INVESTIGATION OF SELECTED MEDICINAL PLANTS AND MARKETED FORMULATION FOR THEIR ANTI-INFLAMMATORY AND ANTI-OSTEOARTHRITIS ACTIVITY

Thesis to be submitted to
The Tamilnadu Dr. M.G.R. Medical University, Chennai,
in partial fulfilment of the requirements for the award of

DOCTOR OF PHILOSOPHY

Submitted by
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Under the guidance of
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MARCH 2010

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DECLARATION

I hereby declare that the thesis entitled **"Investigation of Selected Medicinal Plants and Marketed Formulation for their Anti-inflammatory and Anti-Osteoarthritis Activity"** submitted by me to The Tamilnadu Dr. M.G.R.Medical University, Chennai, for the award of Degree of Doctor of Philosophy in Pharmacy, is the result of my original and independent work carried out at Department of Pharmacology, J.S.S.College of Pharmacy, Ootacamund, under the supervision of **Dr. K.Elango,** Professor, J.S.S.College of Pharmacy, Ootacamund. The thesis or any part thereof has not formed the basis for the award of any degree, diploma, associateship, fellowship, or any other similar title, of this or any other University, previously.

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CERTIFICATE

Title of the Project: Investigation of Selected medicinal plants and ~~mastcated~~ formulation for their Anti-Inflammatory and ~~and~~ anti-Osteo arthritis activity.

Proposal Number: JSSCP|IAEC|PH.D|PH-COLOGY/01|2007-08.

Date received after modification (if any):
Date received after second modification: 10.03.2007.

Approval date: 05.09.07

Animals: Wistar rats/ Albino mice
~~Rabbits~~ / Guinea pigs

Phase-I
Rabbit-1
No. of animals sanctioned: mice-42
Rats 66

Male/Female

Phase-II
Rats-240.

Expiry date (Termination of the Project): 3 Months

Name of IAEC/CPCSEA chairperson: **Prof. K. Elango**

Signature of Chairperson
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Dedicated to my lovable

Mother, Father

&

Lord Sri Venkateswara

ACKNOWLEDGEMENT

It gives me an immense pleasure to put my dissertation work in this form. Let me acknowledge all those, who knowingly or unknowingly contributed this work.

The Prime personality, who is the real man behind this, is my **Dynamic Guide, Dr. K.Elango,** M.Pharm, Ph.D, Principal & Professor of Pharmacology, J.S.S.College of Pharmacy, Ootacamund. His words of wisdom, the profound knowledge and the subject understanding influenced me very well. His keen interest and the untired efforts made me easy to carry out all the work with full zeal and satisfaction. I take this opportunity to say a lot of thanks to him.

I express my profound gratitude to my **Co-Guide, Dr.S.Manimaran**, M.Pharm, Ph.D., Assistant Professor, Department of Phytopharmacy & Phytomedicine, J.S.S.College of Pharmacy, Ootacamund, for his support and encouragement through out my work.

My heartful thanks to **Dr.M.J.Nanjan**, Ph.D., Director, Research Studies & TIFAC CORE HD, J.S.S.College of Pharmacy, Ootacamund for his encouragement and valuable suggestions during my entire work.

I express my sincere thanks to **Dr. M.N.Satish Kumar**, M.Pharm.,Ph.D., Professor & Head, **Prof. V.B.Desai**, M.Pharm., **Mr.R.Vadivelan**, M.Pharm., Assistant Professor and **Mr.A.Shanish Antony,** M.Pharm., Lecturer, Department of Pharmacology, J.S.S.College of Pharmacy, Ootacamund, for their valuable suggestions and constant support.

I am thankful to **Dr. B.Durai Swamy**, M.Pharm, Ph.D., Professor & Head, Department of Pharmacognosy and **Dr. S.P.Dhanabal**, M.Pham, Ph.D, Professor &

Head, Department of Phytopharmacy & Phytomedicine, for their help and suggestions to carry out the phytochemical studies.

I would like to thank **Dr.P.Vijayan**, Ph.D., Head, Department of Pharmaceutical Bio-Technology, **Dr. Raghu Chandrasekhar**, M.Pharm, Ph.D., and **Dr.G.Ashok**, M.Pharm, Ph.D, J.S.S.College of Pharmacy, Ootacamund , for their timely help to carry out the *in vitro* studies.

My sincere thanks to **Prof. K.Chinna Swamy,** M.Sc (Pharm), J.S.S.College of Pharmacy, Ootacamund, for his encouragement and valuable suggestions during my entire work.

I am thankful to **Dr.S.Mahesh Kumar**, M.Pharm, Ph.D., **Dr.B.Praveen**, M.Pham., Ph.D., **Dr.D.Anil Kumar,** M.Pham., Ph.D., **Mr.Saleem,** M.Pharm., and **Mr.Chinni.Santivardan, (**M.Pharm)., for their encouragement and valuable suggestions.

I express my sincere gratitude to **Dr. S.Gopalakrishnan**, Ph.D., Department of Pharmaceutics, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, for his encouragement during my studies.

I express my sincere thanks to **G.V.S.Murthy,** Joint Director, Botanical Survey of India, Coimbatore, for the identification of plant materials.

I would like to thank my colleagues, for their help during my work.

I would like to thank **Mr.S.Putturajappa**, B.Com., Superintendent, J.S.S.College of Pharmacy, Ootacamund, for his support and encouragement.

My sincere thanks to all the supporting staff of this institution for their help and support.

I wish to place my exquisite thanks to my dearest Professor, **Dr. B.Suresh,** M.Pharm, Ph.D., D.Ph., Vice Chancellor, J.S.S.University, Mysore and President, Pharmacy Council of India, New Delhi, for his moral support and timely suggestions during the entire period of my work.

I dedicate this thesis to my lovable **mother, father & Lord Sri Venkateswara**. My parent's moral support, encouragement and the love always made me strong to complete my Doctoral Studies. Also, I owe my thanks to my dearest wife **Sri Devi,** my dearest daughter **Brahma Sripriya**, my dearest son **Brahma Sri Ram**, my dearest sister **Rajeswari** and brother **Suresh** for their nice attitude and emotional support.

Above all, I bow my work in the feet of **'Lord Sri Venkateswara'** who was with me and lead to the actualization of this research work.

I submit my sincere pranams to His Holiness **Jagadguru Sri Sri Shivarathri Deshikendra Mahaswamigalavaru** of Sutturmutt, Mysore.

(Desu Brahma Srinivasa Rao)

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1. INTRODUCTION

1.1 The World Health Organization and traditional medicine

‘DEAD AS THE DODO’

‘Dead as the dodo’. That is what many health professionals and authorities would wish traditional medicine to be and what an even greater number of them believe it to be. Traditional medicine seems, however, to be alive and thriving-to such an extent, indeed, that it has been estimated to be the principal, if not the only, source of medical care for two-thirds of the world’s population. In India alone, there are more than half a million traditional practitioners and some 250 hospitals and 15000 dispensaries offering traditional treatment, and there are just as many Ayurvedic colleges as there are modern medical colleges; in the Philippines, the total number of *Mots* (traditional birth attendants) is approximately 40000, or roughly one for every barangay, the smallest administrative unit, with a population of 1000 to 2000; similarly, there are 20000 *bomohs* (spiritual healers) ministering to the health needs of 12 million Malaysians, while there are fewer than 2500 physicians. [1]

The extensive use of, and reliance on, traditional medicine is not only the result of a lack of modern services. Such services are certainly scarce in many rural parts of the developing world, but even where they do exist they may be poorly utilized, the local population using- and even showing a preference for-the services of traditional healers, with whom they can usually communicate more easily than they can with the personnel of the official health services. [1]

Even in countries deeply permeated by the scientific-technological approach to medicine, the population still cherishes many traditional healing practices and relies on traditional medicine to a greater extent.

The divergence between the two systems of medicine, ‘modern’ and ‘traditional’, almost exactly parallels the division of the world between the rich and the poor. Traditional medicine is identified with socioeconomic underdevelopment and is considered an indication of backwardness. The fact that official health services have generally been reluctant to recognize and evaluate the contribution which traditional medicine is making to the physical, and particularly the psychological, welfare of populations and have tended to disparage rather than cooperate with the traditional systems has not, however, deterred the World Health Organization from promoting traditional medicine because the Organization is

convinced that this constitutes a substantial resource that could be utilized for purposes of primary health care amongst the underserved populations of the world. [1]

Table 1. Ten leading causes of death, 2002. [2]

		% of total deaths
All countries		
1	Ischaemic heart disease	12.6%
2	Cerebrovascular disease	9.6%
3	Lower respiratory infections	6.6%
4	HIV/AIDS	4.9%
5	Chronic obstructive pulmonary disease	4.8%
6	Perinatal conditions	4.3%
7	Diarrhoeal diseases	3.1%
8	Tuberculosis	2.8%
9	Trachea, bronchus, lung cancers	2.2%
10	Malaria	2.1%

Table 2. Ten leading causes of YLD, global estimates for 2002. [2]

		% of total YLD
All countries		
1	Unipolar depressive disorders	11.8%
2	Hearing loss, adult onset	4.6%
3	Cataracts	4.5%
4	Alcohol use disorders	3.3%
5	Maternal conditions	3.3%
6	Schizophrenia	2.8%
7	Perinatal conditions	2.7%
8	**Osteoarthritis**	2.6%
9	Vision loss, age-related and other	2.5%
10	Bipolar affective disorder	2.5%

YLD- Years lived with a disability

1.2 Arthritis

Arthritis (from Greek *arthro-*, joint + *-itis*, inflammation; plural: arthritides) is a group of conditions involving damage to the joints of the body.

There are different forms of arthritis and each has a different cause. The most common form of arthritis, **osteoarthritis** (degenerative joint disease) is a result of trauma to the joint, infection of the joint, or age. Other arthritis forms are **rheumatoid arthritis** and **psoriatic arthritis**, autoimmune diseases in which the body attacks itself. **Septic arthritis** is caused by joint infection. **Gouty arthritis** is caused by deposition of uric acid crystals in the joint, causing inflammation. There is also an uncommon form of gout caused by the formation of rhomboid crystals of calcium pyrophosphate. This gout is known as pseudogout. [3]

Arthritis sufferers include men and women, children and adults. Approximately 350 million people worldwide have arthritis. More than half of those with arthritis are under 65 years of age.

Regardless of the type of arthritis, the goals of arthritis treatment are similar. These include the following:

- Relieve pain / inflammation
- Minimize risks of therapy
- Retard disease progression
- Provide patient education
- Prevent work disability
- Enhance quality of life and functional independence

While the goals are similar they are achieved using different approaches depending on the diagnosis. The effective management includes a combination of conventional medicines, effective alternative treatments, changes in diet and food, rest, exercise, lifestyle changes (e.g., weight loss if needed), and joint protection.Factors involved in decision making

include the diagnosis, the severity of disease, and the patient's response to previous therapies. [4]

1.2.1 Medical Treatment

Not every person with arthritis requires medical attention. For example, some patients with osteoarthritis have minimal or no pain and may not need treatment. However, for those with persisting joint symptoms, the ideal steps to take should lead to a proper diagnosis and an optimal long-term treatment plan. This plan must be customized for each person affected, depending on the joints involved and the severity of symptoms. [4]

1.2.2 Medications

For many patients with arthritis, mild pain relievers such as aspirin and acetaminophen (Tylenol) may be sufficient treatment. Studies have shown that acetaminophen given in adequate doses can often be equally as effective as prescription anti-inflammatory medications in relieving pain in osteoarthritis. Since acetaminophen has fewer gastrointestinal side effects than NSAIDS, especially among elderly patients, acetaminophen is often the preferred initial drug given to patients with osteoarthritis. Pain-relieving creams applied to the skin over the joints can provide relief of minor arthritis pain. Examples include capsaicin, salycin, methyl salicylate, and menthol. [5]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are medications that are used to reduce pain as well as inflammation in the joints. Examples of NSAIDs include aspirin (Ecotrin), ibuprofen (Motrin), nabumetone (Relafen), and naproxen (Naprosyn). It is sometimes possible to use NSAIDs temporarily and then discontinue them for periods of time without recurrent symptoms, thereby decreasing the risk of side effects. This is more often possible with osteoarthritis because the symptoms vary in intensity and can be intermittent. The most common side effects of NSAIDs involve gastrointestinal distress, such as stomach upset, cramping diarrhoea, ulcers, and even bleeding. The risk of these and other side effects increases in the elderly. Newer NSAIDs called cox-2 inhibitors have been designed that have less toxicity to the stomach and bowels.

Some studies, but not all, have suggested that the food supplements glucosamine and chondroitin can relieve symptoms of pain and stiffness for some people with osteoarthritis.

These supplements are available in pharmacies and health-food stores without a prescription, although there is no certainty about the purity of the products or the dose of the active ingredients because they are not monitored by the FDA. The U.S. National Institutes of Health (NIH) is studying glucosamine and chondroitin in the treatment of osteoarthritis. Their initial research demonstrated only a minor benefit in relieving pain for those with the most severe osteoarthritis. Further studies, it is hoped, will clarify many issues regarding dosing, safety, and effectiveness of these products for osteoarthritis. Patients taking blood-thinners should be careful taking chondroitin as it can increase the blood-thinning effect and cause excessive bleeding. Fish oil supplements have been shown to have some anti-inflammation properties, and increasing the dietary fish intake and/or taking fish oil capsules (omega-3 capsules) can sometimes reduce the inflammation of arthritis. [5]

Cortisone is used in many forms to treat arthritis. It can be taken by mouth, given intravenously, and injected directly into the inflamed joints to rapidly decrease inflammation and pain while restoring function. Since repetitive cortisone injections can be harmful to the tissue and bones, they are reserved for patients with more pronounced symptoms.

For persisting pain of severe osteoarthritis of the knee that does not respond to weight reduction, exercise, or medications, a series of injections of hyaluronic acid (Synvisc, Hyalgan, and others) into the joint can sometimes be helpful, especially if surgery is not being considered. These products seem to work by temporarily restoring the thickness of the joint fluid, allowing better joint lubrication and impact capability, and perhaps by directly affecting pain receptors.

Arthritis that is characterized by a misdirected, overactive immune system (such as rheumatoid arthritis or ankylosing spondylitis) frequently requires medications that suppress the immune system. Medications such as methotrexate (Rheumatrex, Trexall) and sulfasalazine (Azulfidine) are examples. Newer medications that target specific areas of immune activation are referred to as biologics (or biological response modifiers). Sometimes combinations of medications are used. All of these medications require diligent, regular dosing and monitoring.

1.2.3 Surgery

Surgery is generally reserved for those patients with arthritis that is particularly severe and unresponsive to the conservative treatments. Surgical procedures can be performed to relieve pain, improve function, and correct deformity. Occasionally, joint tissue is surgically removed for the purpose of biopsy and diagnosis. [5]

Joint surgery using a viewing tube with a cutting instrument is called arthroscopy. Osteotomy is a bone-removal procedure that can help realign some of the deformity in selected patients, usually those with knee disease. Removal of inflamed joint lining tissue is called synovectomy. In some cases, severely degenerated joints are best treated by fusion (arthrodesis) or replacement with an artificial joint (arthroplasty). "Total joint replacement" is a surgical procedure whereby a destroyed joint is replaced with artificial materials. For example, the small joints of the hand can be replaced with plastic material. Large joints, such as the hips or knees, are replaced with metals. Total hip and total knee replacements are now commonplace. These can bring dramatic pain relief and improved function.

1.2.4 Other Therapy

Patients with arthritis may benefit from conservative measures such as rest, exercise, weight reduction, physical and occupational therapy, and mechanical support devices. These measures are particularly important when large, weight-bearing joints are involved, such as the hips or knees. In fact, even modest weight reduction can help to decrease symptoms of osteoarthritis of the large joints, such as the knees and hips. Medications are used in combination with the physical measures. [5]

Resting sore joints decreases stress on the joints, and relieves pain and swelling. Patients are asked to simply decrease the intensity and/or frequency of the activities that consistently cause joint pain.

Exercise usually does not aggravate arthritis when performed at levels that do not cause joint pain. Exercise can be helpful in several ways. First, it strengthens the muscular support around the joints. It also prevents the joints from "freezing up" and improves and maintains joint mobility. Finally, it helps with weight reduction and promotes endurance. Applying

local heat before and cold packs after exercise can help relieve pain and inflammation. Swimming is particularly suited for patients with osteoarthritis because it allows patients to exercise with minimal impact stress to the joints. Other popular exercises include walking, stationary cycling, and light weight training.

Physical therapists can provide support devices, such as splints, canes, walkers, and braces. Toilet seat raisers and jar grippers can assist daily living. These devices can be helpful in reducing stress on the joints. Occupational therapists can assess daily activities and determine whether additional devices may help patients at work or home. Finger splints can support individual joints of the fingers. Splints are commonly used in the treatment of wrist arthritis. Paraffin wax dips, warm water soaks, and nighttime cotton gloves can help ease hand symptoms. Spine symptoms can improve with a neck collar, lumbar corset, or a firm mattress, depending on what areas are involved. [5]

1.3 The main complementary and alternative therapies for arthritis

Complementary and alternative therapies which are widely used and where there is some research supporting their effectiveness in the treatment of arthritis.

1.3.1 Acupuncture

Acupuncture involves inserting fine needles at particular points in the skin. The needles may be stimulated manually, by heat (with a dried herb called moxa) or by a small electrical current (electroacupuncture). The needles are very fine, so having them inserted is rarely painful. Sometimes a sensation of heaviness or tingling may be felt at the insertion site, and this is considered a good sign. [6]

First used in China over 2000 years ago, acupuncture is now widely used in physiotherapy and pain relief. There are two main forms of acupuncture: traditional Chinese and modern Western acupuncture.

- Traditional acupuncture is based on Chinese philosophy; the needles are inserted in 'meridians' – imaginary lines running round the body.

- Western acupuncture is less theoretical and usually involves a smaller number of needles, often inserted close to the painful part.

Acupuncture seems to relieve pain by diverting or changing the painful sensations which are sent to the brain from damaged tissues and by stimulating the body's own pain-relieving hormones (endorphins and encephalins). This pain-relieving effect may only last a short time at the beginning, but repeated treatment (usually weekly for 6 or 8 sessions) can bring long-term benefit, often for 6–9 months or longer. If the pain returns, then some more acupuncture may help for another few months. [6]

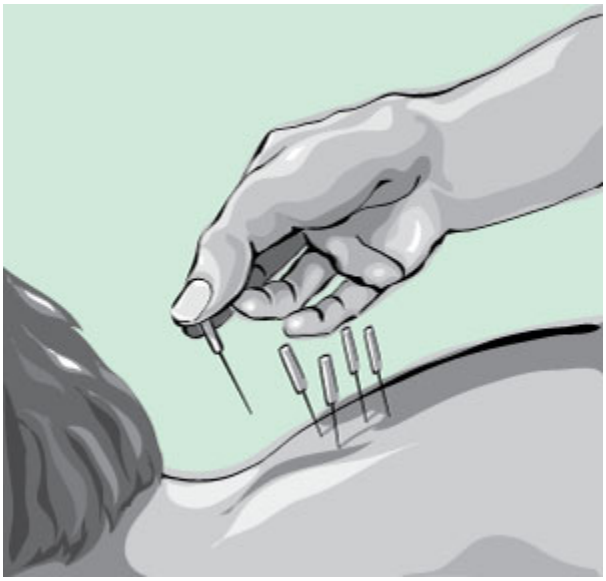


Figure 1. Acupuncture can relieve pain by breaking the pain cycle.

As with all treatments to relieve pain (including physiotherapy and painkilling drugs), breaking the 'pain cycle' sometimes gives permanent relief. To some extent, this depends on the stage of arthritis, although acupuncture can help at almost any stage of the illness. As with many conventional treatments, it cannot cure or reverse the process of arthritis.

Acupuncture generally has a very good safety record, but there are certain risks. It can transmit diseases if single-use needles are not employed every time. It occasionally causes bleeding, and very rarely internal organs may be punctured, including the lung (causing collapse of the lung, known as pneumothorax). Much more common is short-lived dizziness

or faintness after treatment.Acupuncture may help people who cannot tolerate drugs get through a painful episode, or it may be used to manage pain on a long-term basis. Acupuncture is widely accepted as being an effective treatment for pain, and there is now clear scientific evidence that it works for conditions such as osteoarthritis of the knees and low back pain. Some therapies related to acupuncture involve pressure on acupuncture points, but without piercing the skin with needles: these include acupressure and shiatsu. Reflexology is similar but uses pressure on the feet only.

