WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 2, 650-660. Research Article ISSN 2277– 7105

TO STUDY THE *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF *ACALYPHA INDICA* LEAVES.

Yogesh K. Chaudhari[1]*, Dr. Vijay A. Jagtap[1], Dr. Vanita G. Kanase[2], Imtiyaz Ansari[3]

[1]*Smt. S.S. Patil College of Pharmacy. Chopda, Jalgaon (M.S) 425107.

[2]HOD Pharmacology, Oriental College of Pharmacy, Sector 2, Behind Sanpada Railway Station, Sanpada West, Navi Mumbai, Maharashtra 400705.

[3]Dept. of Pharmacology, Oriental College of Pharmacy, Sector 2, Behind Sanpada Railway Station, Sanpada West, Navi Mumbai, Maharashtra 400705.

Article Received on
23 Nov. 2016,

Revised on 13 Dec. 2016,
Accepted on 02 Jan. 2017

DOI: 10.20959/wjpr20172-7684

***Corresponding Author**
Yogesh K. Chaudhari
Smt. S.S. Patil College of Pharmacy. Chopda, Jalgaon (M.S) 425107.

ABSTRACT

The anti-inflammatory activity of the aqueous extract of *Acalypha indica* Linn. Family *Euphorbiaceae*. Leaves were evaluated by in-vitro method. *Acalypha indica* leaves were defatted with petroleum ether (60-80^{0}c) and soaked in water. Aqueous extract were screened for in-vitro activity at the doses of 200mg/kg 400 mg/kg and 600 mg/kg using Mizushima and Kobayashi method. Ibuprofen (100 mg/kg) were used as standard reference drug. The % inhibition of denaturation is produced by *Acalypha indica* linn extract at the dose 200mg/kg, 400 mg/kg and 600 mg/kg were 44.5%, 62.2% and 81.1% respectively when compared to control. The % inhibition of denaturation is produced by *Acalypha indica* linn extract at the dose 600mg/kg were comparable with that produced by Ibuprofen (85.71%) which indicates that *Acalypha indica* Lin. leaves extracts possess significant anti-inflammatory activity.

KEYWORDS: anti-inflammatory, indian acalypha, Kuppaimeni, Mizushima and Kobayashi method, petroleum ether.

INTRODUCTION

Acalypha indica L. known as kuppaimani in Tamil[1] is an annual weed. It belongs to the family euphorbiaceae. It is common weed in many parts of Asia. It grows in the common farmlands gardens, roadside werete lands. Parts used leaves, root, stalk and flowers. Plants are emetic expectorant, laxative and diuretic, useful in bronchitis, pneumonia, asthma and

pulmonary tuberculosis. Leaves are laxative and antiparasiticide, ground with lime juice is used for ringworm and the paste is used as emetic for children A decocation of the leaves is given to children to expel worms; also given in the form of decoction with little garlic. In homeopathy, *Acalypha indica* (English: Indian acalypha, Indian nettle, three-seeded mercury French: Ricinelle des Indes, oreille de chatte, herbe chatt; Tamil: Poonamayakki, Kuppaimeni is a species of plant having catkin type of inflorescence. It occurs throughout tropical Africa and South Africa, in India and Sri Lanka, as well as in Yemen and Pakistan. It has possibly been introduced elsewhere as a weed. In West and East Africa the plant is used as a medicinal plant. It is a common herb growing up to 75 cm tall with ovate leaves. Flowers are green, unisexual found in catkin inflorescence. In West Africa the leaves are cooked and eaten as a vegetable. It is also browsed by cattle. This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body.[1]

Acalypha indica (English: Indian acalypha, Indian nettle, three-seeded mercury French: Ricinelle des Indes, oreille de chatte, herbe chatte Tamil: Poonamayakki, Kuppaimeni is a species of plant having catkin type of inflorescence. It. In West Africa the leaves are cooked and eaten as a vegetable. It is also browsed by cattle. This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body.[2,6]

Botanical Name	*Acalypha indica* L.
Family	EUPHORBIACEAE
Used In	Ayurveda, Folk, Homeopathy and Sidha
Distribution This species is globally distributed from Tropical Africa to Malesia. Within India, it occurs throughout the plains, reaching upto an altitude of 210 m. in Orissa and also found in the Western Ghats. It is a weed in gardens, in werete places and along the roadsides.	

