of experiments in preclinical pharmacology, carrier publication, pg.75.
3) Dr Kurian J.C., Plants That Heal, Vol. 1, Pg no-251-252.
4) Rangari Vinod D., Pharmacognosy and Phytochemistry, Part-2, 1st- Edition, Pg no: 257-259,368-370.
5) Kokate C. K., Purohit A.P., Gokhale S.B., Pharmacognosy Textbook, 27th-Edition, Pg no: 346-347,351-353,395-397.
6) Qadry J.S., Pharmacognosy Textbook, 13th-Edition, Pg no: 193-195,317-319,391-393.
7) Dr. Nadkarni's K.M., Indian Materia Medica, Vol.1, Pg no: 80,965-972.