1.3.2 Diet and nutritional supplements

A good diet is essential for health, and many complementary and alternative therapists advise on diet. Diets can help many people with arthritis, both inflammatory and osteoarthritis. The most important thing for many overweight people who suffer from arthritis of the weight-bearing joints (back, hips, knees, ankles and feet) is to lose weight. A Mediterranean-style diet rich in fruit and vegetables, oily fish, nuts, seeds and olive oil with reduced amounts of red meat may help in the control of rheumatoid arthritis as well as reducing the risk of heart disease. [6]

1.3.3 Food and food supplements for arthritis

A well-balanced diet is essential for health. At least five portions of fruit and vegetables per day are advised for good health.

The omega-3 polyunsaturated fatty acids EPA and DHA (eicosapentaenoic and docosahexaenoic acids) found particularly in oily fish can be helpful for inflammatory arthritis (including rheumatoid arthritis, reactive arthritis, psoriatic arthritis and ankylosing spondylitis, but not gout). In inflammatory type of arthritis other than gout it is a good idea to eat oily fish twice a week. It is very important not to confuse fish oil with fish *liver* oil (cod liver oil or halibut liver oil). Fish *liver* oil is a good source of vitamins A and D, but large doses can be dangerous, especially to unborn babies. In gout oily fish should generally be avoided because of the high purine content. [6]

Omega-6 fatty acids, as found in sunflower and corn oils and in margarines made from these oils, are not beneficial for arthritis and may interfere with the beneficial effects of omega-3, so they are best kept to a minimum. It is important to include in diet plenty of fruit and vegetables high in antioxidants, including vitamin E and selenium. Good sources of vitamin E include wheat germ, sunflower seeds, nuts and avocado. Mild selenium deficiency is quite common and it has been suggested that deficiency may result in a more rapid progression of arthritis, although there is doubt about this. The richest natural source of selenium is Brazil nuts, but meat and fish also contain some.

Slight vitamin D deficiency is not uncommon, especially in winter in the UK. There is some research which suggests that some people who have a low dietary intake of vitamin D may be more likely to develop rheumatoid arthritis. The effect of vitamin D supplements on the progression and pain of knee osteoarthritis is also currently being evaluated. The vitamin D content of most foods is low. The best sources are oily fish, particularly grilled herrings and canned salmon. It is made in the skin by the sun during the summer months, or can be taken as a supplement. [6]

1.3.4 Glucosamine sulphate and chondroitin for osteoarthritis

Osteoarthritis is the commonest form of arthritis. It particularly affects the weight-bearing joints of the legs and back, becoming more common with age. Many people take glucosamine sulphate tablets with or without added chondroitin. Cartilage contains substances related to glucosamine and chondroitin and taking supplements of these natural ingredients may nourish damaged cartilage. The research findings are conflicting, but more evidence is accumulating that some people, at least, will benefit from this therapy. Much of the research has been undertaken on people taking a combined daily dose of 1500 mg of glucosamine and 1200 mg of chondroitin and so this would be a good combination dose. They can also be taken separately. Glucosamine should not be taken by people who have an allergy to shellfish, although there is now a vegetarian version that can be substituted. In some people glucosamine can increase the level of sugar in the blood, so if one has diabetes is sure to check blood sugar and discuss with the doctor if blood sugars seem to be higher. Also if people are taking warfarin their blood-thinning control (international normalized ratio

or INR) may be affected. Patients can try glucosamine and chondroitin supplements for 3 months and if joint pain is much improved they may wish to continue with them. [5, 6]

1.3.5 Homoeopathy

Homoeopathy (from the Greek words meaning 'similar suffering' – now sometimes spelled 'homeopathy') is based on the idea of 'treating like with like'. So for a hot, swollen, tender joint a homoeopath might prescribe apis – made from bee-stings, which can cause hot, swollen, tender swellings. The controversial aspect of homoeopathy is its use of extremely dilute medicines (sometimes called remedies).[5,6]

Figure 2. In homoeopathy, only tiny doses of the active ingredient are used.

Homoeopathy is not a regulated profession, so people need to be cautious in selecting a practitioner. Some doctors have studied homoeopathy in depth and may use these therapies in addition to conventional medicines in a truly complementary manner.

Homoeopaths often advise changes in lifestyle, which could include changing diet, more relaxation or exercise. Homoeopathy is generally very safe, although sometimes the right

medicine can cause an 'aggravation' – a temporary flare-up of symptoms, usually a good sign.

A number of carefully controlled trials have been carried out with homoeopathic medicine, some of them involving arthritis. Overall the research suggests that homoeopathy is better than a placebo (a 'dummy' drug with no active ingredient) for conditions including osteoarthritis and fibromyalgia. Homoeopathic remedies need to be prescribed on an individual basis, so there is no particular remedy for arthritis, but rather for the person who has arthritis.

1.3.6 Magnet therapy

One of the biggest surprises thrown up by recent research is the finding that magnets can be helpful for pain, including low back and knee pain. Magnets can be used in different ways, including bracelets, magnets taped to joints, and various magnetic devices, including mattress pads! Unfortunately the evidence is too fragmented to make clear recommendations on the kind of magnet therapy. But it is very safe, so it may be worth experimenting. [6]

1.3.7 Manipulative therapies: chiropractic, osteopathy and manual medicine

Manipulative therapies include chiropractic, osteopathy and manual medicine. They are used mainly for musculoskeletal problems, including spine, neck and shoulder disorders, joint, posture and muscle problems, sciatica, sports injuries, whiplash, and repetitive strain injury. The best-known technique is the 'high-velocity thrust' – a short, sharp motion, usually applied to the spine, which often produces the sound of a joint 'cracking' – but many other methods are also used. These procedures are undertaken by many health care professionals including doctors, physiotherapists, and osteopaths and chiropractors, who are now registered health professionals in the UK. Such treatments should include not only hands-on manipulative therapy but also advice on diet, exercise and lifestyle. [6]

Various kinds of manipulation are effective for low back and neck pain. For the best results one need to combine manipulation with exercise and other lifestyle changes.One should not use manipulative therapies if they have a circulatory problem affecting the spine, severe

osteoporosis, malignant or inflammatory spine conditions or recent fractures or dislocations, or if they are on anti-clotting drugs. The most serious risks of osteopathy and chiropractic are stroke and spinal cord injury after manipulation of the neck. Estimates of how often such severe problems occur vary, but they are very rare. Osteopaths and chiropractors should look out for the risk factors. Slight discomfort at the site of manipulation for a few hours afterwards is quite common.

1.3.8 Relaxation and hypnosis

There are many forms of relaxation and meditation techniques. Hypnosis is a deeply relaxed state, induced by a practitioner, in which patients are given therapeutic suggestions to encourage changes in behavior or relief of symptoms. Hypnosis for someone with arthritis might include a suggestion that the pain can be turned down like the volume of a radio.There are several methods that involve progressive relaxation of the muscles. Visualization involves achieving a relaxed state through picturing healing images. In autogenic training, one has to concentrate on experiencing physical sensations, such as warmth and heaviness, in different parts of the body in a learnt sequence. Meditation involves concentration on breathing or a sound (called a mantra). Yoga, which originated in India, and tai chi and qigong, both Chinese in origin, combine meditation with slow, gentle movement. Most relaxation techniques need to be practiced daily. Typically, one would learn the method in a course of 8 weekly classes lasting an hour or so, and practice by themselves for 15–30 minutes a day.

There is quite good evidence that these techniques can help with pain and associated symptoms such as anxiety. Some may also help with movement and flexibility. They are safe, although there are a few reports of problems associated with extreme yoga positions. [5, 6]

1.3.9 The Alexander technique

At the end of the nineteenth century the Australian actor F Matthias Alexander developed the technique known by his name in order to improve his voice. The Alexander technique educates the sense of body position and movement, eliminating bad habits of posture, muscle tension and movement. It is really a’re-education' method rather than a therapy, and

practitioners call themselves teachers. It may be helpful in preventing problems such as low back pain and repetitive strain injury (RSI). There is little published research on the Alexander technique for arthritis, but many people report benefit and it is safe. [5, 6]

1.3.10 Aromatherapy

Aromatherapy is the therapeutic use of scented essential oils. The oils may be inhaled, used in the bath, or massaged into the skin. When used for massage they are diluted in a 'carrier' oil. Many different oils can be used. For back pain, for instance, an aroma therapist might select lavender or marjoram to relieve muscle spasm or ginger if there is a circulatory problem. Other oils such as rosemary or peppermint are considered to have stimulating properties.

The oils are very concentrated, and should never be applied to the skin undiluted. They may be harmful in large quantities, particularly to pregnant women. In practice aromatherapy is very safe, apart from occasional allergic reactions. There is little evidence that it is effective in arthritis, although there is some evidence that it is beneficial in other painful conditions, and helpful with anxiety. Many people with chronic pain do report that an aromatherapy massage gives several weeks' relief. [5, 6]

1.3.11 Copper bracelets

Many people with arthritis wear copper bracelets. Research has shown that people with arthritis do have enough copper in their bodies for normal health. So it is difficult to understand what effect these bangles can have. There is no current research supporting the use of copper bangles. [6]

1.3.12 Healing

Healing may take many forms, such as faith healing, the 'laying on of hands', spiritual healing, lay healing, or 'distance' ('absent') healing. Healing has close links with specific belief systems, which may be religious, spiritual, social or cultural.

In a healing session, the healer will try to assess patient's 'energy field' and then try to pass energy to their body by way of a gentle touch or by sweeping their hands near to their body. Distance healing tries to achieve this at a distance, through thought, meditation or prayer. The impact and effect of these forms of healing depend upon patient beliefs. [6]

1.3.13 Massage

Massage has been around for thousands of years. There are many systems of massage now practiced in the UK. They all use a manual technique in which a rhythmic movement uses a variety of strokes, kneading or tapping to move the muscles and soft tissue of the body. Massage can be stimulating or sedating, vigorous or gentle, and include the whole body or only part. Oils, creams, lotions or talcum powder may be used. [5, 6]

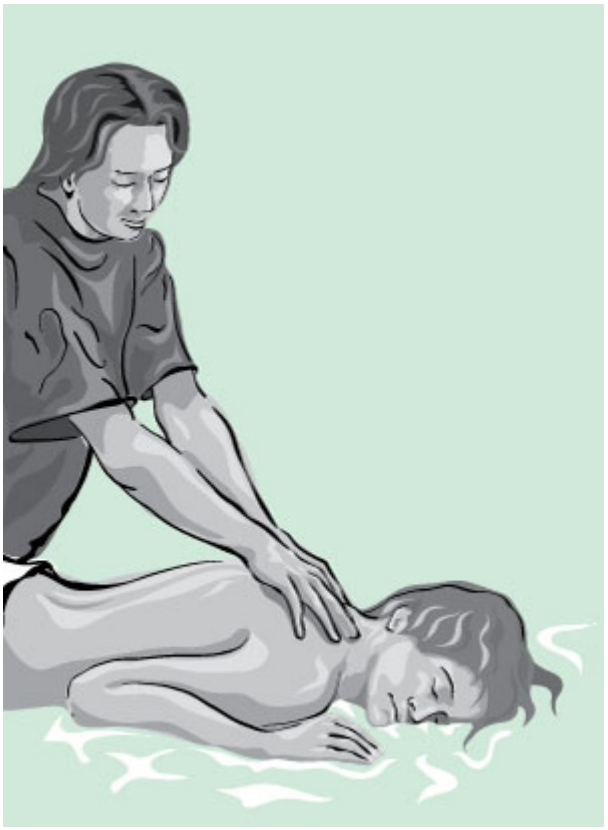


Figure 3. Massage can be soothing.

Massage can reduce patient's anxiety and stress levels, relieve muscular tension and fatigue, improve circulation, and thus reduce pain levels. It is generally very safe and relaxing, but a

trained massage therapist will always follow strict guidelines to avoid endangering the person being massaged.

1.3.14 Herbal medicine

Herbal medicine is the use of plants and plant extracts to treat disease, something mankind has always done. Herbal medicine exists in many local varieties depending on the regional flora. Many modern drugs were originally extracted from plant sources, even if they are now made synthetically, and many other drugs are descended from plant substances. For instance, aspirin, the original non-steroidal anti-inflammatory drug (NSAID) and 'grandfather' of a large family of such drugs, was originally extracted from willow bark. Whereas conventional medicine tries to isolate the active ingredient of a plant, herbal remedies use the whole plant. Herbalists believe that the natural chemical balance in the whole plant has a better effect on the body than if just the active ingredients are given. [6]

Some of the most promising herbs for arthritis, all of which are backed by some research, are devil's claw (made from a plant which grows in Namibia), *Boswellia* (from the frankincense tree) and rosehip. Generally speaking they are safe.

Boswellia serrata tree is commonly found in India. The therapeutic value of its gum (guggulu) has been known. It posses good anti-inflammatory, anti-arthritic and analgesic activity.

In this study, two plant species i.e., ***Strobilanthes kunthianus*** (family: Acanthaceae) and ***Strobilanthes cuspidatus*** (family: Acanthaceae) were selected and were compared with the marketed formulation **Shallaki** (*Boswellia serrata* extract).

2. SCOPE AND OBJECTIVE

The use of medicinal plants in both crude and prepared forms has increased greatly. The world health organization has estimated that 80% of the global population relies chiefly on traditional medicine for health care and there are reports that about 51% of all drug preparations in industrialized countries derive from plants, acting as sources of therapeutic agents or models for new synthetic compounds, or as raw material for semi-synthetic production of highly complex molecules.

Analgesics and anti-inflammatory therapy has been the major treatment for osteoarthritis in the past, and NSAIDS (Non-steroidal anti-inflammatory drugs) still continue 92% of the drugs used against osteoarthritis even though their serious side effects are well done along with their moderate efficacy in long term. Intra-articular injections of corticosteroids are useful. Both classes of drugs are reviewing with regard to their function leads to a more critical approach in drug selection. So-called chondroprotective drugs, mainly sulphated polysaccharides, played a certain role in the pharmacotherapy of osteoarthritis, but failed to demonstrate clinical efficacy, and now have lost significance. Anti-oxidative enzymes or drugs such as superoxide dismutase or diacerin are also considered to influence osteoarthritic conditions, recently, matrix-metalloprotease- inhibitors (MMP-I), originally designed to inhibit tumor cells invasion, have shown promising results in counteracting the progressive enzymatic cartilage degradation, and some compounds treatment gaining interest are derivatives of hyaluronic acid, which are applied intra-articularly in a series of injections. Most of these preparations have been failed as devices rather than drugs, claiming a visco-supplementation with anti-inflammatory, analgesic and chondroprotective properties. [7]

Hence, progress in the development of new analgesic, anti-inflammatory and anti-osteoarthritic agents using medicinal plants are essential and also scientific validations of traditional medicines are necessary. In contrast to chemically defined drugs, herbal medicinal products contain complex mixtures of different compounds with active and synergistic substances.

There are several herbs used in the indigenous system of medicine for the treatment of inflammation and joint disorders. As per the literature survey information, column chromatographic fractionation of various extracts of *Strobilanthes callosus* (Family- Acanthaceae) showed some known compounds like long chain alkane dotricontane, a triterpene alcohol - lupeol, stigmasterol, and β-D-glucopyranoside. Complex phenyl propanoid glycosides, verbacoside and crassifoiloside have been isolated along with a trisaccharide raffinose.[8] The methanol extract of leaf of *Strobilanthes cusia* (Family- Acanthaceae) showed antinociceptive, anti-inflammatory and antipyretic effects**.** [9] The plant *Strobilanthes ciliatus* (Family- Acanthaceae) a traditional herb reported for anti-inflammatory activity and used in joint disorders. [10]

The plants *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* are also belongs to the Family Acanthaceae. Although there is no record of these plants being used for medicinal purposes, the tribal people of Nilgiri hills have been used in joint pains and inflammations. Due to a strong chemo taxonomical relationship among species of Strobiolanthes, the two species *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* were selected for the present study to investigate the analgesic, anti-inflammatory and anti-osteroarthritic activity and will be compared with the marketed herbal formulation **Shallaki** which contains *Boswellia serrata* extract.

3. REVIEW OF LITERATURE

Agarwal et al., (2003) reported the phytochemical investigation and anti-inflammatory and anti-arthritic activities of essential oil of *Strobilanthus ixiocephala* Benth. Column chromatographic fractionation of essential oil obtained by hydro distillation from the flowering tops of *S. ixiocephala* resulted in the isolation of beta-caryophyllene, fenchyl acetate, T-cadinol and a new sesquiterpene alcohol for which a name ixiocephol has been proposed. The beta-caryophyllene and fenchyl acetate were identified by Co-TLC with authentic samples whereas T-cadinol and ixiocephol were structurally elucidated by UV, IR, 1H NMR, 13C NMR and Mass spectral data. The GC-MS analysis of the essential oil has also revealed the presence of various monoterpenoids and sesquiterpenoids. The essential oil of *S. ixiocephala* demonstrated a dose dependant anti-inflammatory activity in carrageenan-induced rat paw edema. It has also revealed good activity in cotton pellet granuloma and adjuvant induced arthritis model in rats. [11]

Akindele et al., (2007) was evaluated the anti-inflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus* using carrageenan and egg albumin induced rat paw edema, xylene induced mouse ear edema and formaldehyde induced arthritis , inflammation tests. The extract administered orally at doses of 50, 100, 200 and 400 mg/kg body weight produced a significant dose dependent inhibition of edema formation in all four methods used. The results obtained suggested that the aqueous leaf extract of *B. coccineus* is endowed with effective anti-inflammatory activity mediated via either inhibition of phospholipase A_2 (PLA_2) activity or cyclooxygenase cascade and by blocking the release of vasoactive substances (histamine, serotonin and kinins). These findings seem to justify the use of the plant in traditional African medicine
in the treatment of inflammation, including arthritic conditions. [12]

Ali et al., (2003) reviewed the pharmacological and toxicological properties of *Nigella sativa.* The seeds of *Nigella sativa* Linn. (Ranunculaceae), commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma,

diarrhoea and dyslipidaemia. The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil, but which is also present in the fixed oil. The pharmacological actions of the crude extracts of the seeds (and some of its active constituents, e.g. volatile oil and thymoquinone) that have been reported include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. The seeds/oil has anti-inflammatory, analgesic, anti-pyretic, anti-microbial and anti-neoplastic activity. The oil decreases blood pressure and increases respiration. Treatment of rats with the seed extract for up to 12 weeks has been reported to induce changes in the haemogram that include an increase in both the packed cell volume (PCV) and haemoglobin (Hb), and a decrease in plasma concentrations of cholesterol, triglycerides and glucose.The seeds are characterized by a very low degree of toxicity. Two cases of contact dermatitis in two individuals have been reported following topical use. Administration of either the seed extract or its oil has been shown not to induce significant adverse effects on liver or kidney functions.

It would appear that the beneficial effects of the use of the seeds and thymoquinone might be related to their cytoprotective and anti-oxidant actions, and to their effect on some mediators of inflammation. [13]

Altman et al., (2001) evaluated the efficacy and safety of a standardized and highly concentrated extract of 2 ginger species, *Zingiber officinale* and *Alpinia galanga* (EV.EXT 77), in patients with osteoarthritis (OA) of the knee. Two hundred sixty-one patients with OA of the knee and moderate-to-severe pain were enrolled in a randomized, double-blind, placebo-controlled, multicenter, parallel-group, 6-week study. After washout, patients received ginger extract or placebo twice daily, with acetaminophen allowed as rescue medication. The primary efficacy variable was the proportion of responders experiencing a reduction in "knee pain on standing," using an intent-to-treat analysis. A responder was defined by a reduction in pain of ≥15 mm on a visual analog scale.

In the 247 evaluable patients, the percentage of responders experiencing a reduction in knee pain on standing was superior in the ginger extract group compared with the control

group (63% versus 50%;). Analysis of the secondary efficacy variables revealed a consistently greater response in the ginger extract group compared with the control group, when analyzing mean values: reduction in knee pain on standing (24.5 mm versus 16.4 mm), reduction in knee pain after walking 50 feet (15.1 mm versus 8.7 mm), and reduction in the Western Ontario and McMaster Universities osteoarthritis composite index (12.9 mm versus 9.0 mm). Change in global status and reduction in intake of rescue medication were numerically greater in the ginger extract group. Change in quality of life was equal in the 2 groups. Patients receiving ginger extract experienced more gastrointestinal (GI) adverse events than did the placebo group (59 patients versus 21 patients). GI adverse events were mostly mild.