Language (No. of Names)	Vernacular Name
English	indian acalypha
Hindi	khokali, khokla, khokli, kholi, kuppi, kuppikhokli, kuppu
Marathi	khajoti, khojoti, khokalee, khokali, khokla, khokli, kupameni, kupi, kuppi, mamjarshejari, petari, pitari, shendri
Sanskrit	aristamanjari, arittamanjarie, arittamunjari, arittamunjariye, arittamunjayrie, haritamanjari, manshinka, muktavarcca

GEOGRAPHIC DISTRIBUTION

Acalypha indica occurs widely throughout the tropics of the Old World. In Africa it occurs in Nigeria in West Africa and further widely throughout tropical Africa and the Indian Ocean islands. It also occurs in India, South East Asia and Oceania. It has been introduced to areas of the new world with favorable climates.

CHEMICAL CONSTITUENTS

The chemical compounds acalyphine and triacetoneamine have been extracted from *A. indica*. Other chemical constituents include cyanogenic glucosides and alkaloids.[1]

USES IN TRADITIONAL MEDICINE:- (*acalypha indica*)[4]

Acalypha indica is used as a purgative for which the plant is boiled and the extract is drunk. It is a very good remedy in the treatment of piles. The roots are used to remove intestinal parasites in children and given in the morning empty stomach. It work as mild laxative and also expels worms. Its leaves juice is given to children for getting relief from cold, cough and congestion. Leaves with turmeric are used getting relief from acne and pimples. The tribes in the Nilgiri hills uses its leaves paste to wereh and clean their bodies.

Indian Acalypha leaves juice can be applied externally in scabies and other skin diseases. Its leaves are also useful in bed sores. In congestive headache a piece of cotton saturated with its leaves juice can be inserted into each nostril. In cases of obstinate constipation of children the leaves ground into a paste and made into a ball and introduced into the rectum, relax the sphincter ani and produces free motions.[5]

A drug having a marked action on the alimentary canal and respiratory organs. It is indicated in incipient phthisis, with hard, racking cough, bloody expectoration, arterial hemorrhage, but no febrile disturbance. Very weak in the morning, gains strength during day. Progressive

emaciation. All pathological hemorrhages having notably a MORNING worse. WORSE in morning.

INTRODUCTION OF INFLAMMATION

Inflammation: A localized reaction that produces redness, warmth, swelling and pain as a result of infection, irritation, or injury. Inflammation can be external or internal.[6]

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 and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. Without inflammation, wounds and infections would never heal. Similarly, progressive destruction of the tissue would compromise the survival of the organism.[7,8]

Pain, heat, redness and swelling (dolor, calor, rubor, tumor) are the classic manifestations of the inflammatory process. Abnormalities of the joints of the spine, associated muscles, tendons, ligaments and bone structural abnormalities can all result in pain and need for neurosurgical consultations. Typically, patients will not require immediate surgical intervention and therefore require treatments to reduce pain and enhance quality of life activities.

In most cases, the genesis of pain is inflammatory, regardless of the etiology. With the elucidation of the role of inflammatory cytokines, there is now a clear understanding of the pathways by which many anti-inflammatory drugs can alleviate inflammation and relieve pain.[9]

TYPES OF INFLAMMATION

ACUTE INFLAMMATION

Starts rapidly (rapid onset) and quickly becomes severe. Signs and symptoms are only present for a few days, but in some cases may persist for a few weeks.[9]

Examples of diseases, conditions, and situations which can result in acute inflammation include:

- Acute bronchitis

- Infected ingrown toenail
- Sore throat from a cold or flu
- A scratch/cut on the skin
- Exercise (especially intense training)
- Acute appendicitis
- Acute dermatitis
- Acute tonsillitis
- Acute infective meningitis
- Acute sinusitis
- A blow.

CHRONIC INFLAMMATION

This means long-term inflammation, which can last for several months and even years. It can result from:

- Failure to eliminate whatever were causing an acute inflammation
- An autoimmune response to a self antigen - the immune system attacks healthy tissue, mistaking it (them) for harmful pathogens
- A chronic irritant of low intensity that persists.

Examples of diseases and conditions with chronic inflammation include:

- Asthma
- Chronic peptic ulcer
- Tuberculosis
- Rheumatoid arthritis
- Chronic periodontitis
- Ulcerative colitis and Crohn's disease
- Chronic sinusitis
- Chronic active hepatitis

THE FIVE CARDINAL SIGNS OF ACUTE INFLAMMATION - "PRISH"[10]