A highly purified and standardized ginger extract had a statistically significant effect on reducing symptoms of OA of the knee. This effect was moderate. There was a good safety profile, with mostly mild GI adverse events in the ginger extract group. [14]

Balian et al., (2006) reported that the methanolic extract of leaf and leaf callus of *Silybum marianum* posses a potent anti-inflammatory activity, that could inhibit the acute inflammation in rat paw, induced either by carrageenan or formalin. The *in vitro* culture-generated callus extract showed maximum inhibition in rat paw edema, which is due to presence of higher amount of secondary metabolites, as compared to natural plant leaf. [15]

Cheeke et al., (2006) reviewed the anti-inflammatory and anti-arthritic effects of yucca schidigera. *Yucca schidigera* is a medicinal plant native to Mexico. According to folk medicine, yucca extracts have anti-arthritic and anti-inflammatory effects. The plant contains several physiologically active phytochemicals. It is a rich source of steroidal saponins, and is used commercially as a saponin source. Saponins have diverse biological effects, including anti-protozoal activity. It has been postulated that saponins may have anti-arthritic properties by suppressing intestinal protozoa which may have a role in joint inflammation. Yucca is also a rich source of polyphenolics, including resveratrol and a number of other stilbenes (yuccaols A, B, C, D and E). These phenolics have anti-inflammatory activity. They are inhibitors of the nuclear transcription factor NFkappaB. NFkB stimulates synthesis of inducible nitric oxide synthase (iNOS), which causes

formation of the inflammatory agent nitric oxide. Yucca phenolics are also anti-oxidants and free-radical scavengers, which may aid in suppressing reactive oxygen species that stimulate inflammatory responses. Based on these findings, further studies on the anti-arthritic effects of *Yucca schidigera* are warranted. [16]

Choi et al., (2002) screened the effects of SKI 306X, a new herbal agent, on proteoglycan degradation in cartilage explant culture and collagenase induced rabbit osteoarthritis model. [17]

Coelho et al., (**2004)** reported the anti-arthritic effect and subacute toxicological evaluation of *Baccharis genistelloides* aqueous extract. They studied the potential subacute toxicological effects of the aqueous extract of *Baccharis genistelloides* (AEBg) and demonstrated a new anti-arthritic therapeutic effect. The treatment of the collagen-induced arthritis (CIA) group with 4.2 mg/kg AEBg induced an important decrease (75%) in CIA severity in all animals, while the 42 mg/kg dose treated only 50% of animals. After AEBg treatment, no significant differences were observed in body weight, aspect, color and relative weight of liver, kidneys, thymus or lungs between CIA groups. CIA and healthy AEBg groups treated with both doses did not show genotoxic effects to liver and kidney cells by the Comet assay, compared to its own control group. The augmented AST in the CIA group, compared to healthy control one was regularized by the AEBg treatment with 4.2 mg/kg but not with 42 mg/kg. No other significant difference was found on serum biochemical parameters, as well as on spontaneous or stimulated lymphocyte proliferation between CIA groups. The treatment of healthy animals with AEBg 4.2 mg/kg did not change the aspect, color or relative weight of kidneys, liver or lungs but reduced the body weight, the thymus and popliteal lymph node (PLN) relative weight and serum glucose and triglyceride levels. These results indicate an anti-arthritic effects of AEBg without liver and kidney subacute toxicity and hypoglycemic and hypotriglyceridemic actions on healthy animals. [18]

Didem et al., (2007) investigated the i*n vivo* anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. The ethanolic

extract was shown to possess significant inhibitory activity against inflammatory models (i.e., carrageenan-induced and PGE_1-induced hind paw edema models, as well as on acetic acid-induced increase in a capillary permeability model) and on a pain model based on the inhibition of *p*-benzoquinone-induced writhing in mice. Hexane, chloroform, ethyl acetate, *n*-butanol and the remaining water fractions were obtained through bioassay-guided fractionation. Ethyl acetate and *n*-butanol fractions displayed potent anti-inflammatory and antinociceptive activities at a dose of 919 mg/kg without inducing acute toxicity. [19]

Dilip et al., (2008) studied the pharmacological activity of Indian black tea (leaf variety) in acute and chronic inflammatory conditions**.** Infusions of Indian black tea (BTI), when administered orally, produced significant inhibition of rat paw edema, induced with carrageenan (pre and post treatment) and arachidonic acid. BTI was also found to inhibit peritoneal capillary permeability and caused a marked reduction of lipopolysaccharide induced PGE_2 generation. In these models, the observed anti-edema effect was similar to that of BW755C (a dual inhibitor of cyclooxygenase and 5-lipoxygenase enzymes). BTI was found to scavenge superoxide and hydroxyl radicals, and also protected rat erythrocytes from the damaging effects of hydrogen peroxide. In chronic studies, BTI inhibited granuloma formation along with the reduction of both lipid peroxidation and hydroxyproline content (in the granuloma tissue). Significant antiarthritic activity was observed with regular administration of BTI in the Freund's adjuvant induced model of arthritis. Chronic treatment with BTI (in arthritic rats) resulted in a decrease of paw diameter and tissue lipid peroxidation, along with a restoration of GSH, catalase and superoxide dismutase levels. [20]

Dimo et al., (2006) evaluated the acute and chronic anti-inflammatory properties of leaf extracts of *Kalanchoe crenata* in rats. The methylene chloride/methanol extract of *K. crenata* was extracted by using hexane, methylene chloride, ethyl acetate, and n-butanol. The anti-inflammatory profile of these extracts was investigated on the basis of paw edema induced by carrageenan. The n-butanol fraction (most potent) was further assessed through acute inflammatory models induced by histamine, serotonin, and formalin. The

chronic anti-inflammatory and the ulcerogenic activities of the n-butanol fraction were also examined. They found that oral administration of n-butanol fraction (600 mg/kg) caused a maximum inhibition of about 45% in paw edema induced by carrageenan. The n-butanol fraction also exhibited acute anti-inflammatory activity on paw edema induced by histamine (47.51%), serotonin (54.71%), and formalin (40.00%). In the chronic inflammation model, this extract showed maximum inhibition of 61.26% on the ninth day of treatment. The ulcerogenic assessment showed that ulcer indices after oral treatment with n-butanol fraction were zero. On the basis of these findings, they inferred that *K. crenata* is an anti-inflammatory and anti-arthritic agent that may blocks histamine and serotonin pathways. The results are in agreement with the traditional use of the plant in inflammatory conditions. [21]

Dinesh et al., (2007) reviewed the numerous agents derived from plants can suppress the cell signaling intermediates, including curcumin (from turmeric), resveratrol (red grapes, cranberries and peanuts), tea polyphenols, genistein (soy), quercetin (onions), silymarin (artichoke), guggulsterone (guggul), boswellic acid (salai guggul) and withanolides (Ashwagandha). Several preclinical and clinical studies suggest that these agents have potential for arthritis treatment. Although gold compounds are no longer employed for the treatment of arthritis, the large number of inexpensive natural products that can modulate inflammatory responses, but lack side effects, constitute 'goldmines' for the treatment of arthritis. [22]

Fernandez et al., (2004) investigated the effect of ITB (a novel COX-2 inhibitor) on the production of catabolic or anti-inflammatory mediators in osteoarthritis (OA) cartilage. In osteoarthritis cartilage explants, ITB inhibited the production of prostaglandin E_2 (PGE_2), tumor necrosis factor- α (TNF-α) and matrix metalloproteinase – 13 (MMP-13) in a concentration dependent manner, whereas nitrite was partially reduced. These results suggested that ITB may be useful in the prevention of cartilage degeneration. [23]

Ficarra et al., (1995) reported the analgesic and anti-inflammatory activities of leaf extracts of some cordia species as well as their chromatographic analysis. The analgesic,

anti-inflammatory and anti-arthritic activities in the rat of different extracts of C. francisci, C. martinicensis, C. myxa, C. serratifolia and C. ulmifolia leaves were studied. The results obtained showed that the petroleum ether and alcoholic extracts especially of C. francisci, C. myxa and C. serratifolia leaves have a significant analgesic, anti-inflammatory and anti-arthritic activity in the rat. The flavonoids and phenolic derivative content of the five species of genus Cordia leaves was investigated by TLC and determined by reversed-phase HPLC with an acetonitrile/water/acetic acid buffer solvent gradient. UV detection was carried out at 255 and 280 nm. Four flavonoid glycosides, robinin, rutin, datiscoside and hesperidin, one flavonoid aglycone, dihydrorobinetin, two phenolic derivatives, chlorogenic and caffeic acid, were evidenced and determined. [24]

Gokhale et al., (2002) evaluated the anti-inflammatory and anti-arthritic activity of S. *lappa*, *A. speciosa* and *A. aspera*. The ethanolic extracts of the plants at the doses of 50, 100 and 200 mg/kg, p.o. were screened for their effect on acute and chronic inflammation induced in mice and rats. *S. lappa* and *A. speciosa* were found to significantly inhibit paw edema induced by carrageenan and Freund's complete adjuvant and to prevent accumulation of inflammatory cells in carrageenan-induced peritonitis at doses of 50–200 mg/kg. *A. aspera* inhibited these inflammatory responses at doses of 100–200 mg/kg. The studies revealed that the ethanolic extracts of *S. lappa*, *A. speciosa* and *A. aspera* possess anti-inflammatory, anti-arthritic activity and support the rationale behind the traditional use of these plants in inflammatory conditions. [25]

Herman et al., (1987) reported the modulation of cartilage destruction by select nonsteroidal anti-inflammatory drugs. Non-enzymatic factors produced by synovial tissue can potentially mediate cartilage destruction by inducing the synthesis and release of matrix-degrading proteinases from chondrocytes. Pharmacologic control of this process is of potential clinical relevance. The *in vitro* effect of therapeutic concentrations of select nonsteroidal anti-inflammatory drugs on the synthesis and activity of catabolism-inducing cytokines produced by 6-day explant cultures of osteoarthritic and rheumatoid synovial tissue were studied. Piroxicam regularly suppressed such factor synthesis by both types of tissue without significantly affecting total protein synthesis. This did not occur using sodium salicylate or indomethacin in osteoarthritis tissue cultures and was

observed only occasionally in rheumatoid arthritis cultures. None of the nonsteroidal anti-inflammatory drugs studied consistently blocked catabolism-inducing activity in osteoarthritis tissue, whereas piroxicam more consistently inhibited activity produced by rheumatoid arthritis tissue. [26]

John (2006) investigated the analgesic, anti-inflammatory and hypoglycemic effects of *Zingiber officinale* dried rhizomes ethanol extract (ZOE) in mice and rats. The analgesic effect of ZOE was evaluated by "hot-plate" and "acetic acid" analgesic test methods in mice; while the anti-inflammatory and hypoglycemic effects of the plant extract was investigated in rats, using fresh egg albumin-induced pedal edema, and streptozotocin (STZ)-induced diabetes mellitus models. Morphine (MPN, 10 mg/kg), diclofenac (DIC, 100 mg/kg) and chlorpropamide (250 mg/kg) were used as reference drugs for comparison. ZOE (50-800 mg/kg, i.p.) produced dose-dependent, significant (p< 0.05-0.001) analgesic effects against thermally and chemically induced nociceptive pain in mice. The plant extract (ZOE, 50-800 mg/kg, p.o.) also significantly (p< 0.05-0.001) inhibited fresh egg albumin-induced acute inflammation, and caused dose-related, significant (p<0.05-0.001) hypoglycemia in normal (normoglycemic) and diabetic rats. The findings of this experimental animal study indicated that *Zingiber officinale* rhizomes ethanol extract possesses analgesic, anti-inflammatory and hypoglycemic properties; and thus lend pharmacological support to folkloric, ethno medical uses of ginger in the treatment and/or management of painful, arthritic, inflammatory conditions, as well as in the management and/or control of type 2 diabetes mellitus in some rural Africa communities. [27]

John (2005) studied the analgesic and anticonvulsant properties of *Tetrapleura tetraptera* (Taub) (Fabaceae) fruit aqueous extract in mice. Morphine (MPN, 10 mg/kg i.p.), diclofenac (DIC, 100 mg/kg i.p.), phenobarbitone (20 mg/kg i.p.) and diazepam (0.5 mg/kg i.p.) were used, respectively, as reference analgesic and anticonvulsant agents for comparison. *T. tetraptera* fruit aqueous extract (TTE, 50-800 mg/kg i.p.) produced dose-dependent, significant (p < 0.05-0.001) analgesic effects against thermally and chemically induced pain in mice. Like the standard anticonvulsant agents

(phenobarbitone and diazepam) used, *T. tetraptera* fruit aqueous extract (TTE, 50-800 mg/kg i.p.) significantly ($p < 0.05$-0.001) delayed the onset of, and antagonized, pentylenetetrazole (PTZ)-induced seizures. Aqueous extract of the fruit (TTE, 50-800 mg/kg i.p.) also profoundly antagonized picrotoxin (PCT)-induced seizures, but only partially and weakly antagonized bicuculline (BCL)-induced seizures. However, the results of this experimental animal study indicate that *Tetrapleura tetraptera* (Taub) fruit aqueous extract (TTE) possesses analgesic and anticonvulsant properties. These findings lend pharmacological support to the suggested folkloric uses of the plant's fruit in the management and/or control of painful, arthritic, inflammatory conditions, as well as for the management and/or control of epilepsy and childhood convulsions in some tropical African countries. [28]

Kim et al., (2005) demonstrated that SKI 306X a herbal mixture, clinically approved for the treatment of osteoarthritis, did not produce any significant damage up to dose of 2g/kg and was effective in significantly protecting the damage associated to diclofenac – induced gastric ulcerations. SKI 306 X could spare the gastric mucosa through significantly suppressing gastric leukotriene (LT) synthesis. [29]

Kim et al., (2000) provided scientific evidence that *Cimicifuge rhizoma*, a traditional herbal medicine used to treat fever, pain and inflammation in Korea and China exert analgesic and anti-inflammatory effects by inhibiting bradykinin/ histamine mediated actions and inhibiting 6-keto-$PGF_{1\alpha}$ induction. [30]

Kimmatkar et al., (2003) observed the efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee. A randomized double blind placebo controlled crossover study was conducted to assess the efficacy, safety and tolerability of *Boswellia serrata* extract (BSE) in 30 patients of osteoarthritis of knee, 15 each receiving active drug or placebo for eight weeks. After the first intervention, washout was given and then the groups were crossed over to receive the opposite intervention for eight weeks. All patients receiving drug treatment reported decrease in knee pain, increased knee flexion and increased walking distance. The frequency of swelling in the knee joint

was decreased. Radiologically there was no change. The observed differences between drug treated and placebo being statistically significant, are clinically relevant. BSE was well tolerated by the subjects except for minor gastrointestinal ADRs. So, BSE is recommended in the patients of osteoarthritis of the knee with possible therapeutic use in other arthritis. [31]

Kulkarni et al., (2008) carried the anti-oxidant and anti-inflammatory activity of *Vitex negundo.* The total methanol extract of the plant was standardized in terms of total polyphenols. The standardized extract in a dose of 100 mg/kg caused a comparable reduction in edema with that of diclofenac sodium (25 mg/kg) when evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema method. The extract also exhibited a strong free radical scavenging activity by 1, 1-diphenyl-2-picrylhydrazyl method and caused a significant reduction in the formation of thiobarbituric acid reacting substances when evaluated for its lipid peroxidation inhibitory activity. The results strongly suggested that radical quenching may be one of the mechanisms responsible for its anti-inflammatory activity. [32]

Kulkarni et al., (2005) reported that coxibs were the widely prescribed drugs (nearly 8 million people round the globe take these drugs) until the recent set back with rofecoxib, which was withdrawn from the market by the innovator due to increased risk of heart attacks and strokes observed with its long-term use. [33]

Mary Latha et al., (1998) tested the anti-inflammatory effect of an alcoholic extract from the flower of *Vernonia cinerea* (Asteraceae) in adjuvant arthritic rats. They found that changes in paw volume, body and tissue weights and serum and tissue enzyme activities of ALT, AST, ALP and cathepsin-D in adjuvant rats were reversed by oral administration of 100 mg/kg body weight (BW) of the flower extract. The extract also reversed the major histopathological changes in the hind paws of the arthritic rats. Phytochemical studies revealed the presence of alkaloids, saponins, steroids and flavonoids. They concluded that the extract contains as yet unidentified anti-inflammatory principle(s). [34]

Mathew et al., (2004) evaluated the anti-inflammatory and wound healing activity of *Gentiana lutea* rhizome extracts in animals. They examined the effectiveness of alcohol and petrol ether extracts of rhizomes of *Gentiana lutea* at 500 and 1000 mg/kg doses orally in the carrageenan-induced rat paw edema, xylol-induced mouse ear edema and cotton pellet-induced chronic inflammatory models. Both extracts showed significant dose-dependent anti-inflammatory activities in all of these models. Both extracts exhibited significant wound healing activity at 300 and 500 mg/kg, p.o., in excision, resutured incision and dead space wound models. [35]

Naresh Singh et al., (2008) proved that *Piper longum* Linn. extract inhibits TNF-*α*-induced expression of cell adhesion molecules by inhibiting NF-*κ*B activation and microsomal lipid peroxidation. They have evaluated the effect of *Piper longum* chloroform extract (PlCE) on TNF-*α*-induced expression of ICAM-1 on endothelial cells and on NADPH-catalyzed rat liver microsomal lipid peroxidation with a view to identify modulators for the expression of CAMs and demonstrated that PlCE inhibits adhesion of neutrophils to endothelial monolayer. This inhibition is due to the ability of PlCE to significantly block the TNF-*α*-induced expression of CAMs, i.e. ICAM-1, VCAM-1 at 17.5 µg/ml concentrations and E-selectin at 15 µg/ml concentration on human umbilical vein endothelial cells. The inhibition of ICAM-1, VCAM-1 and E-selectin by PlCE is mediated through inhibition of NF-*κ*B in endothelial cells. To demonstrate the antioxidant activity of PlCE, they showed that PlCE inhibited the NADPH-catalyzed rat liver microsomal lipid peroxidation significantly. These results suggested a possible mechanism of anti-inflammatory as well as antioxidant activity of PlCE. [36]

Ojewole (2004) has been reported that *Sclerocarya birrea* stem bark aqueous extract possess analgesic, anti-inflammatory and hypoglycemic properties in experimental animal studies. These experimental findings lend pharmacological support to the suggested folkloric uses of the plant's stem-bark in the management and/or control of pain, inflammatory conditions, and adult-onset, type-2 diabetes mellitus in some communities of South Africa. [37]

Osadebe et al., (2003) evaluated the anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves using egg-albumen-induced rat hind paw edema as a model of inflammation. The crude extract was subjected to acute toxicity test. Fraction A2, which exhibited the most promising anti-inflammatory effect, was also subjected to analgesic and ulcerogenic tests. Phytochemical analysis of the extracts showed the presence of terpenes, sterols, flavonoids, tannins, carbohydrates, glycosides, saponins and traces of alkaloids. The LD_{50} of the aqueous ME was found to be 1131.4 mg/kg. The crude ME (50 mg/kg) gave anti-inflammatory activity which was significant ($P<0.05$) at all the observation times (1–3 h). The different solvent fractions exhibited varying degrees of anti-inflammatory activities, with terpenoid fraction (A2) and the tannin-containing multi-component fraction (D) showing very high and significant ($P<0.01$) activity at 100 mg/kg, with percentage inhibition of edema value of 87.69 each. They found that aqueous ME of *Alchornea cordifolia* leaves could be beneficial in the management of different inflammatory disease states. Its anti-inflammatory activity may not be attributed only to the terpenoid content. [38]

Perez et al., (1995) was examined the ethanol extract of the plant *Hippocratea excelsa* for its anti-inflammatory effects using several animal models. It produced significant inhibition of carrageenan- induced paw edema and reduced the weight of cotton pellet-induced granuloma at doses of 25-100 mg/kg. The extract was found to exert a protective effect on heat-induced erythrocyte lysis at concentrations of 25, 50 and 100 μg/ml. In chronic models of formaldehyde and adjuvant arthritis, its antiarthritic activity was found to be less than that of phenylbutazone. [39]

Perumal Samy et al., (2006) tested the effect of aqueous extract of *Tragia involucrata* Linn. On acute and sub acute inflammation. Anti-inflammatory activity of aqueous extract of *Tragia involucrata* was tested on carrageenan-induced hind paw edema and cotton pellet granuloma models in albino rats. In the subacute model, cotton pellet granuloma was produced by implantation of 10 mg sterile cotton in the axilla under ether anesthesia. The animals were administered an aqueous extract at various concentrations of 50, 100, 200, 300 and 400 mg/kg. Phenyl butazone (80 mg/kg) was used as a standard

drug. The paw diameter was measured at different time intervals and the dry granuloma weight was taken after the treatment. The aqueous leaf extract (400 mg/kg) showed the maximum inhibition (84.23%) of edema at the end of 3 h following carrageenan-induced rat paw edema. In sub acute inflammation, the extract showed 76.25% reduction in granuloma weight. The results proved that the aqueous leaf extract showed highest anti-inflammatory activity in acute and subacute inflammation and also support the usage of traditional claims. [40]