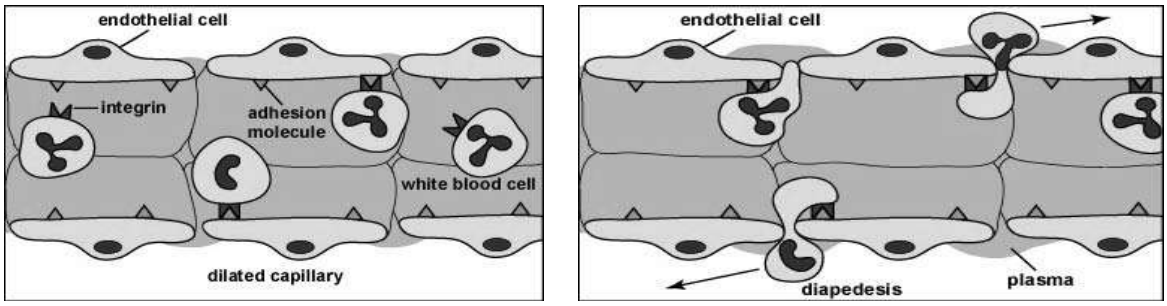
- **Pain** - the inflamed area is likely to be painful, especially when touched. Chemicals that stimulate nerve endings are released, making the area much more sensitive.
- **Redness** - this is because the capillaries are filled up with more blood than usual
- **Immobility** - there may be some loss of function
- **Swelling** - caused by an accumulation of fluid

- **Heat** - as with the reason for the redness, more blood in the affected area makes it feel hot to the touch.

The plants are one of the most important sources of medicines. India is known due to availability of several thousands of medicinal plants in the different bioclimatic zones anti-inflammatory diseases.

NATURAL COMPOUNDS FOR INFLAMMATION

Because of the significant side effect profiles of steroidal and NSAID medications, there is a greater interest in natural compounds, such as dietary supplement and herbal remedies, which have been used for centuries to reduce pain and inflammation. Many of these natural compounds also work by inhibiting the inflammatory pathways in a similar manner as NSAIDs. In addition to the COX pathway, many natural compounds act to inhibit nuclear factor-kB (NF-kB) inflammatory pathways.[12]



Cells are called out to the area of inflammation in a process called chemotaxis.

ANTI-INFLAMMATORY PLANTS AND HERBS

Chronic inflammation contributes to many painful conditions, including osteoarthritis and back pain. Anti-inflammatory medicines such as aspirin, ibuprofen and naproxen work well because they block the enzymes that trigger both swelling and pain.[11]

"There are natural alternatives to NSAIDs that have a similar mechanism," says Leopold. They include turmeric, green tea, ginger, rosemary, cat's claw, devil's claw and willow bark. Which pain supplement is best? Leopold and other experts single out turmeric. "It works really well," says Tanya Edwards, MD, medical director of the Center for Integrative Medicine at the Cleveland Clinic. "I've had patients with arthritis who start using turmeric and are able to go off their NSAIDs entirely." Look for turmeric in standardized capsules.

Because they work in the same way as NSAIDs,[12] these pain supplements do pose some of the same risks, such as bleeding and stomach upset. However, the side effects tend to be less severe. Leopold still suggests that people on blood-thinning drugs check with a doctor before using any of these pain supplements.

MATERIALS AND METHODS

The plant materials of *Acalypha indica* were collected from the region of satpuda and nearby territory. The plant were authenticated by Dr. Gautam P. Vadnere, Principal and Head, PG Dept. of Pharmacognosy, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda.

PREPARATION OF AQUEOUS EXTRACTS

The freshly collected leaves of this plant were chopped. Shed dried and coarsely powdered. The powder were defatted with petroleum ether ($60\text{-}80^0$c) and soaked in water. The aqueous extracts were dried under reduced pressure using a rotary vacuum evaporator. The percentage yield were 7% w/w for aqueous extract (CFA). The extracts were kept in refrigerator for further use.

ANTI-INFLAMMATORY ACTIVITY

All leaves extracts were screened for *in-vitro* anti-inflammatory activity using inhibition of albumin denaturation technique[12] which were studied according to Mizushima and Kobayashi[13,14] with slight modification. Dimethyl formamide (DMF) were used as solvent. No drug were added as control. Each experiment were done in triplicate and average were taken. Ibuprofen were used as standard drug. All the leaves extracts screened for *in-vitro* anti-inflammatory activity exhibited significant to moderate result. Compound N-cyclohexyl-5,6,7,8-tetrahydrobenzothiopheno[2,3-d]pyrimidin-4-amine (**4e)** showed highest percentage of denaturation as compare to standard ibuprofen. Result of anti-inflammatory activity is tabulated (Table 1).

PRESENT WORK

Inflammation involving the innate and adaptive immune systems is a normal response to infection. However, when allowed to continue uncheeked, inflammation may result in autoimmune or autoin-flammatory disorders. Neurodegenerative disease, or caner. A variety of safe and effective antiinflammatories agents are available, including aspirin and other nonsteroidal anti-inflammtories with many more drugs under development. Other anti-inflammatories. Currently in use or under development include statins, histone deacetylase

inhibitors, PPAR agonists and small RNAs. Anti-inflammtory[15] and Antioxidant activites of the aqueous (CFA) were assayed in wistar albino rats. The extracts were found to possess significant anti-inflammatory effect in both acute and chronic models. *Acalypha indica* Linn has many therapeutic uses. Therefore, we aimed to study its anti-inflammatory activity.[16] The aqueous extract of dired leaves of *Acalypha indica* Linn were prepared. The anti-inflammatory activity of these extracts were investigated using the in-vitro method. It were observed that aqueous extract showed maximum anti-inflammatory activity at600 mg/kg dose.[17,18,19] It showed maximum perccntage mhibinon of 84.69% which were comparable with the positive control. Ibuprofen, which showed 85% inhibition. Thus it could be concluded that *Acalypha indica* leaves extracts possess significant anti-inflammatory and anti oxidant properties.[20]

SCREENING FOR ANTI-INFLAMMATORY ACTIVITY (*IN-VITRO* MODELS*)*

The leaves extracts are screened for anti-inflammatory[21] activity by using inhibition of albumin denaturation technique which were studied according to Mizushima and Kobayashi with slight modification. The standard drug and test leaves extracts were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions were less than 2.0%. Test solution (1 ml) containing different concentrations of drug were mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$C in water bath for 10 min. After cooling the turbidity were measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation[22,23] was calculated from control where no drug were added. Each experiment was done in triplicate and average was taken. The Ibuprofen were used as standard drug. Results are tabulated in table no. 1.

$$\% \text{ of inhibition} = 100 \times - \left[1 - \frac{Vt}{Vc} \right]$$

Where,
Vt = Mean absorbance of test.
Vc = Mean absorbance of control.

Table No. 1: *In-vitro* Anti-Inflammatory Activity

Sr. No.	Treatment	Absorbance Value (Mean ± SE)	Inhibition of Denaturation (in %)
01	Control	0.098 ± 0.007	----
02	Ibuprofen	0.182 ± 0.002	85.71
03	200 mg/kg	0.141 ± 0.008	43.87
04	400 mg/kg	0.168 ± 0.003	71.42
05	600 mg/kg	0.181 ± 0.004	84.69

RESULT AND DISCUSSION

In the present study, leaves extract of *Acalypha indica*, were studies for anti-inflammatory activity by in-vitro method. The extracts were found to possess significant activity at both selected doses i.e. 200, 400 and 600 mg/kg body weight, *Acalypha indica* showed comparable results with the standard drug. Aqueous extracts showed significant anti-inflammatory activity. This aqueous extracts showed maximum anti-inflammatory activity at 600 mg/kg dose. It showed maximum percentage inhibition of 84.69%, which were comparable with the positive control, ibuprofen, which showed 85.71% inhibition.

CONCLUSION

Combinatorial chemistry[27,28] should be approached to the tremendous impact of the identification of new leads. Its primary goal is to design and produce highly diversified molecular libraries to be screened on selected and vast majorities have definitely caught the attention of pharmaceutical companies. It offers higher productivity with lower expenses. These technique has definitely decreased the cost associated with the drug research and increased the chances of finding new lead molecule.

REFERENCES

1. Thomas M.Walter* Review of Acalypha indica, Linn in Traditional Siddha Medicine. indian Materia Medica by Dr. K.M. Nadkarni, Volume I, pages: 17-19, http://indiabiodiversity.org/species/show/32852.
2. Materia Medica (Vegetable section), Volume I, by Dr. Murugesa Muthaliar, pages: 359, publisher, Tamilnadu Siddha Medical Council, Chennai. Fourth edition, 1988.
3. Seebaluck R[1], Gurib-Fakim A[2], Mahomoodally F[3] Medicinal plants from the genus Acalypha (Euphorbiaceae)--a review of their ethnopharmacology and phytochemistry. J Ethnopharmacol. 2015 Jan 15; 159: 137-57. doi: 10.1016/j.jep.2014.10.040. Epub 2014 Oct 30.
4. Kirtikar KR and Basu BD, Indian Medicinal Plants, Orient Longman Ltd, Dehra Dun 1998.
5. Vame JR and Botting RM, New insight in to the mode of actionof anti-inflammatory drugs, *inflamm* Res, 1995; 44: 1-10.
6. Iwueke AV, Nwodo Of and Okoli CO, Evalution of the anti-inflammatory and analgesic activities of *Vitex doinana leaves*, *African J Biotech*, 2006; 5: 1929-35.