Puntero et al., (1997) studied the anti-inflammatory and anti-ulcer activity of *Teucrium buxifolium*. In this work, phytochemical screening was carried out to ascertain the qualitative composition of this species. They found that this species has exhibited potent anti-inflammatory properties against experimentally-induced arthritis and carrageenan induced paw edema. Additionally, *Teucrium buxifolium* species have displayed significant anti-ulcer and cytoprotective activity. [41]

Raju et al., (2006) studied the anti-inflammatory and free radical scavenging activity of *Ricinus communis* root extract in Wistar albino rats. The methanolic extract at doses 250 and 500 mg/kg p.o. exhibited significant ($P < 0.001$) anti-inflammatory activity in carrageenan-induced hind paw edema model. The extract at the dose of 500 mg/kg p.o. also exhibited significant ($P < 0.001$) anti-inflammatory activity in cotton pellet granuloma model. The methanolic extract showed significant free radical scavenging activity by inhibiting lipid peroxidation initiated by carbon tetrachloride and ferrous sulphate in rat liver and kidney homogenates. The extract enhanced free radical scavenging activity of stable radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\bullet}$), nitric oxide and hydroxyl radical in *in vitro* assay methods. The results of the study indicated that the methanolic extract of *Ricinus communis* root possessed significant anti-inflammatory activity in acute and chronic inflammatory models in rats. The observed pharmacological activity may be due to the presence of phytochemicals like flavonoids, alkaloids and tannins present in the plant extract with various biological activities. [42]

Rasool et al., (2007) evaluated the anti-inflammatory effect of the Indian Ayurvedic herbal formulation Triphala on adjuvant-induced arthritis in mice. The arthritis was induced by intradermal injection of complete Freund's adjuvant (0.1 mL) into the right hind paw of Swiss albino mice. Triphala (1 g/kg/body weight) and indomethacin (3 mg/kg/body weight) were administered orally for 8 days (from day 11 to 18) after adjuvant injection. The levels of lysosomal enzymes, tissue marker enzymes, glycoproteins and paw thickness were increased in adjuvant-induced arthritic animals. The body weight was found to be reduced when compared with the control animals. These physical and biochemical changes observed in arthritic animals were altered significantly to near normal conditions after oral administration of Triphala (1 g/kg/body weight). The results obtained clearly indicated the fact that the Indian Ayurvedic herbal formulation Triphala has promising anti-inflammatory activity. [43]

Rasool et al., (2007) investigated the protective effect of *Withania somnifera* root powder in relation to lipid peroxidation, anti-oxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats. Results were compared with those for Indomethacin, a nonsteroidal anti-inflammatory drug. Arthritis was induced by intradermal injection of complete Freund's adjuvant (0.1 mL) into the right hind paw of Wistar albino rats. *Withania somnifera* root powder (1000 mg/kg/day) and Indomethacin (3 mg/kg/day) were orally administered for 8 days (from 11th to 18th day) after adjuvant injection. The anti-arthritic effect of *W. somnifera* root powder was assessed by measuring changes in lipid peroxidation, anti-oxidant status, and glycoprotein levels in plasma and spleen of arthritic animals. In addition, cartilage degradation was also assessed by estimating bone collagen and urinary constituents in arthritic animals. Results showed significant increase in the level of lipid peroxides, glycoproteins, and urinary constituents with the depletion of anti-oxidant status and bone collagen in arthritic animals. These biochemical alterations observed were ameliorated significantly by oral administration of *W. somnifera* root powder (1000 mg/kg body weight) in arthritic animals. The results of this study clearly indicated that *W. somnifera* root powder is capable of rectifying the above biochemical changes in adjuvant arthritis. [44]

Sabino et al., (1999) tested the anti-arthritic effect of a hydro alcohol extract of *Pterodon pubescens* (HEPp) seeds using collagen-induced arthritis (CIA) in DBA1/J mice treated with daily oral doses of HEPp in different schedules. The preventive treatment significantly reduced both the arthritic index (AI) and the CIA incidence. Using a therapeutic protocol, only the lower dose of HEPp induced a decrease in both parameters. These results provide a scientific foundation for the popular use of *Pp* seed infusions in rheumatoid arthritis (RA) treatment. [45]

Schmid et al., (2001) studied the efficacy and tolerability of a standardized willow bark extract in patients with osteoarthritis. Willow bark extract, in a dose corresponding to 240 mg salicin/day, was compared with placebo in a 2-week, double-blind, randomized controlled trial. The primary outcome measure was the pain dimension of the WOMAC Osteoarthritis Index. Secondary outcome measures included the stiffness and physical function dimensions of the WOMAC, daily visual analogue scales (VAS) on pain and physical function, and final overall assessments by both patients and investigators. A total of 78 patients (39 willow bark extract, 39 placebos) participated in the trial. A statistically significant difference between the active treatment and the placebo group was observed in the WOMAC pain dimension (d = 6.5 mm, 95% C.I. = 0.2-12.7 mm); the WOMAC pain score was reduced by 14% from the baseline level after 2 weeks of active treatment, compared with an increase of 2% in the placebo group. The patient diary VAS confirmed this result, and likewise the final overall assessments showed superiority of the willow bark extract over the placebo. It was concluded that the willow bark extract showed a moderate analgesic effect in osteoarthritis and appeared to be well tolerated. [46]

Shahavi et al., (2008) studied the methanolic extract of *Butea monosperma* flowers (MEBM) for anti-inflammatory activity against carrageenan induced paw edema and cotton pellet granuloma in albino rats. In carrageenan induced paw edema, MEBM at oral doses of 600 mg/kg and 800 mg/kg, dose-dependently inhibited the paw edema. In cotton pellet induced granuloma, MEBM at the same doses was found to significantly inhibited granuloma tissue formation, including significant reduction in levels of serum lysosomal enzymes (SGOT, SGPT and ALP) and lipid peroxides as compared to control. [47]

Sharma et al., (1996) investigated the immunomodulatory activity of boswellic acids (Pentacyclic triterpene acids) from *Boswellia serrata* on cell mediated and humoral components of the immune system and the immunotoxicological potential. A single oral administration of BA (50-200 mg/kg) inhibited the expression of the 24 h delayed type hypersensitivity (DTH) reaction and primary humoral response to SRBC in mice. The secondary response was appreciably enhanced at lower doses. In a multiple oral dose schedule BA (25, 50 and 100 mg/kg) reduced the development of the 24 h DTH reaction and complement fixing antibody titres and slightly enhanced the humoral antibody synthesis. In concentrations greater than 3.9 μg/mL BA produced almost similar and dose related inhibition of proliferative responsiveness of splenocytes to mitogens and alloantigen. Preincubation of macrophages with different concentrations of BA enhanced the phagocytic function of adherent macrophages. Prolonged oral administration of BA (25-100 mg/kg/d×21 days) increased the body weight, total leukocyte counts and humoral antibody titres in rats. It is not cytotoxic nor does it cause immunosuppression. [48]

Singh et al., (2008) revealed the boswellic acids: A leukotriene inhibitor also effective through topical application in inflammatory disorders. Boswellic acids (BA), a natural mixture isolated from oleo gum resin of *Boswellia serrata* comprised of four major pentacyclic triterpene acids: *β*-boswellic acid (the most abundant), 3-acteyl-*β*-boswellic acid, 11-keto-*β*-boswellic acid, and 3-acetyl-11-keto-*β*-boswellic acid, is reported to be effective as anti-inflammatory, immunomodulatory, anti-tumor, anti-asthmatic and in Chron's disease. It inhibits pro-inflammatory mediators in the body, specifically leukotrienes via inhibition of 5-lipoxygenase, the key enzyme of leukotriene synthesis, is the scientifically proved mechanism for its anti-inflammatory/anti-arthritic activity. All previous work on BA for its biological activity has been done through the systemic application but no pre-clinical data reported for its anti-inflammatory activity by topical application. They reported the anti-inflammatory activity of BA through this route by applying different acute and chronic models of inflammation i.e., arachidonic acid and croton oil-induced mouse ear edema, carrageenan-induced rat paw edema and adjuvant-induced developing arthritis in rats. The results of the study revealed that the effect

observed through this route is in accordance to the study conducted with the systemic route, thus establishing that BA when used through topical application is as effective as through the systemic route. [49]

Singh et al., (2008) evaluated the gastric ulcer protective effect of boswellic acids, a leukotriene inhibitor from *Boswellia serrata*, in rats. The reason for the study was that, the known non-steroidal anti-inflammatory drugs (NSAIDs) are full of side effects especially ulceration which is at the top. BA, although, used as an anti-arthritic agent yet it is not only devoid of ulcer production but protective also. The activity evaluation was done by pyloric ligation, ethanol–HCl, acetylsalicylic acid, indomethacin and cold restrained stress-induced ulceration in rats. Results of the study revealed that BA possesses a dose dependent antiulcer effect against different experimental models. It showed different degree of inhibition of the ulcer score towards different ulcerogenic agents. The ulcer score against various ulcer inducing agents viz., pyloric ligation, ethanol/HCl, (acute and chronic) acetylsalicylic acid, indomethacin and cold restraint stress, was inhibited by 39%, 38%, 51%, 31%, 37% and 42% respectively at 250 mg/kg. From the data it was concluded that BA inhibited ulcer production non-specifically in all the experimental models. [50]

Singh et al., (2002) investigated the anti-inflammatory and anti-microbial activities of the 95% ethanol extract, benzene fraction and isolated triterpenoids of *Strobilanthes callosus*. In the carrageenan-induced paw edema inflammation model, the taraxerol showed a high reduction of edema, but the anti-microbial effect observed was lower at the two doses employed. These results confirmed the use of this plant in folk medicine as an anti-inflammatory and anti-microbial herbal drug. [51]

Venil et al., (2008) tested the effects of aqueous extracts of *Withania somnifera* (Ashwagandha) root and glucosamine sulphate (GlcS) on the levels of nitric oxide (NO) and glycosaminoglycans (GAGs) secreted by knee cartilage from chronic osteoarthritis (OA) patients using a validated explant model of *in vitro* cartilage damage. *W. somnifera* extracts significantly decreased NO release by explants from one subset of patients (anti-inflammatory response) and significantly increased levels of NO and GAGs released by

explants from the second subset ("non-responders"). This was the first study showing direct, statistically significant, anti-inflammatory effects of *W. somnifera* on human OA cartilage. It also confirmed that glucosamine sulphate exhibited statistically significant, anti-inflammatory and chondroprotective activities in human OA cartilage. [52]

Vignon et al., (1993) reported the effect of nonsteroidal anti-inflammatory drugs on proteoglycanase and collagenase activity in human osteoarthritic cartilage. Here, the effect of several nonsteroidal anti-inflammatory drugs, used at concentrations achievable in synovial fluid, on human osteoarthritic (OA) cartilage metalloprotease activity in vitro was studied. Acetaminophen and ketoprofen had no effect; sodium salicylate, indomethacin, and diclofenac slightly decreased proteoglycanase activity. Piroxicam and tenoxicam suppressed proteoglycanase activity by 48.2% and 68.3%, respectively, and suppressed collagenase activity by 19.1% and 36.8%, respectively. Use of these NSAIDs may help to decrease cartilage catabolism in patients with OA. [53]

Vongtau et al., (2004) was studied the anti-nociceptive activity of the methanolic extract of *Neorautanenia mitis* in mice and rats. Five experimental models of nociception employed were: acetic acid-induced abdominal constriction, hot-plate test in mice, formalin-induced pain, analgesy-meter and Randall-Selitto tests in rats. The study showed that *Neorautanenia mitis* possesses both peripherally and centrally mediated antinociceptive activity. The peripheral mediated action may be linked partly to lipoxygenase and/ or cycl-oxygenases, while the central antinociception is likely to be mediated via opioid receptors in the CNS. [54]

Wittenberg et al., (1993) examined the *in vitro* release of prostaglandins and leukotrienes from synovial tissue, cartilage, and bone in degenerative joint diseases. Release of prostaglandin E_2 (PGE_2), 6-keto-$PGF_{1\alpha}$, leukotriene B_4 (LTB_4), and LTC_4 was measured in supernatants of synovial tissue, cartilage, and bone incubates from patients with osteoarthritis, active rheumatoid arthritis (RA), inactive RA, and pseudogout. Radioimmunoassay (RIA) was used to determine the levels of the eicosanoids. Addition of the divalent cation ionophore A23187 resulted in significant release of all eicosanoids

measured from synovial tissue, but not from cartilage, cortical bone, or cancellous bone. PG release was significantly inhibited by the addition of indomethacin or diclofenac. The amount of LTC_4 released from cartilage and bone was only slightly above the detection limit of the RIA, whereas large amounts were released from synovial tissue. Neither indomethacin nor diclofenac had an effect on LTC_4 release. LTC_4 release from synovial tissue of patients with inactive RA was significantly decreased in comparison with the levels from synovial tissue of patients with the other joint diseases. There was no significant difference in PG release among patients in the various disease groups. [55]

4. PLAN OF WORK

The plan of work is as follows:

I. Phytochemical studies

1. Collection of plant materials
2. Identification and authentication of collected plants
3. Preparation of ethanolic extracts
4. Qualitative phytochemical analysis of the ethanolic extracts

II. *In-vitro* studies

1. Anti-inflammatory activity by Human RBC membrane stabilization
2. Anti-osteoarthritis activity by cartilage explants culture

III. *In-vivo* studies

1. Behavioral & toxicity Studies:

i. Acute toxicity Study

2. Efficacy studies:

i. Analgesic activity:

a. Acetic acid induced writhing test

b. Randall-selitto assay

ii. Anti-inflammatory activity:

a. Carrageenan induced rat paw edema method

b. Cotton pellet induced granuloma formation

iii. Anti-osteoarthritis activity:

a. Iodoacetate induced osteoarthritis

3. Safety studies:

i. Sub acute toxicity studies

5. MATERIALS & METHODS

CHEMICALS USED

Analytical / laboratory grade chemicals were used for the studies which are procured from the following manufacturers:

- Himedia laboratories Pvt. Ltd., Bombay.
- Ranbaxy laboratories Ltd., chemical division, S.A.S.Nagar, Punjab.
- S.D.Fine chemicals Ltd., Biosar.
- Fisher inorganic and aromatic, Chennai.
- Qualigen Fine Chemicals, Mumbai.
- E-merck (India) Ltd., Mumbai.
- Biocolor Ltd., New Delhi.
- Wilson laboratories, Mumbai.
- Sigma chemical company, New Delhi.

5.1 PLANT PROFILE

5.1.1 *Strobilanthes kunthianus*

Family: Acanthaceae

Morphological Description

Bushy shrub, 1-2 m high, in stray clumps or gregarious; stems numerous, erect, quadrangular; nodes prominent. Leaves elliptic-ovate, ca 5 x 2.5 cm, acute at base, crenate serrate at margin, acute at apex, coriaceous, scab rid above, white-villous between veins beneath; secondary veins 8 or 9 pairs, prominent; petioles ca 5 mm long. Inflorescences spikes, uninterrupted, sometimes branched, ca 8 cm long, supported by leafy bracts, white-villous; bracts elliptic-ovate, ca 1.2 cm long, acute at apex, white villous, floccose all over, adhering to peduncle at base; bracteoles lanceolate, ca 1 cm long, floccose at margin and middle. Calyx ca 1.2 cm long, floccose-villous; segments linear-lanceolate, connate almost half way from base. Corolla tubular ventricose, ca 2.5 cm long, blue, mauve or white; cylinder base very short, ca 3 mm long; ventricose portion gradually expanding from base, hairy inside; lobes 5, orbicular, entire. Stamens 2, included, monadelphous; filaments ca 7 mm long, pilose; staminal sheath extending just above cylinder base. Ovary glabrous but hairy at tip; style ca 1.5 cm long, included, hairy. Capsules oblong, ca 1.2 cm long, 4-seeded; seeds orbicular, ca 1.5mm, complanate, densely hairy and hairs spreading when wet except on basal circular areole. [56]

Flower & Fruit December-February, Flowers once in 12 years.

Habitat Moist deciduous forests and on grassy slopes, 500 – 2000 m.

Distribution Tamil Nadu: Nilgiri, Coimbatore, Dindigal, Madurai, Salem and Tirunelveli. Karnataka: Mysore. Kerala: Idukki, Kottayam and Palghat.

Ethno medical information Traditionally the plant is used to treat joint pains and inflammations.

Figure 4. The plant ***Strobilanthes kunthianus***

5.1.2 *Strobilanthes cuspidatus*

Family: Acanthaceae

Morphological Description

Shrub, 1 m high; branch lets appressed-white-woolly or somewhat tawny, sticky. Leaves ovate, ca 12 x 5 cm, acute or cuneate at base, crenulate at margin, cuspidate-acuminate at apex, membranous, glabrous above, white-silky beneath; petioles ca 3 cm long. Inflorescences spikes, in terminal or upper axils, usually single or in 3s, ca 7 cm long, interrupted, glandular; peduncles ca 3 cm long; bracts linear-lanceolate, ca 1.5 cm long, acuminate, recurved at apex, glandular-hairy; bracteoles lanceolate, shorter, ca 8 mm long, acute, glandular-hairy. Calyx ca 1 cm long, glandular-hairy; lobes unequal, divided about base in flower, linear-lanceolate, ca 8 mm long, divided to base in fruit, accrescent, ca 1.5 cm long. Corolla tubular-ventricose, ca 1.5 cm long, glabrous or puberulous outside, long-hairy inside, blue; cylindric base small, ca 3 mm long; ventricose portion ca 1.2 cm long; lobes ovate-acute. stamens 2, included; staminal sheath extending to base of ventricose portion; filaments ca 8 mm long, sparsely hairy; anthers ca 2 mm. Ovary on a disc, ca 3 mm long; style ca 1.2 cm, hairy at base. Capsules with a minute stalk, ca 1.2 cm long, 4-seeded; seeds obliquely triangular, ca 2.5 mm long complanate, acute at apex, thin, hairy except at areole on both faces. [56]

Flower & Fruit December- April. Flowers once in 7 years.

Habitat 1000 – 2000 m in the Nilgiris; Foothills to 900 m in the Palnis and 100 m in Jukumari hills in East Godavari; always along streams but not generally rooting in water and seldom under much shade.

Distribution Tamil Nadu: Nilgiri, Chengalpattu, Coimbatore, Dindugal, Madurai, Salem and Tirunelveli. Andhra Pradesh: East Godavari and Visakhapatnam. Kerala: Idukki and Palghat. Orissa: Ganjam and Kalahandi.

Ethno medical information Traditionally the plant is used to treat joint pains and inflammations.

Figure 5. The plant ***Strobilanthes cuspidatus***

5.2 PHYTOCHEMICAL STUDIES

5.2.1 *Strobilanthes kunthianus*

5.2.1.1 Collection

Strobilanthes kunthianus (F. Acanthaceae) leaves were collected from Kalliti (10 kms from Ootacamund) in the Nilgiri hills, Tamilnadu, India, in Dec 2006.

5.2.1.2 Identification & Authentication

The leaves of *Strobilanthes kunthianus* (F. Acanthaceae) were identified and authenticated by the Director, Botanical Survey of India, Coimbatore, Tamilnadu, India and sample specimens were deposited, at the Department of Pharmacology, JSS College of Pharmacy, Ootacamund, Tamilnadu, India.

5.2.1.3 Extraction

Strobilanthes kunthianus leaves were air-dried in the shade and crushed to coarse powder. The crude ethanolic extract was obtained from the powdered botanical material by maceration in cold 80% ethanol with occasional agitation, for 7 days at room temperature (28-30^{0}c). The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure and stored in the dark at +4^{0}c until tested. [57] Henceforth, the ethanolic extract of *Strobilanthes kunthianus* will be called as **SKE**.

5.2.1.4 Qualitative phytochemical analysis

The following qualitative chemical tests were carried out for the extract of SKE to identify the presence of various chemical constituents. [58]

- **Test for alkaloids**
    - Mayer's reagent
    - Drangendroff's reagent
    - Wagner's reagent
    - Hager's reagent
- **Test for saponins**
    - Foam test
- **Test for carbohydrates**
    - Molisch's test
    - Fehling's test
    - Benedict's test
- **Test for glycosides**
    - Borntrager's test
    - Modified Borntrager's test
- **Test for steroids / terpenoids**
    - Libermann Burchard test
- **Test for fixed oils and fat**
    - Spot test
    - Saponification test
- **Test for tannins and phenolic compounds**
    - With dilute ferric chloride
    - With lead acetate
    - With gelatin
    - With aqueous bromine solution
    - Shinoda's test (Mg/HCl test – Magnesium turnings test)

- **Test for proteins and amino acids**
    - Biuret test
    - Ninhydrin test
- **Test for gums and mucilages**
    - Precipitation with alcohol
    - Molisch's test

Test for volatile oils

Steam distillation

5.2.2. *Strobilanthes cuspidatus*

5.2.2.1 Collection

S*trobilanthes cuspidatus* (F. Acanthaceae) leaves were collected from Kalliti (10 kms from Ootacamund) in the Nilgiri hills, Tamilnadu, India, in Dec 2006.