7. Paul A. Insel; Analgesic, antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout; Goodman and Gillman's The pharmacological basis of therapeutics, 9th Int Ed.; McGraw Hill health professions division-New York; 1995; 615-657.
8. Gandhism R, Thamaraichelvan A and Baburaj, Anti-inflammatory action of *Lannea coromandelica* HRBC membrane stabilization, Fitotherapia, 1991; 62: 82-83.
9. Chou CT, The anti-inflammatory effects of *Tripterygium wilfordii* Hook. On adjuvant induced paw edema in rats and inflammatory mediators release, *phyrother Res*, 1997; 11: 152.
10. Sreenivasa Rao D, Jayachandran E, Sreenivasa GM and Shivakumar B. Inhibition of albumin denaturation and anti-inflammatory activity of 2-[N-4-Tolyl sulphon hydrazino]-6-fluoro-7-substituted (1,3) benzothiazoles Oriental Journal of Chemistry, 2005; 21(1): 113-116.
11. Mizushima Y and Kobayashi M; Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins; J Pharm Pharmacol; 1968; 20: 169.
12. Y. Mizushima; Inhibition of protein aggregation by vitamin E and selenium; The Lancet; 1965; 2: 169.
13. M Aminur Rahman1, Sitesh C Bachar2 And Mohammed Rahmatullah1* Analgesic And Antiinflammatory Activity Of Methanolic Extract Of *Acalypha Indica* Linn. Pakistan Journal Of Pharmaceutical Sciences. July 2010.
14. Ghani A, Medicinal plants of Banglades, chemical constituents and usesm 2nd ed, The Asiatic Society of Bangladesh, Dhaka, 2003; 63-438.
15. Shivayogi PH, Rudresh K, Shrishailapa B, Saraswati BP and Somnath RP, Post-coital antifertility activity of *Acalypha indica* L., *J Ethnopharmacol*, 1999; 67: 253-58.
16. Annie S, Rajendran K, Ramgopal B and Dinesh Kumar C, Neutrali Zation potential of *Viper russelli russelli* (Russell's viper) Venom by ethanol leaf extract of *Acalypha indica*, *J Ethnopharmacol*, 2004; 94: 267-273.
17. Suresh Reddy J, Rafgeswara Rao P and Mada SR, Wound healing effects of *Heliotropium indicum*, *plumbago zeylanicum* and *Acalypha indica* in rats, J *Ethnopharmacol*, 2002; 79: 249-251.
18. Ruchi GM, Majekodunmi OF, Ramla M, Gouri BV, Hussain A and Suad Khamis SB, Antioxidants capacity of some edible and wound healing plants in Oman, *Food chem*., 2007; 101: 465-470.

19. LAD Williams et al; The *in-vitro* denaturation effects induced by natural products and non-steroidal compounds in heart treated (Immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process; West Indian Med J; 2008; 57(4): 327-331.
20. Mihana Vamsi N, Venkata Sunil Kumar M, Kidabdaram N and Padamanabha Reddy Y, Evaluation of anti-inflammatory activity of *Acalypha indica*, *Indian Pharm*, 2008; 7: 89-91.
21. Elias G and Rao M N A.; Inhibition of albumin denaturation and anti-inflammatory Singh DAP, Raman M, Saradha V, Jayabharathi P and Kumar VRS, Acaricidal property of kuppaimeni (*Acalypha indica*) against natural *Psoroptes cuniculi* infestation imbroiler rabbits, Indian J Anim Sci, 2004; 74: 1003-1006.
22. Das AK, Ahmed F, Biswere NN, Dev S and Masud MM, Diuretic bacxtivity of *Acalypha indica*, *Dhaka Univ JPharm sci*, 2005; 4: 1-2.
23. Govindarajan M, jebanesan A, Reetha D, Amsath R, Pushpanathan T and Samidurai K, Antibacterrial activity of *Acalypha indica* L., *Eur Rev Medpharmacol Sci*, 2008; 12: 299-302.
24. Henrick R Honsen C, Combinatorial chemistry CHEM 553 Winter 2003.
25. Dongre S K, Sharma S V & Suresh B, Application in drug discovery, IJPE, 2004; 38.
26. Srinivasa G.M, Jayachandran E, Shivakumar B, Sreenivasa Rao D. Synthesis and pharmacological screening of 2-[3-amino,5-s-met carboxamido pyrazol-1-yl]6-fluoro,7-substituted (1,3)benzothiazole; Oriental Journal of Chemistry, 2004; 20(1): 103-110.
27. Rang H. P., Dale M. M., Ritter J. M.; Anti-inflammatory and immuno-suppresant drugs pharmacology; 3 Int Student Ed; 1995; 246-266.