5.2.2.2 Identification & Authentication

The leaves of S*trobilanthes cuspidatus* (F. Acanthaceae) were identified and authenticated by the Director, Botanical survey of India, Coimbatore, Tamilnadu, India and sample specimens were deposited, at the Department of Pharmacology, JSS College of Pharmacy, Ootacamund, Tamilnadu, India.

5.2.2.3 Extraction

Strobilanthes cuspidatus leaves were air-dried in the shade and crushed to coarse powder. The crude ethanolic extract was obtained from the powdered botanical material by maceration in cold 80% ethanol with occasional agitation, for 7 days at room temperature (28-30^{0}c). The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure and stored in the dark at +4^{0}c until tested. [57] Henceforth, the ethanolic extract of S*trobilanthes cuspidatus* will be called as **SCE**.

5.2.2.4 Qualitative phytochemical analysis

The qualitative chemical tests done for SKE were also carried for the extract of SCE to identify the presence of various chemical constituents. [58]

5.3 *IN-VITRO* STUDIES

5.3.1 Anti-inflammatory studies

5.3.1.1 Human RBC membrane stabilization method

Human Red Blood Cells membrane stabilization was used as a method to study the anti-inflammatory activity. Blood was collected from a healthy volunteer who was not taken any NSAIDs for 2 weeks prior to the experiment; and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of SKE, SCE & Shallaki were prepared (10, 50 and 100µg/mL), and to each concentration, 1mL of phosphate buffer, 2 mL of hypo saline and 0.5 mL of HRBC suspension were added and incubated the mixture at 56^0 c for 30 min.The tubes were cooled under running water for 20 min, the mixture was centrifuged. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac (50 µg /mL) was used as a reference standard and distilled water as control in this study. [59] The percentage of HRBC membrane stabilization was calculated (by assuming the haemolysis produced in distilled water as 100%) using the formula:

$$\% \text{ protection} = \frac{100 - \text{optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

Statistical analysis

Results were analyzed by student's t-test, 'p' value less than 0.05 were taken as significant.

5.3.2 Anti-Osteoarthritis studies

5.3.2.1 Rabbit cartilage explants culture method

Articular cartilages from hock joints of 5-week-old rabbit were removed immediately after the animal was sacrificed. The care and handling of the animal was in accordance with the guidelines of Institutional Animal Ethics Committee. After the articular surfaces were exposed surgically under sterile conditions, approximately 200-220 mg of articular surfaces per joint were dissected and submerged into complete medium (DMEM, supplemented with heat inactivated 5% FBS; penicillin 100U/ml; streptomycin 100 µg/ml). They were then rinsed several times with the complete medium and incubated for 1 to 2 days at 37^0C in a humidified 5% CO_2/95% air incubator for stabilization. The complete medium was replaced with a basal medium (DMEM, supplemented with heat-inactivated 1% FBS, 10mM HEPES, and penicillin 100U/ml, streptomycin 100 µg/ml). Approximately 50 to 60 mg cartilage pieces were placed in 24-well plates and treated with 10,50,100 and 200 µg/ml concentrations of SCE and SKE, shallaki (50 µg/ml), diclofenac (50 µg/ml) and celecoxib (50 µg/ml). After pretreatment for 1 h, 5 ng/mL of rhIL-1α was added to the culture medium and further incubated at 37^0C in a humidified 5% CO2/95% air incubator. The culture medium was collected 60 h later and stored at -20^0C until assay. [60]

Measurements of glycosaminoglycans (GAG)

The amount of sulphated GAGs in the medium at the end of reaction reflecting the amount of proteoglycan (PG) degradation was determined through 1,9-dimethyl-methylene blue method using a commercially available kit (The Blyscan proteoglycan & glycosaminoglycan assay kit, Biocolor Ltd., New Delhi). The amount of glycosaminoglycan release was estimated spectrophotometrically at 656 nm. [60]

Statistical analysis

Results were analyzed by student's t-test, 'p' value less than 0.05 were taken as significant.

5.4 *IN-VIVO* STUDIES

Experimental animals

Adult Wistar albino rats (150-180 g) and Swiss albino mice (25-30 g) of either sex were procured from the laboratory animal house, J.S.S. College of pharmacy, Ootacamund, Tamilnadu, India and used in the study. The animals were kept under standard environmental conditions of room temperature ($22^0 \pm 2^0$C), relative humidity (50% ± 5%) and 12 h light and dark cycle. The animals were housed in the colony cages (either three rats or six mice per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Brook bond Lipton India limited, Bangalore) and water *ad libitum.*

All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of Ootacamund No. JSSCP/IAEC/PH.D/PH.COLOGY/01/2007-08.

5.4.1 Behavioral & toxicity studies

5.4.1.1 Acute toxicity study

3 groups were formed with 10 rats in each group. All the animals were fasted over night before the test.

The treatment was given in the following manner:

Group-I - Control (Saline 0.9% Nacl; p.o)

Group-II- SKE (2000 mg/kg; p.o)

Group-III- SCE (2000 mg/kg; p.o)

In all the cases the dosing volume was fixed at 10 ml/kg body weight. The suspensions of SKE & SCE were prepared using saline (0.9% Nacl) and were administered by oral gavage.

First group was given equivolumes of saline, second group was given 2000 mg/kg body weight of freshly prepared SKE and the third group was given SCE.

The animals were observed for 0 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr and there after every day for 14 days. At the end of 14^{th} day the animals were sacrificed with excessive ether anesthesia and dissected for examination of vital organs like brain, liver, kidney, lungs and heart for pathological changes. [61]

5.4.2 Efficacy studies

5.4.2.1 Analgesic activity

5.4.2.1.1 Acetic acid induced writhing in mice

7 groups were formed with 6 mice in each group.

Mice were pretreated with test agents in appropriate volumes; all administered orally.

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/ kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Aspirin (100 mg/kg; p.o)

After 30 minutes pre treatment time each mouse was administered 0.7% of an aqueous solution of acetic acid (10 ml/kg) intraperitoneally and the mice were then placed in transparent Perspex observation boxes for observation. After a 5 min lag period post administration of acetic acid, the numbers of abdominal constrictions were counted for each mouse for 10 minutes. [62] The percentage inhibition of constrictions for the test agents (SKE, SCE), shallaki, standard (Aspirin), and control were calculated.

Statistical analysis

Results were analyzed by one way ANOVA, followed by Turkey's multiple comparison test, 'p' value less than 0.05 were taken as significant.

5.4.2.1.2 Randall – selitto assay

7 groups were formed with 6 wistar rats in each group.

Edema was induced by administration of 0.1ml of 20% suspension of brewers yeast (subcutaneous) to the planter surface of the left hind paw. One hour after injection of brewers yeast, test agents (SKE, SCE), shallaki and aspirin (300mg/kg; p.o) were administered. One and three hour later a pressure stimulus was applied to the inflamed paw with an analgesiometer (Ugo basile, Italy). The strength of stimulus, which produced a pain reaction in the rat, was taken as the pain threshold. [63] Increase in the pain threshold in drug treated group was compared with that of control group.

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)
II Group – SKE (100 mg/kg; p.o)
III Group - SKE (200 mg/kg; p.o)
IV Group - SCE (100 mg/kg; p.o)
V Group - SCE (200 mg/kg; p.o)
VI Group – Shallaki (50 mg/kg; p.o)
VII Group – Aspirin (300 mg/kg; p.o)

Statistical analysis

Results were analyzed by one way ANOVA, followed by Turkey's multiple comparison test, 'p' value less than 0.05 were taken as significant.

5.4.2.2 Anti – Inflammatory activity

5.4.2.2.1 Carrageenan induced rat paw edema method

7 groups were formed with 6 wistar rats in each group.

Test agents (SKE, SCE), shallaki and diclofenac (100mg/kg) were administered (p.o) 30 minutes before 1% carrageenan injection (0.1ml) into the sub plantar area of the right hind paw. The volumes of injected and contralateral paws were measured 1, 2 and 3 hours after induction of edema by using plethysmometer (Ugo basile, Italy). [64]

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Diclofenac (100 mg/kg; p.o)

Statistical analysis

Results were analysed by one way ANOVA, followed by Turkey's multiple comparison test, 'p' value less than 0.05 were taken as significant.

5.4.2.2.2 Cotton pellet induced granuloma formation in rats

7 groups were formed with 6 rats in each group.

A sterilized cotton pellet weighing 20 mg was introduced subcutaneously into the groin region of rats after the rats have been anaesthetized with ketamine. Following the implantation of the cotton pellet, the animals in the control, test group and reference groups were treated once daily for 4 days with saline, test agents (SKE, SCE), shallaki and diclofenac respectively. All the animals were sacrificed on the fifth day with an overdose of phenobarbitone sodium (40 mg/kg; i.p.) and the pellet surrounded by granuloma tissue was dissected out carefully and dried overnight in an oven at 60^0C to a constant weight. The mean weight of the granuloma tissue formed in each group was obtained and the percentage inhibition was determined by comparing the mean weight in the control group. [65]

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Diclofenac (10mg/kg; p.o)

Statistical analysis

Results were analyzed by one way ANOVA, followed by Turkey's multiple comparison test, 'p' value less than 0.05 were taken as significant.

5.4.2.3 Anti-osteoarthritis activity

5.4.2.3.1 Iodoacetate induced osteoarthritis

8 groups were formed with 10 rats in each group.

The rats were anaesthetized with ketamine and arthritis was induced by 25 µl of intra-articular injection of 10mg/ml concentration of iodoacetate into the left knee joint of rats. Then the rats were treated with test agents (SKE, SCE), shallaki and standard (diclofenac, celecoxib) for a period of 21 days. After 21 days the animals were sacrificed by excess dose of phenobarbitone sodium (40mg/kg, i.p.) and left joint was immediately disarticulated and fixed in 10% buffered formalin for 24-48 h prior to capturing the image. [66]

The treatment was given in the following manner:

I Group – Control (Normal saline 10 ml/kg; p.o)

II Group – SKE (100 mg/kg; p.o)

III Group - SKE (200 mg/kg; p.o)

IV Group - SCE (100 mg/kg; p.o)

V Group - SCE (200 mg/kg; p.o)

VI Group – Shallaki (50 mg/kg; p.o)

VII Group – Diclofenac (100mg/kg; p.o)

VIII Group - Celecoxib (100mg/kg; p.o)

Articular cartilage lesion score

After fixation, an image of the tibial cartilage was captured using an Optimas image analysis system. The tibial plateau was utilized for image analysis because it provided a relatively flat surface compared with the femoral condyles, allowing the image analysis camera to focus on the entire cartilage surface.

Cartilage damage was assessed using a scale of 0-4 of increasing severity [0=normal; 4=maximum severity]. [66]

Statistical analysis

Results were analyzed by Mann-Whitney test, ‘p’ value less than 0.05 were taken as significant.

5.4.3 Sub acute toxicity study

The study incorporated 35 male and 35 female wistar rats. Five animals from each sex were randomly placed into each of the seven treatment groups (10 total animals per treatment group: 5 male and 5 female) representing control, low, mid and high dose levels.

Animals were administered 0 mg (Control), 200 mg (low-dose), 400 mg (mid-dose) and 800 mg (high-dose) of SKE and SCE / kg b.w/ day in a solution of normal saline by oral gavage for four weeks.

The treatment was given in the following manner.

I Group – Control (Normal saline; p.o)
II Group - SKE (200 mg/kg; p.o)
III Group - SKE (400 mg/kg; p.o)
IV Group - SKE (800 mg/kg; p.o)
V Group - SCE (200 mg/kg; p.o)
VI Group - SCE (400 mg/kg; p.o)
VII Group - SCE (800 mg/kg; p.o)

Clinical observations

Mortality was assessed twice daily, whereas examinations regarding food consumption, body weight, reflexes (i.e., pain and corneal reflexes), behavior and general condition were observed weekly. Ophthalmic, hearing and dental examinations were conducted prior to the first substance administration and during the test week 4. The eyes were examined with a focused light beam, the ears were checked with a simple noise test and teeth were visually inspected.

Hematology and biochemical estimations

Hematology and biochemical assessments were conducted at the end of study. [67] Blood samples were obtained from the retro orbital venous plexus of one eye. EDTA (Merck, Mumbai, India) was used as an anticoagulant during the assessment of hematological

parameters. Hematological parameters assessed [Cell analyzer, Boule Medical AB, Sweden) were, erythrocytes (RBC), hemoglobin, haematocrit, leucocytes (WBC) and thrombocytes (Platelets).

The serum was separated from whole blood samples, and subsequently used for biochemical assessments. Parameters assessed [Auto analyzer, Micro lab 200, Merck, India.] include aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase, total proteins, albumin, total bilirubin, urea, glucose, creatinine, total cholesterol, and triglycerides.

Pathology

At the end of the treatment period, the animals were anaesthetized with thiopentone sodium (40 mg/ kg, i.p) and sacrificed by exsanguinations. All animals were subjected to full gross necropsy, which included examination of the external surface of the body, orifices and the cranial, thoracic and abdominal cavities and their contents.

The liver, kidney, adrenals, testes/ ovary, spleen, brain, lungs and heart of all the animals were trimmed of any adherent tissues and their wet weight were recorded (Sartorius, India) after the dissection. Brain, heart, lungs, liver and kidneys were collected and fixed in a 10% buffered formalin solution. All the fixed organs from animals in the control and low-dose, mid-dose, high-dose groups were embedded in the paraffin wax, sectioned and subsequently stained with haemotoxylin and eosin (H&E). All sample slides were examined microscopically for pathological observations. [67]

Statistical analysis

Values were expressed as mean ± SEM. The treatment groups were statistically compared with the vehicle treated rats of individual sex using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, values $P < 0.05$ were considered as statistically significant.

6. RESULTS & DISCUSSION

6.1 PHYTOCHEMICAL STUDIES

6.1.1 Plant material and extraction

6.1.1.1 *Strobilanthes kunthianus*

The air-dried and finely ground leaves of *Strobilanthes kunthianus* was extracted by maceration with cold 80% ethanol, at 28-30^{0}c for 7 days, when filtered and concentrated under reduced pressure gave a yield of 11.4% w/w. This was kept in the dark at +4^{0}c until tested. Hence forth, the extract of *Strobilanthes kunthianus* will be called as **SKE** (Table 3).

6.1.1.2 *Strobilanthes cuspidatus*

The air-dried and finely ground leaves of *Strobilanthes cuspidatus* was extracted by maceration with cold 80% ethanol, at 28-30^{0}c for 7 days, when filtered and concentrated under reduced pressure gave a yield of 10.8% w/w. This was kept in the dark at +4^{0}c until tested. Hence forth, the extract of *Strobilanthes cuspidatus* will be called as **SCE** (Table 3).

Table 3. Percentage yield of ethanolic extracts of leaves of *Strobilanthes kunthianus* (SKE) and *Strobilanthes cuspidatus* (SCE)

S.No.	Name of the plant	Percentage yield (w/w)
1	*Strobilanthes kunthianus*	11.4%
2	*Strobilanthes cuspidatus*	10.8%

6.1.2 Qualitative phytochemical analysis

6.1.2.1 *Strobilanthes kunthianus*

Preliminary phytochemical screening revealed the presence of carbohydrates, phytosterols, triterpenoids, flavonoids and tannins in SKE (Table 4).

6.1.2.2 *Strobilanthes cuspidatus*

Preliminary phytochemical screening revealed the presence of carbohydrates, phytosterols, triterpenoids, flavonoids and tannins in SCE (Table 4).

Table 4. Preliminary qualitative phytochemical analysis of SKE and SCE

TEST	SKE	SCE
Alkaloids	-	-
Carbohydrates	+	+
flavonoids	+	+
Saponins	-	-
Proteins & amino acids	-	-
Phytosterols	+	+
Triterpenoids	+	+
Fixed oils and fats	-	-
Tannins	+	+
Gums and mucilages	-	-
Volatile oil	-	-

6.2 *IN-VITRO* STUDIES

6.2.1 Anti-Inflammatory activity

6.2.1.1 Human RBC membrane stabilization method

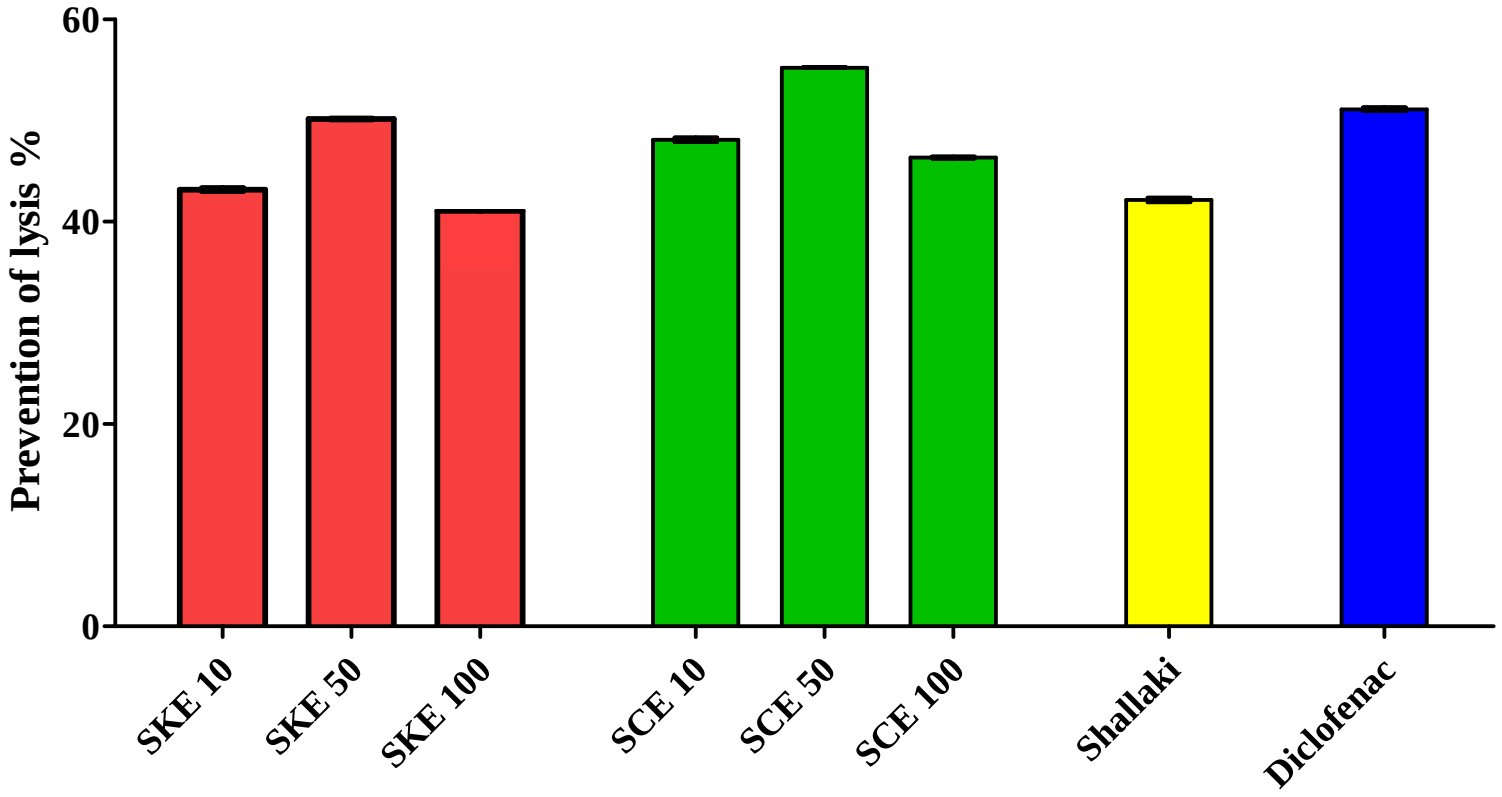
The membrane stabilizing activity of two ethanolic extracts SKE and SCE at concentrations 10, 50, and 100 µg/ml were studied on heat induced lysis of human red blood cell membrane (HRBC membrane). The extracts SKE and SCE at a concentration of 50 µg/ml shown highest protection (50.16% and 55.24% respectively) and the effect was equipotent to diclofenac – 50 mg/kg (51.11%). Further, the protective effect of both extracts SKE and SCE at a concentration of 100 µg/ml was lesser (41.1% and 46.35% respectively) than the effect produced at concentration 50 µg/ml showing that the increase in concentration is producing the decreased activity. Moreover, both the extracts at concentration of 50 µg/ml shown 7% much effect than 10 µg/ml showing that the extracts are possessing the dose dependent activity upto 50 µg/ml. (Table 5 and Graph 1)

Table 5. Effect of SKE and SCE on human RBC Membrane stabilization

S.No.	Treatment	Concentration (µg/ml)	Activity (Prevention of lysis %) (Mean ± SD)
1	SKE	10	43.18 ± 0.08
		50	50.16 ± 0.05
		100	41.10 ± 1.14
2	SCE	10	48.11 ± 0.09
		50	55.24 ± 0.02
		100	46.35 ± 0.04
3	Shallaki	50	42.16 ± 0.09
4	Diclofenac	50	51.11 ± 0.07

Values are mean ± SD (n=6 trials per group)

Graph No. 1 Effect of SKE & SCE on Human RBC Membrane Stabilization



6.2.2. Anti-Osteoarthritis activity

6.2.2.1 Rabbit cartilage explants culture

The protective activity of two ethanolic extracts SKE and SCE at concentrations 10, 50, 100, and 200 µg/ml on rhIL-1α induced degradation of proteoglycan in rabbit cartilage explants culture was studied and the percentage inhibition of proteoglycan degradation was calculated. Treatment with SKE and SCE at a concentration of 100 µg/ml shown significant ($p<0.05$) protective effect (71% and 78.3% respectively) in inhibiting the proteoglycan degradation and the effect was equipotent to celecoxib – 50 µg/ml (80.9%). Moreover, the activity of diclofenac – 50 µg/ml (45.4%) and shallaki - 50 µg/ml (52.6%) were found to be lesser when compared to SKE (100 µg/ml) and SCE (100 µg/ml). Further, the treatment with 200 µg/ml of both extracts SCE and SKE shown a lesser activity (51.9% and 55.9% respectively) shows that increase in concentration decreased the activity. Moreover, the protective effect of both extracts at concentrations 10, 50, and 100 µg/ml possess dose dependent activity. (Table 6 and Graph 2).

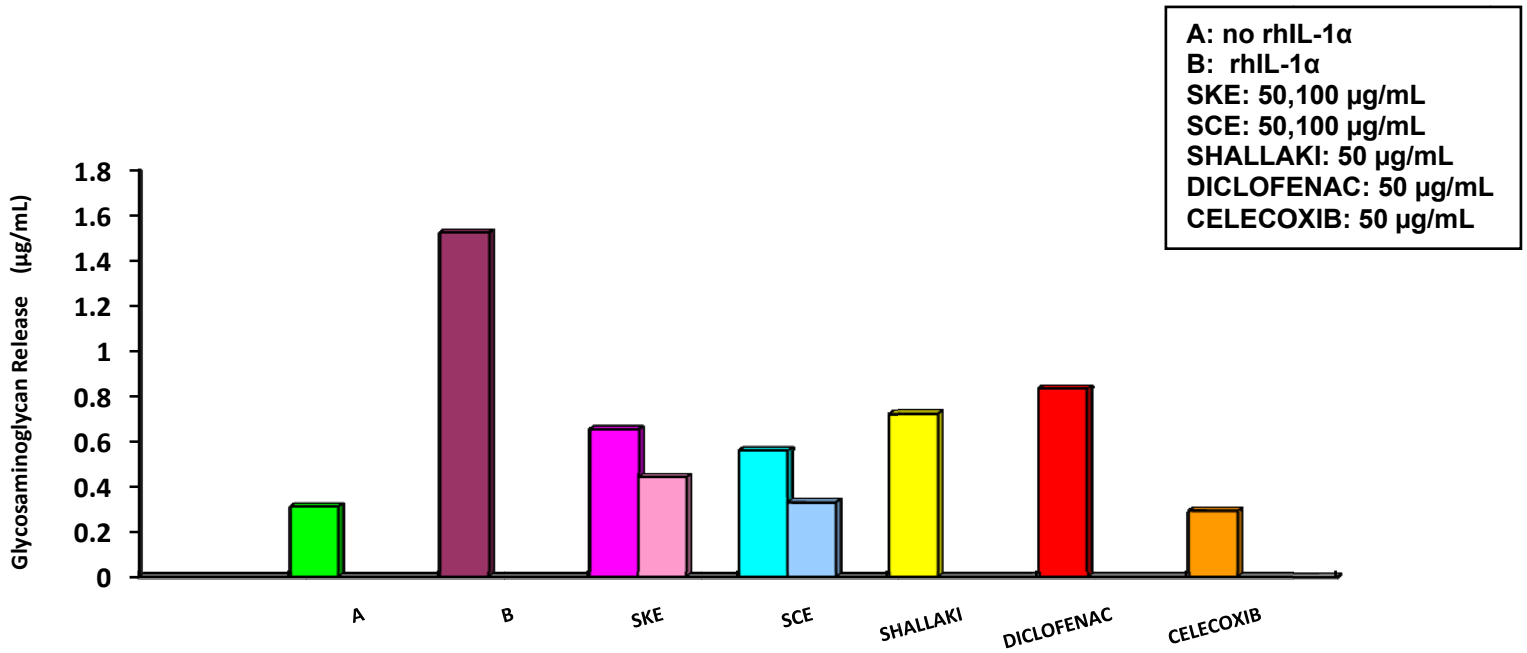
Table 6. Effect of SKE and SCE on rhIL-1α induced degradation of proteoglycan in rabbit cartilage explants culture

S.No.	Treatment	Concentration (μg/ml)	Amount of glycosaminoglycan released (μg/ml) (mean ± SD)	% inhibition of proteoglycan degradation
1	Vehicle	-	0.31 ± 0.04	-
2	Control	-	1.52 ± 0.06	-
3	SKE	10	1.03 ± 0.09[b]	32.2
		50	0.65 ± 0.02[c]	57.2
		100	0.44 ± 0.01[c]	71.0
		200	0.73 ± 0.06[c]	51.9
4	SCE	10	0.93 ± 0.05[c]	38.8
		50	0.56 ± 0.01[c]	63.1
		100	0.33 ± 0.03[c]	78.3
		200	0.67 ± 0.08[c]	55.9
5	Shallaki	50	0.72 ± 0.03[c]	52.6
6	Diclofenac	50	0.83 ± 0.07[c]	45.4
7	Celecoxib	50	0.29 ± 0.03[c]	80.9

Values are Mean ± SD (n=6 trials per group)
a-P<0.05, b-P<0.01, c-P<0.001 when compared to control by student't' test

GRAPH 2

Graph-2: rhIL – 1α induced degradation of proteoglycan in rabbit cartilage explants culture



6.3 *IN-VIVO* STUDIES

6.3.1 Behavioral & toxicity studies

6.3.1.1 Acute toxicity study

SKE

The ethanolic extract of leaves of *Strobilanthes kunthianus* (SKE) was found to be safe and no mortality of the rats was observed at dose of 2000 mg/kg for 14 days in acute toxicity study.

SCE

The ethanolic extract of leaves of *Strobilanthes cuspidatus* (SCE) was found to be safe and no mortality of the animals (female Wistar rats) was observed at dose of 2000 mg/kg for 14 days in acute toxicity study.

6.3.2 Efficacy studies

6.3.2.1 Analgesic activity

6.3.2.1.1 Acetic acid induced writhing test

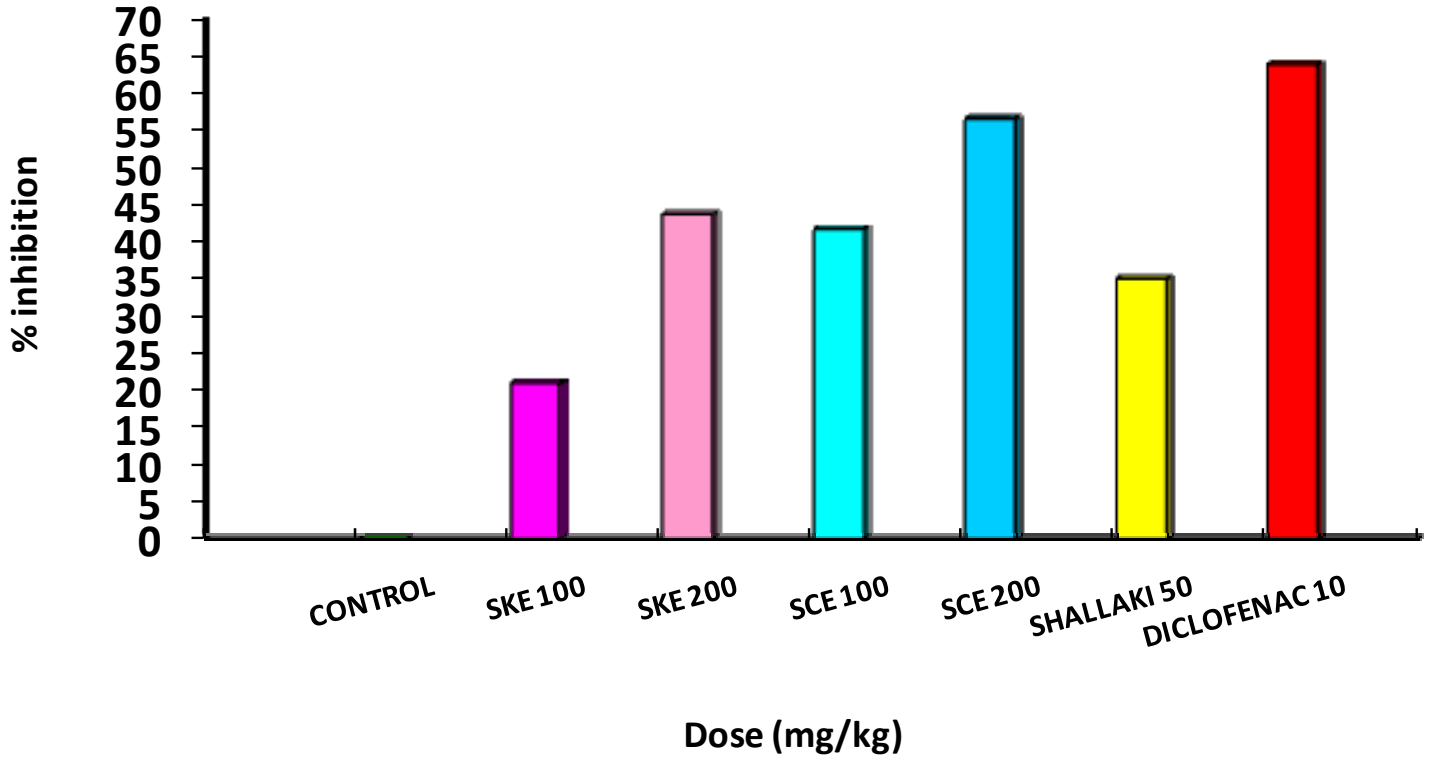
The effect of two ethanolic extracts SKE and SCE at two dose levels 100 mg/kg and 200 mg/kg in acetic acid induced writhing in mice was studied and the percentage inhibition was calculated. The study revealed that pretreatment of mice with SKE (200 mg/kg) and SCE (100 mg/kg and 200 mg/kg) showed similar significance ($P<0.001$) in inhibiting the writhing responses. The extract SKE at 100 mg/kg shown lesser activity ($P<0.05$). In particular, the treatment with SKE (200 mg/kg) showed significant ($P<0.05$) inhibition of wriths when compared to SKE (100 mg/kg) showing the dose dependent activity and the similar kind of difference in activity was not observed with two doses of SCE. Further, the activity with SCE (200 mg/kg) was equipotent with aspirin (100 mg/kg). (Table 7 and Graph 3)

Table 7. Effect SKE and SCE on acetic acid induced writhing in mice

S.No.	Treatment	Dose (mg/kg)., p.o	Number of wriths (10 min)	% inhibition of wriths
1	Control (Saline)	10 ml/ kg	29.33 ± 0.88	-
2	SKE	100	23.33 ± 2.64[a]	20.45
3	SKE	200	16.66 ± 0.95[cd]	43.19
4	SCE	100	17.16 ± 0.94[c]	41.49
5	SCE	200	12.83 ± 0.87[c]	56.25
6	Shallaki	50	19.16 ± 0.83[c]	34.67
7	Aspirin	100	10.66 ± 0.88[c]	63.65

Values are mean ± SEM (n=6)
a-P<0.05, b-P<0.01, c-P<0.001 when compared control vs. treatment groups
d-P<0.05, e-P<0.01, f-P<0.001 when compared SKE 100 vs. SKE 200
g-P<0.05, h-P<0.01, i-P<0.001 when compared SCE 100 vs. SCE 200
by one-way ANOVA followed by Turkey's multiple comparison test.

Graph-3: Effect of SKE and SCE On Acetic Acid Induced Writhing in Albino Mice



6.3.2.1.2 Randall– Selitto assay

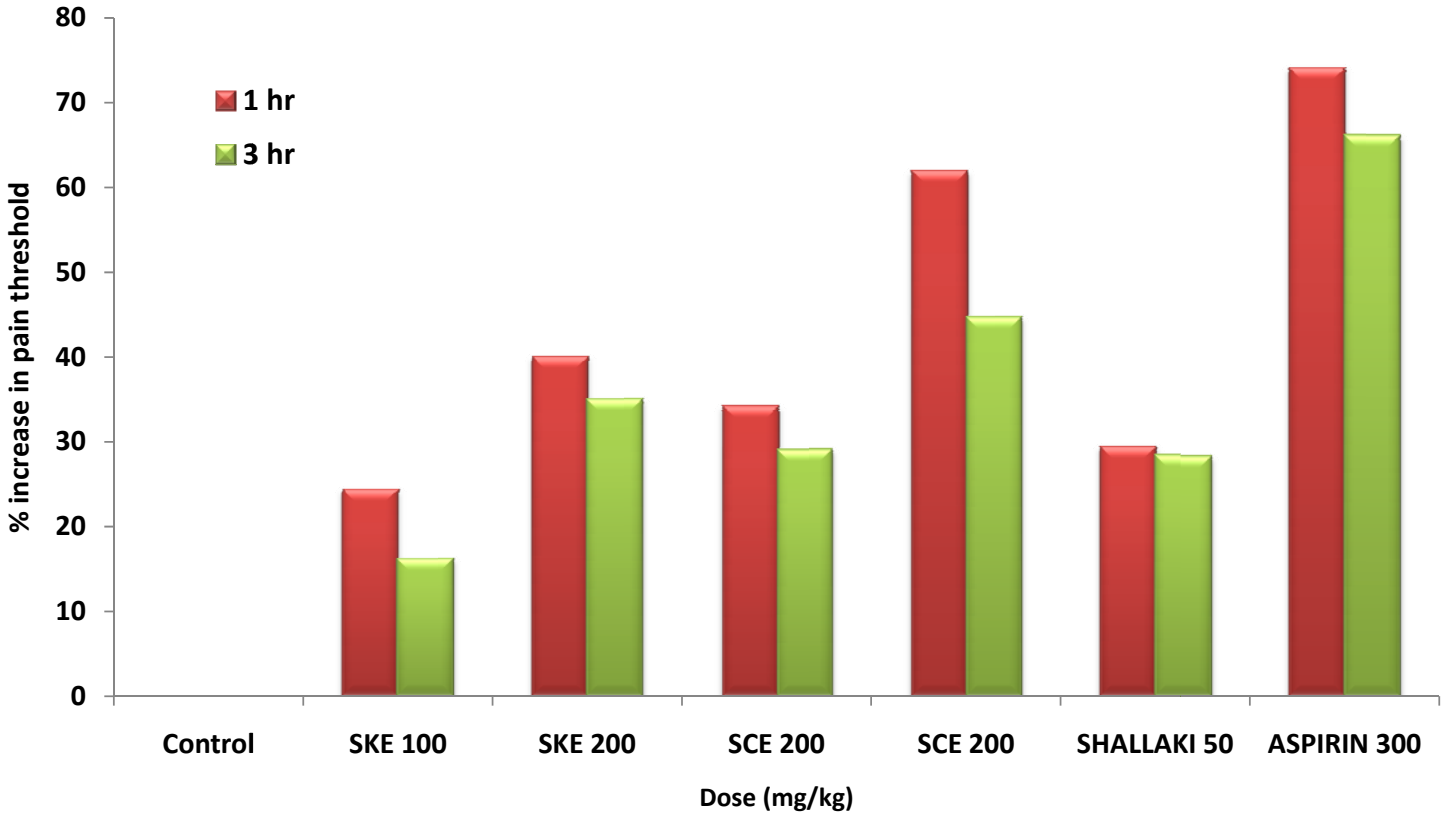
The effect of two ethanolic extracts SKE and SCE at two dose levels 100 mg/kg and 200 mg/kg in brewer's yeast induced pain in rats was studied and the percentage increase in pain threshold was calculated. The study revealed that pretreatment of rats with 200 mg/kg of both extracts of SKE and SCE showed significant ($P<0.001$) increase in pain threshold at the end of 1st hour and the response was continued upto 3 hours. The extract SCE (100 mg/kg) shown lesser activity ($P<0.01$) and it is equipotent to shallaki - 50 mg/kg ($P<0.01$). In particular, the treatment with SKE (100 mg/kg) showed significant ($P<0.05$) increase in pain threshold by the end of 1st hour but the response was not continued upto 3rd hour. Moreover, the treatment with SCE (200 mg/kg) produced significant ($P<0.05$) increased response when compared to SCE (100 mg/kg) showing the dose dependent activity and the similar kind of difference in activity was not observed with two doses of SKE. (Table 8 and Graph 4)

Table 8. Effect of SKE and SCE on Brewer's yeast induced pain threshold in paw pressure test in Wistar rats (Randall-selitto assay)

S.No.	Treatment	Dose (mg/kg)., p.o	Pressure on paw 'g'		
			Oh	1 h	3 h
1	Control (Saline)	10 ml/ kg	73.16 ± 4.07	77.66 ± 4.02	67.33 ± 2.31
2	SKE	100	69.83 ± 2.70	96.5 ± 3.06[a] (24.25)	78.16 ± 2.08 (16.08)
3	SKE	200	68.33 ± 2.65	108.66 ± 5.92[c] (39.91)	90.83 ± 4.36[c] (34.90)
4	SCE	100	72.33 ± 2.74	104.16 ± 4.72[b] (34.12)	86.83 ± 2.58[b] (28.96)
5	SCE	200	70.83 ± 2.70	125.66 ± 3.62[cg] (61.80)	97.33 ± 3.53[c] (44.55)
6	Shallaki	50	70.16 ± 3.73	100.33 ± 3.28[b] (29.19)	86.33 ± 3.60[b] (28.21)
7	Aspirin	300	74 ± 3.93	135.16 ± 3.87[c] (74.04)	111.83 ± 4.85[c] (66.09)

Values are mean ± SEM (n=6)
The figures in parenthesis indicate the percent increase in pain threshold in comparison with control group.
a-P<0.05, b-P<0.01, c-P<0.001 when compared control vs. treatment groups
d-P<0.05, e-P<0.01, f-P<0.001 when compared SKE 100 vs. SKE 200
g-P<0.05, h-P<0.01, i-P<0.001 when compared SCE 100 vs. SCE 200
by one-way ANOVA followed by Turkey's multiple comparison test.

Graph-4 Effect of SKE And SCE on brewer's yeast induced pain threshold in paw pressure test in wistar rats (Randall-selitto assay)



6.3.2.2 Anti-inflammatory activity

6.3.2.2.1 Carrageenan induced rat paw edema

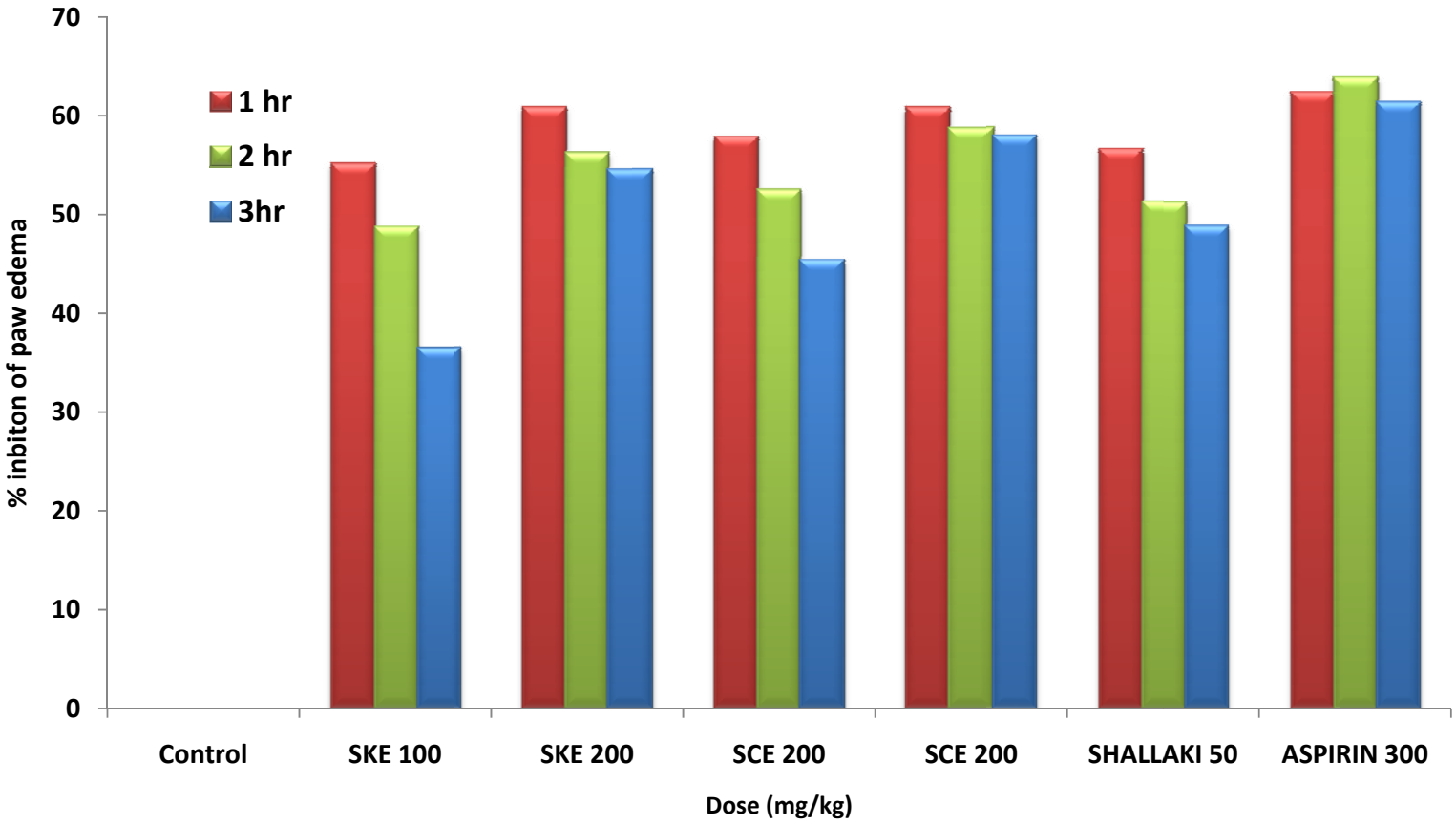
The effect of two ethanolic extracts SKE and SCE at two dose levels 100 mg/kg and 200 mg/kg in carrageenan induced rat paw edema was studied and the percentage inhibition of paw edema was calculated. The study revealed that pretreatment of rats with SKE (200 mg/kg) and SCE (100 mg/kg and 200 mg/kg) shown similar significance ($P<0.001$) in inhibiting the development of paw edema upto 3 hours. In particular, pre-treatment with SKE (100mg/kg) was also significantly ($P<0.001$) inhibited the paw volume upto 2 hours but the response was decreased ($P<0.01$) at the end of 3rd hour. Further, SKE (200 mg/kg) and SCE (200 mg/kg) shown equipotent response with shallaki (50 mg/kg) and diclofenac (100 mg/kg) in inhibiting the paw edema by the end of 3rd hour. (Table 9 and Graph 5)

Table 9. Effect of SKE and SCE on carrageenan induced rat paw edema

Treatment	Dose (mg/kg)., p.o	Mean increase in paw volume (mL)			
		Time in hours			
		0	1	2	3
Control (Saline)	10 ml/kg	0.27 ± 0.12	0.69 ± 0.07	0.80 ± 0.11	0.88 ± 0.15
SKE	100	0.25 ± 0.11	0.31 ± 0.05[c] (55.1)	0.41 ± 0.02[c] (48.7)	0.56 ± 0.04[a] (36.4)
SKE	200	0.23 ± 0.07	0.27 ± 0.02[c] (60.8)	0.35 ± 0.03[c] (56.2)	0.40 ± 0.02[c] (54.5)
SCE	100	0.25 ± 0.12	0.29 ± 0.08[c] (57.9)	0.38 ± 0.03[c] (52.5)	0.48 ± 0.02[c] (45.4)
SCE	200	0.23 ± 0.01	0.27 ± 0.04[c] (60.8)	0.33 ± 0.01[c] (58.7)	0.37 ± 0.01[c] (57.9)
Shallaki	50	0.23 ± 0.06	0.30 ± 0.04[c] (56.5)	0.39 ± 0.04[c] (51.2)	0.45 ± 0.02[c] (48.8)
Diclofenac	100	0.24 ± 0.02	0.26 ± 0.06[c] (62.3)	0.29 ± 0.01[c] (63.8)	0.34 ± 0.03[c] (61.3)

Values are Mean ± SEM (n=6)
a-P<0.05, b-P<0.01, c-P<0.001 when compared control vs. treatment groups
d-P<0.05, e-P<0.01, f-P<0.001 when compared SKE 100 vs. SKE 200
g-P<0.05, h-P<0.01, i-P<0.001 when compared SCE 100 vs. SCE 200
by one-way ANOVA followed by Turkey's multiple comparison test.
Each value in parentheses indicates the percentage inhibition rate.

Graph-5 Effect of SKE and SCE on Carrageenan induced rat paw edema



6.3.2.2.2 Cotton pellet induced granuloma formation

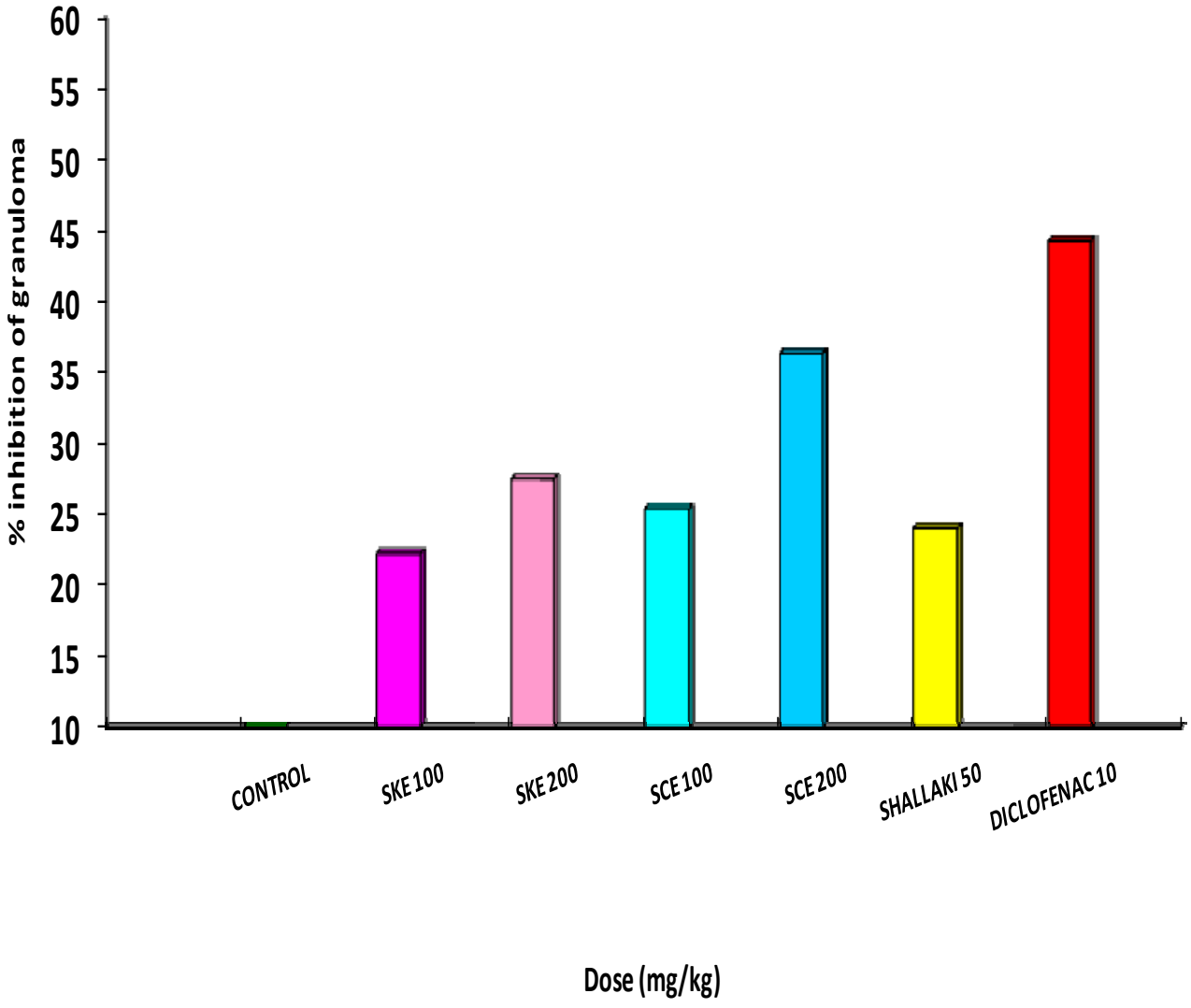
The effect of two ethanolic extracts SKE and SCE at two dose levels 100 mg/kg and 200 mg/kg in cotton pellet induced granuloma formation in rats was studied and the percentage inhibition of granuloma was calculated. The study revealed that treatment of rats with SKE - 200 mg/kg ($P<0.01$), SCE - 100 mg/kg ($P<0.05$) and SCE - 200 mg/kg ($P<0.001$) showed significant reduction in weight of cotton pellet induced granuloma. The extract SKE (200 mg/kg) inhibited the granuloma formation (27.5%) and the effect was equipotent with shallaki – 50 mg/kg (23.95%). Treatment with SCE (100 mg/kg and 200 mg/kg) inhibited the granuloma upto 25.47 % and 36.39 % respectively. Whereas, the extract SCE (200 mg/kg) was found to be similarly effective ($P<0.001$) in reducing the granuloma formation when compared to diclofenac (100 mg/kg). (Table 10 and Graph 6)

Table 10. Effect of SKE and SCE on cotton pellet induced granuloma in rats

S.No.	Treatment	Dose (mg/kg).,p.o	Increase in weight of pellet (mg)	% inhibition
1	Control (Saline)	10 ml/kg	49.26±3.18	-
2	SKE	100	38.31±2.11	22.22
3	SKE	200	35.7±2.83[b]	27.52
4	SCE	100	36.71±2.64[a]	25.47
5	SCE	200	31.38±2.48[c]	36.29
6	Shallaki	50	37.46±2.12[a]	23.95
7	Diclofenac	10	27.41±1.74[c]	44.35

Values are Mean ± SEM (n=6)
a-P<0.05, b-P<0.01, c-P<0.001 when compared control vs. treatment groups
d-P<0.05, e-P<0.01, f-P<0.001 when compared SKE 100 vs. SKE 200
g-P<0.05, h-P<0.01, i-P<0.001 when compared SCE 100 vs. SCE 200
by oneway ANOVA followed by Turkey's multiple comparison test.
Each value in parentheses indicates the percentage inhibition rate.

Graph-6: EFFECT OF SKE AND SCE ON COTTON PELLET INDUCED GRANULOMA IN WISTAR RATS



6.3.2.3 Anti-Osteoarthritis activity

6.3.2.3.1 Iodoacetate induced osteoarthritis in rats

The effect of two ethanolic extracts SKE and SCE at two dose levels 100 mg/kg and 200 mg/kg in iodoacetate induced cartilage damage in rats was studied and the percentage reduction of cartilage damage was calculated. The study revealed that treatment of rats with SKE (100 mg/kg and 200 mg/kg) and SCE (100 mg/kg and 200 mg/kg) showed similar significance ($P<0.05$) in reducing the damage of articular cartilage. The extract SKE (200 mg/kg) significantly ($P<0.05$) inhibited the cartilage damage (31.2%) when compared to SKE - 100 mg/kg (15.8%). Treatment with SCE (200 mg/kg) significantly ($P<0.05$) reduced the cartilage destruction (43.6%) when compared to SCE - 100 mg/kg (21.06%) showing the dose dependent activity. The percentage reduction of joint damage with treatment of shallaki (50 mg/kg) was found to be 6.4% and 18.8% lesser when compared to SKE (200 mg/kg) and SCE (200 mg/kg) respectively. (Table 11, Graph 7 and Figure 6)

Table 11. Effect of SKE and SCE on joint damage in the rat iodoacetate model

S.No.	Treatment	Dose (mg/kg).,p.o	Cartilage lesion score (Mean ± SEM)	% reduction
1	Control (Saline)	10 ml/kg	2.66 ± 0.21	-
2	SKE	100	2.24 ± 0.17*	15.8
3	SKE	200	1.83 ± 0.16*†	31.20
4	SCE	100	2.10 ± 0.14*	21.06
5	SCE	200	1.50 ± 0.11*#	43.60
6	Shallaki	50	2.0 ± 0.13*	24.81
7	Diclofenac	100	2.12 ± 0.24*	20.31
8	Celecoxib	100	1.40 ± 0.12*	47.36

Values are expressed as the mean joint damage or cartilage lesion score ± SEM from 10 animals.

* P<0.05 when compared control vs. treatment groups

† P<0.05 when compared SKE 100 vs. SKE 200

# P<0.05 when compared SCE 100 vs. SCE 200

by using Mann-Whitney test.

Graph-7: Effect of SKE and SCE on joint damage in the rat iodoacetate model

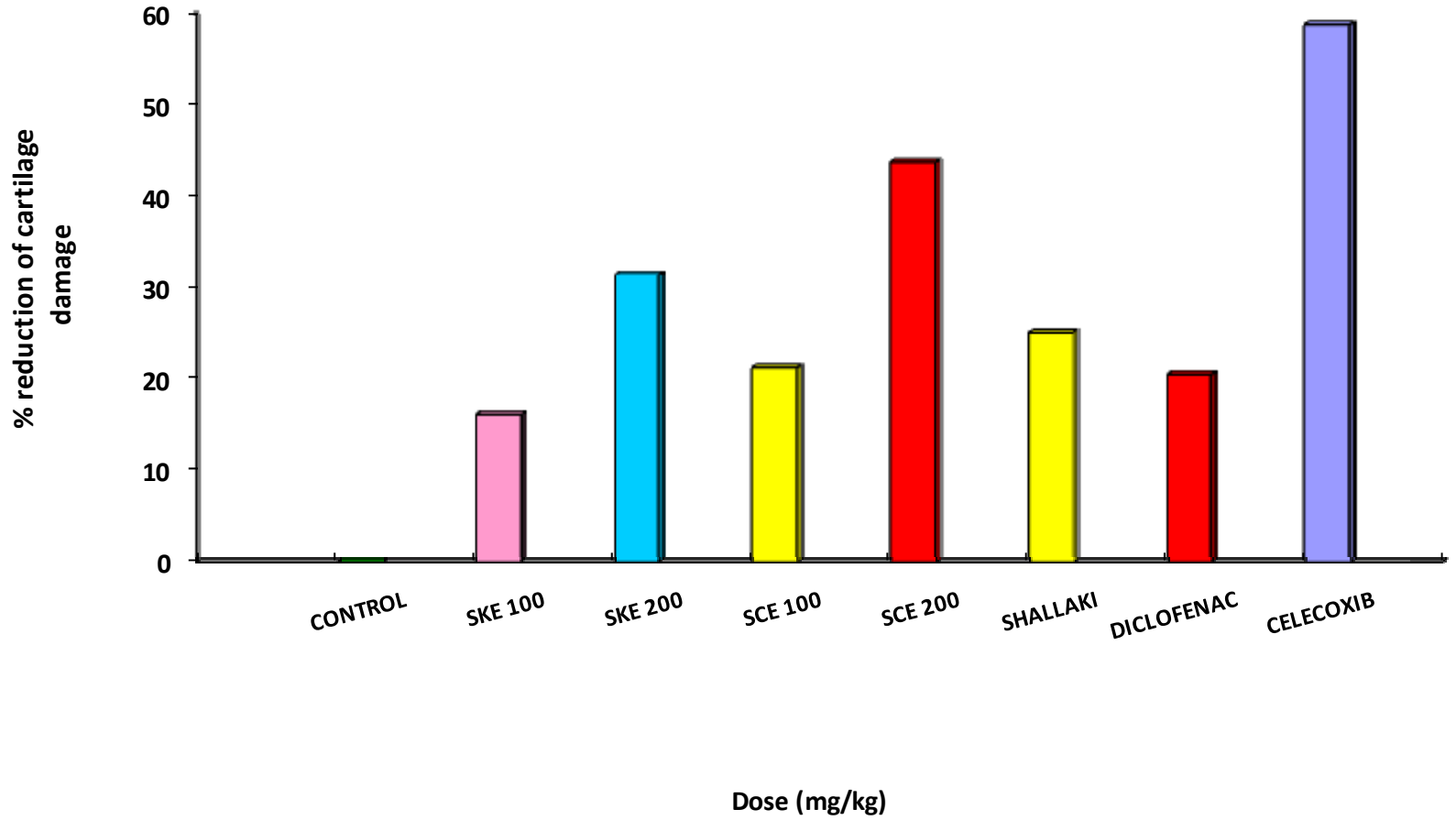
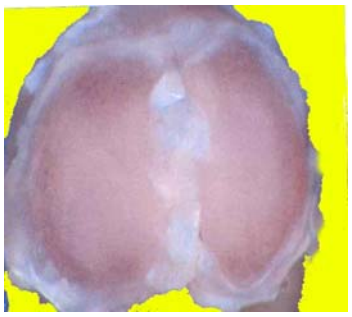
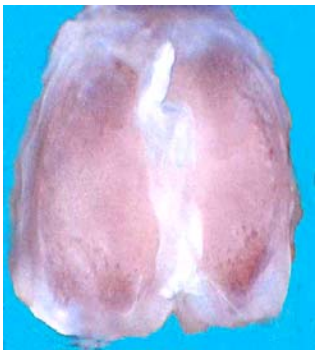


Figure 6. Macroscopic scoring of cartilage lesions

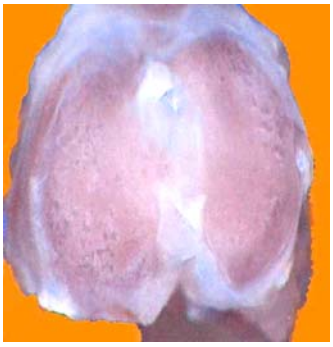
NORMAL



GRADE 1



GRADE 2



GRADE 3

GRADE 4

6.3.3. Safety studies

6.3.3.1 Sub acute toxicity studies

The oral administration of SKE and SCE at 200, 400 and 800 mg/kg body weight/day for 4 weeks to male and female rats did not show any adverse effects. In particular, there was no significant difference between control and treated animals with respect to body weight gain, feed consumption, hematological parameters, biochemical parameters (Table 12-16) and pathological changes.

Table 12. Effect of SKE and SCE on hematological parameters in rats after daily oral administration for 28 days

Treatment	Dose (mg/kg)	RBC (10^6/mm^3)	Hemoglobin (g/dl)	Haematocrit (%)
Control	0	9.55 ± 0.32	16.28 ± 0.33	53.06 ± 1.24
SKE	200	9.12 ± 0.23	15.91 ± 0.25	48.45 ± 1.07
	400	8.80 ± 0.36	15.87 ± 0.38	48.15 ± 0.82
	800	9.01 ± 0.26	15.61 ± 0.36	46.62 ± 1.18
SCE	200	8.41 ± 0.80	15.22 ± 0.32	45.91 ± 1.35
	400	9.51 ± 0.19	15.89 ± 0.29	48.57 ± 1.07
	800	8.32 ± 0.16	15.10 ± 0.35	45.76 ± 1.10

Values are Mean ± SEM (n=10)
Comparisons were made between control and treatment groups using one-way ANOVA followed by Dunnett's test.

Table 13. Effect SKE and SCE on hematological parameters in rats after daily oral administration for 28 days

Treatment	Dose (mg/kg)	Platelets ($10^3/mm^3$)	WBC ($10^3/mm^3$)
Control	0	920.74 ± 44.62	9.46 ± 1.16
SKE	200	936.60 ± 42.8	9.72 ± 0.94
	400	968.18 ± 48.13	9.18 ± 0.76
	800	954.57 ± 40.91	10.15± 0.83
SCE	200	926.11 ± 52.67	9.78 ± 1.30
	400	949.28 ± 47.89	9.84 ± 0.71
	800	941.40 ± 28.95	10.02± 0.63

Values are Mean ± SEM (n=10)
Comparisons were made between control and treatment groups using one-way ANOVA followed by Dunnett's test.

Table 14. Effect of SKE and SCE on certain biochemical parameters in serum of rats after daily oral administration for 28 days

Treatment	Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Proteins (g/dL)
Control	0	57.13 ± 4.96	35.19 ± 1.58	247.38 ± 25.14	6.30 ± 0.85
SKE	200	58.22 ± 3.61	36.37 ± 1.27	257.12 ± 33.30	6.41 ± 0.22
	400	57.80 ± 4.85	35.50 ± 1.62	250.21 ± 27.89	6.38 ± 0.31
	800	60.24 ± 2.66	36.40 ± 1.70	248.25 ± 23.14	6.50 ± 0.05
SCE	200	60.37 ± 3.39	38.26 ± 1.83	245.90 ± 30.14	6.34 ± 0.42
	400	58.65 ± 4.88	39.37± 1.36	260.01 ± 29.52	6.61 ± 0.49
	800	61.98 ± 5.23	38.25 ± 1.28	258.64 ± 26.05	6.59 ± 0.08

Values are Mean ± SEM (n=10)
Comparisons were made between control and treatment groups using One-way ANOVA followed by Dunnett's test.

Table 15. Effect of SKE and SCE on certain biochemical parameters in serum of rats after daily oral administration for 28 days

Treatment	Dose (mg/kg)	Albumin (g/dL)	Total Bilirubin (mg/dL)	Urea (mg/dL)
Control	0	4.53 ± 0.21	0.30 ± 0.03	34.56 ± 2.24
SKE	200	4.59 ± 0.18	0.30 ± 0.02	32.28 ± 1.08
	400	4.64 ± 0.11	0.33 ± 0.02	32.16 ± 1.29
	800	4.73 ± 0.15	0.29 ± 0.16	33.87 ± 2.35
SCE	200	4.68 ± 0.08	0.31 ± 0.03	31.33 ± 2.58
	400	4.82 ± 0.13	0.34 ± 0.04	32.87 ± 1.30
	800	4.55 ± 0.06	0.33 ± 0.02	36.26 ± 1.10

Values are Mean ± SEM (n=10)
Comparisons were made between control and treatment groups using oneway ANOVA followed by Dunnett's test.

Table 16. Effect of SKE and SCE on certain biochemical parameters in serum of rats after daily oral administration for 28 days

Treatment	Dose (mg/kg)	Glucose (mg/dL)	Creatinine (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
Control	0	90.87 ± 3.06	0.50 ± 0.02	49.19 ± 4.16	43.70 ± 3.58
SKE	200	87.34 ± 3.50	0.50 ± 0.03	44.55 ± 3.51	39.85 ± 3.66
	400	85.16 ± 3.96	0.53 ± 0.03	45.10 ± 3.86	40.17 ± 3.32
	800	89.93 ± 4.28	0.48 ± 0.02	48.79 ± 4.63	46.0 ± 2.78
SCE	200	88.15 ± 3.27	0.52 ± 0.02	41.29 ± 4.18	35.75 ± 4.91
	400	90.33 ± 5.01	0.50 ± 0.01	46.02 ± 4.37	48.70 ± 3.65
	800	85.04 ± 4.21	0.52 ± 0.03	42.15 ± 2.85	37.59 ± 3.44

Values are Mean ± SEM (n=10)
Comparisons were made between control and treatment groups using one-way ANOVA followed by Dunnett's test.

DISCUSSION

The air-dried and finely ground leaves of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus were* subjected to cold maceration with 80% ethanol, yielded 11.4% W/W and 10.8% W/W respectively.

Phytochemical analysis in both the plant extracts showed similar phytoconstituents viz. carbohydrates, triterepenoids, phytosterols, flavonoids, and tannins. Several phytoconstituents like flavonoids [24], phytosterols [34], triterpenoids [51] and tannins [41] are known to have anti-inflammatory and anti-arthritic properties.

Earlier [8] it was reported that column chromatographic fractionation of various extracts of *Strobilanthes callosus* and *strobilanthes ixiocephala* contained some known compounds like long chain alkane dotricontane, a triterpene alcohol - lupeol, stigmasterol, and β-D-glucopyranoside. Complex phenyl propanoid glycosides, verbacoside and crassifoiloside have been isolated along with a trisaccharide raffinose. [8] The herbs *S.kunthianus* and *S.cuspidatus* also belongs to family Acanthaceae and a chemotoxonomical relationship has been observed among species of Strobilanthes Further, the nature of phyto constituents present in all the plants were found to be same.

The red blood cell stability test is based on the finding that a number of non-steroidal anti-inflammatory agents inhibit heat – induced lysis of erythrocytes, presumably by stabilizing the membrane of the cell. Agents that can prevent the rupture of the latter, and there by prevent damage to the tissue caused by the release of the hydrolytic enzymes contained within the lysosome may be expected to alleviate some symptoms of inflammation**.** [68] It is well known that lysosomes and their contents (hydrolytic enzymes and / or cationic proteins) play an important role in inflammation and inflammatory disorders. [69, 70, 71]

It has been demonstrated that certain herbal preparations were capable of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-

inflammatory activity. [72, 73] The results showed a strong and dose related stabilization of HRBC by the extracts SKE and SCE. This suggests that membrane stabilization, especially the lysosomes, may constitute the observed anti-inflammatory activity of SKE and SCE in *in vitro*.

Rabbit articular cartilage explants culture was treated with recombinant human interleukin 1α (rhIL-1α) to induce proteoglycan degradation. The amount of glycosaminoglycan released into the medium was measured as an index of proteoglycan degradation. IL-1 is known to be a key cytokine in the pathogenesis of arthritis. IL-1 is known to generate reactive oxygen species and promote PGE_2 production in human chondrocytes. [74] IL-1 mediated induction of COX-2 produces high levels of PGE_2 which mediates cartilage resorption by decreasing proliferation of chondrocytes, enhancing matrix maetalloproteinase activity, and inhibiting aggrecan synthesis in chondrocytes. [75] The results revealed that the extracts SKE and SCE significantly reduced proteoglycan degradation in a dose dependent manner. This result may be attributed that the extracts (SKE and SCE) have multifactorial features such as antioxidant capacity against diverse reactive oxygen species (ROS), down-regulation of PGE_2 generation, inhibition of PLA_2. In a clinical sense, suppression of the upstream stimulation of proinflammatory factors through neutralization of excessive ROS together with inhibition of the downstream degenerative inflammatory consequences by a natural nontoxic agent in combination with current modalities of treatment can be of therapeutic value for effective treatment of both inflammatory and degenerative joint disorders.

In acute toxicity studies, SKE and SCE did not showed any toxic symptoms or caused death of rats even after 14 days at 2000mg/kg of the extracts.

Nociception can be induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called abdominal constriction. The acetic acid – induced abdominal constriction method is very sensitive and able to detect anti-nociceptive effects of compounds and dose levels that may appear inactive in other methods like the tail-flick test. The mouse writhing assay is a useful test

to evaluate mild analgesic NSAIDs. Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, PGs, bradykinin and substance P that stimulate pain nerve endings. [76] Local peritoneal responses are postulated to be partly involved in abdominal constriction response. [77, 78] The method has been associated with increased levels of prostanoids such as PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products. [79, 80] Effects of the extracts SKE and SCE on acetic acid induced abdominal writhing in mice suggested that they might inhibit or modify responses to pain mediated by nociceptors peripherally. The results reveal that one of the mechanisms of action of the extracts may be linked to cycloxygenases and lipoxygenases.

Randall-selitto test for measuring analgesic activity is based on the principle that inflammation increases the sensitivity to pain and this sensitivity is susceptible to modification by analgesics. [81] Inflammation decreases the pain reaction threshold and this low pain reaction threshold is readily elevated by analgesics.[7] Peripherally acting analgesics, such as NSAIDs, increase only the threshold of the inflamed paw where as opiate analgesics also increases the threshold of the intact paw. Brewer's yeast has been used as an inducer for inflammation which increases pain after pressure. [54] The results indicate that the extracts SKE and SCE increased the nociceptive threshold in inflammed paws thereby further strengthening the evidence of peripherally mediated anti-nociceptive activity. This study has shown that the ethanolic extract of SKE and SCE possesses anti-nociceptive activity which may be peripherally mediated. The peripherally mediated action may be linked partly to lipoxygenases and/or cycloxygenases.

Carrageenan induced rat paw edema has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. Carrageenan induced inflammatory process is believed to be biphasic. [82] The initial phase seen at the first hour is attributed to the release of histamine and serotonin. [83] The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. [84] So, the extracts SKE and SCE probably acts by the inhibition of the release of the inflammatory mediators specially prostaglandin, bradykinin and lysozyme. The result obtained from the carrageenan induced paw edema was markedly inhibited by the

oral administration of the extracts SKE and SCE, thus indicating that the extracts can inhibit acute inflammatory process and that the extracts are orally active. This is because carrageenan induced paw edema is an acute model of inflammation [85] and has been reported to be active in detecting orally active anti-inflammatory drugs. [86]

The extracts were further evaluated by cotton pellet induced granuloma formation to understand their potential in the chronic inflammatory phase. Cotton pellet granuloma is the index of proliferative phase of inflammation. Chronic inflammation involves proliferation of macrophages and neutrophils which are modulators of granuloma formation. Therefore, decrease in granuloma weight indicates the suppression of proliferative phase. [87] The results of the present study indicate the extracts SKE and SCE effectively suppressed granuloma formation induced by cotton pellets. These results suggest that the extracts SKE and SCE inhibited the acute and chronic phase of inflammation in a dose dependent manner.

The injection of iodoacetate into the knees of rats provides a model where lesions resembling some aspects of human osteoarthritis can be quickly produced and has been suggested as a model for the study of chondroprotective drugs. [88,89] The injection of iodoacetate induces the loss of cartilage proteoglycan. Proteoglycan loss was followed by a severe thinning of the cartilage and the development of lesions in the region of the subchondral bone and calcified cartilage consisting of fibrous tissue, infiltrating mononuclear cells and blood vessels. The extracts SKE and SCE significantly reduced the cartilage damage in a dose dependent manner. Both the extracts SKE (200 mg/kg) and SCE (200 mg/kg) showed better response than the herbal positive control. The inhibition of joint damage in this model by the extracts SKE and SCE supports the further evaluation of the therapeutic potential in human osteoarthritis.

Stigmasterol is found to be anti-inflammatory and anti-osteoarthritis by inhibiting the IL-1β, MMP-3 mRNA, and PGE_2 in human and mouse.[90] The ethanolic extracts of *S.kunthianus* and *S.cuspidatus* showed anti-inflammatory and anti-osteoarthritis effect which may be due to the presence of phytosetrols.

The extracts (SKE and SCE) have analgesic, anti-inflammatory and anti-osteoarthritis potential may be due to the presence of multiple phytoconstituents such as flavonoids, phytosterols, triterpenoids and tannins.

The short-term toxicity of SKE and SCE were investigated in rats. At all the doses tested (200 mg/kg, 400 mg/kg, and 800 mg/kg) by the oral administration of both SKE and SCE did not show any adverse effects till 28 days of study. In particular, there was no significant difference between solvent control and the extracts treated animals with respect to body weight gain, feed consumption, hematological parameters, biochemical parameters and pathological changes. This suggests that both the ethanolic leaf extracts of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* under the present experimental conditions, at the dose levels tested were found to be safe even after repeated administration in long-term use.

7. SUMMARY AND CONCLUSION

There are several herbs used in the indigenous system of medicine for the treatment of inflammation and joint disorders. As per the literature survey information, the plant *Strobilanthes callosus* (Family-Acanthaceae) a traditional herb reported for anti-inflammatory and anti-arthritic activity. Due to a strong chemotaxonomical relationship among species of Genus Strobiolanthes, the two species i.e., *Strobilanthes kunthianus* (Family -Acanthaceae) and *Strobilanthes cuspidatus* (Family -Acanthaceae) were selected for the present study. The leaves of *S. kunthianus* and *S. cuspidatus* were collected from Kalliti, Nilgiri hills.

The effects of ethanolic extracts of *S. kunthianus* (100 mg/kg and 200 mg/kg) and *S. cuspidatus* (100 mg/kg and 200 mg/kg) for their analgesic, anti-inflammatory, and anti-osteoarthritic activity were studied in different animal models using Wistar albino rats and Swiss albino mice *in vivo* and *in vitro* assays such as human RBC membrane stabilization and rabbit cartilage explants culture were also performed.

The extracts SKE and SCE produced various changes in abdominal constriction (writhes), tolerance to pain, paw volume, granuloma formation and cartilage lesions when tested *in vivo* and also produced changes in lysis of human RBC and glycosaminoglycan release when tested *in vitro.*

The extracts SKE and SCE were also studied for their safety in acute and sub acute toxicity models using Wistar rats, both the extracts did not produced any adverse effects or death in acute toxicity study and also there were no hematological changes, biochemical changes, pathological changes and death in sub acute toxicity study. This indicates that the extracts SKE and SCE were safe to use.

The results obtained in the present investigation are consistent with the ethno medical claim made for the use of genus Strobilanthes in inflammation and joint disorders. Interestingly, shallaki showed lesser analgesic, anti-inflammatory and anti-

osteoarthritis activity when compared to SKE and SCE. The potential therapeutic activity of the extracts *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* may be attributed to the presence of multiple phytoconstituents such as flavonoids, phytosterols, triterpenoids and tannins.

Although more wide-cut understanding of the mechanism by which SKE and SCE mediates their beneficial effects needs further investigation on the action of prostaglandins, cycloxygenases, lipoxygenases, matrix-metalloproteases and reactive oxygen species, other animal models (Collagenase induced osteoarthritis and incapacitence test) and to isolate the phytoconstituents from the ethanolic extracts which are responsible for the therapeutic activity. Studies with varying dose levels and isolated phytoconstituents can also be performed. Work in this direction is progress in the institution.

8. BIBLIOGRAPHY

1. Hannu Vuori. The World Health Organization and traditional medicine. Community Med. 1982; 4 : 129-37.

2. World Health Organization. Global burden of disease [Document on internet].Data sources, methods and results; [Cited on 2009 June 10]. Available from: www.who.int/healthinfo/paper54.pdf.

3. Wikipedia. Arthritis [Document on internet]. Definition, causes and types; [Cited on 2009 June 15]. Available from: http://en.wikipedia.org/wiki/Arthritis.

4. Arthritis treatment [Document on internet]. Goals of treatment; [Cited on 2009 June 24]. Available from: http://www.arthritis-treatment-and-relief.com/arthritis-treatment.html.

5. Emedicinehealth. Arthritis [Document on internet]. Medications, surgery and other therapy; [Cited on 2009 July 1]. Available from: http://www.emedicinehealth.com/arthritis/article_em.htm.

6. Arthritis research campaign [Document on internet]. Complementary and alternative medicine; [Cited on 2009 July 15]. Available from: http://www.arc.org.uk/arthinfo/patpubs/6007/6007.asp.

7. Gerhard Vogel H. Drug discovery and evaluation, pharmacological assays. 2nd ed. New York: Springer-Verlag Berlin Heidelberg; 2002. p. 669- 787.

8. Ramesh A, Vinod R. Comparative phytochemical studies of *Strobilanthus callosus* and *Strobilanthus ixiocephala* roots. Indian Drugs. 2001 ; 38 (12): 646-8.

9.Yu-Ling HO, Kuo-ching Kao, Huei-Yann Tsai, Fu-Yu Chueh, Yuan-Shiun chang.

Am J Chin Med. 2003 ; 31 (1): 61-9.

10. Indian Medicinal Plants, A compendium of 500 species. Madras: Orient- Longmann Ltd; 1997. p. 366-7 (vol. 4).

11. Agarwal RB, Rangari VD. Phytochemical investigation and evaluation of anti-inflammatory and anti-arthritic activities of essential oil of *Strobilanthus ixiocephala* Benth. Indian J Exp Biol. 2003 ; 41 (8) : 890-4.

12. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus*. Fitoterapia. 2007 ; 78 (1) : 25-8.

13. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res. 2003 ; 17 (4) : 299- 305.

14. Altman RD, Marcussen KC. Evaluation of efficacy and safety of a standardized and highly concentrated extract of 2 ginger species, *Zingiber officinale* and *Alpinia galanga*, in patients with osteoarthritis of the knee. Arthritis Rheum. 2001 ; 44 (11) : 2531-8.

15. Balian S, Ahmed S, Zafar R (2006). Anti-inflammatory activity of leaf and leaf callus of *Silybum marianum* in albino rats. Indian J Pharmacol. 38 (3) : 213-4.

16. Cheeke PR, Piacente S, Oleszek W. Anti-inflammatory and anti-arthritic effects of *Yucca schidigera*: A review. J Inflamm. 2006 ; 3 : 6.

17. Choi JH, Kim DY, Yoon JH, Yoon HY, Yi JB. Effects of SKI-306X, a new herbal agent, on proteoglycan degradation in cartilage explants culture and Collagenase-induced rabbit osteoarthritis model. Osteoarthritis Cartilage. 2002 ; 10 : 471-8.

18. Coelho MGP, Reis PA, Gava VB, Marques PR, Gayer CR, Laranja GAT. Anti-arthritic effect and sub acute toxicological evaluation of *Baccharis genistelloides* aqueous extract. Toxicol Lett. 2004 ; 154 (1-2) : 69-80.

19. Didem DO, Ali H, Esra K, Erdem Y. *In vivo* anti-inflammatory and anti-nociceptive activity of the crude extract and fractions from *Rosa canina L.* fruits. J Ethnopharmacol. 2007 ; 112 (2) : 394-400.

20. Dilip KR, Mani Senthil Kumar KT, Sanmoy K, Siddhartha P, Samir KS, Dipan A. Pharmacological studies on Indian black tea (leaf variety) in acute and chronic inflammatory conditions. Phytother Res. 2008 ; 22 (6) : 814–9.

21. Dimo T, Agathe LF, Nguelefack TB, Asongalem EA, Kamtchiouing P. Anti-inflammatory activity of leaf extracts of *Kalanchoe crenata*. Indian J Pharmacol. 2006 ; 38 (2) : 115-9.

22. Dinesh K, Gautham S, Kwang SA, Manok KP, Ajaikumar B, Kunnumakkara. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol. 2007 ; 7(3) : 344-51.

23. Fernandez P, Guillen MI, Gomar F, Aller E, Molina P, Alcaraz MJ. A novel cyclo-oxygenase-2 inhibitor modulates catabolic and anti-inflammatory mediators in osteoarthritis. Biochem Pharmacol. 2004 ; 68 : 417-21.

24. Ficarra R, Ficarra P, Tommasini S, Calabro ML, Ragusa S, Barbera R. Leaf extracts of some cordia species: Analgesic and anti-inflammatory activities as well as their chromatographic analysis. Farmaco. 1995 ; 50 (4) : 245-56.

25. Gokhale AB, Damre AS, Kulkarni KR, Saral MN. Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa, A. speciosa and A. aspera*. Phytomedicine. 2002 ; 9 (5) : 433-7.

26. Herman JH, Appel AM, Hess EV. Modulation of cartilage destruction by selective non-steroidal anti-inflammatory drugs. Arthritis Rheum. 1987 ; 30 (3) : 257-65.

27. John AOO. Analgesic, anti-inflammatory and hypoglycemic effects of ethanol extract of *Zingiber officinale* (roscoe) rhizomes (zingiberaceae) in mice and rats. Phytother Res. 2006 ; 20 (9) : 764-72.

28. John AOO. Analgesic and anti-convulsant properties of *Tetrapleura tetraptera* (Fabaceae) fruit aqueous extract in mice. Phytother Res. 2005 ; 19 (12) : 1023-9.

29. Kim JH, Rhee HI, Jung IH, Ryn K, Jung K, Han CK, et al. SKI306X, an oriental herbal mixture, suppresses gastric leukotriene B4 synthesis without causing mucosal injury and the diclofenac –induced gastric lesions. Life Sci. 2005 ; 77 : 1181-93.

30. Kim SJ, Kim MS. Inhibitory effects of *Cimicifugae rhizoma* extracts on histamine, bradykinin and COX-2 mediated inflammatory actions. Phytother Res. 2000 ; 14 : 596-600.

31. Kimmatkar N, Thawani V, Hingorani L, Khiyani R. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee-a randomized double blind placebo controlled trial. Phytomedicine. 2003 ; 10 : 3-7.

32. Kulkarni RR, Virkar AD, Mello PD. Anti-oxidant and anti-inflammatory activity of *Vitex negundo*. Indian J Pharm Sci. 2008 ; 70 (6) : 838-40.

33. Kulkarni SK, Jain NK. Coxibs: The new super aspirins or unsafe pain killers. Indian J Pharmacol. 2005 ; 37 (2) : 86-9.

34. Mary Latha R, Geetha T, Varalakshmi P. Effect of *Vernonia cinerea* Less flower extract in adjuvant-induced arthritis. Gen Pharmacol. 1998 ; 31 (4) : 601-6.

35. Mathew A, Taranalli AD, Torgal SS. Evaluation of anti-inflammatory and wound healing activity of *Gentiana lutea* rhizome extracts in animals. Pharm Biol. 2004 ; 42 (1) : 8-12.

36. Naresh Singh, Sarvesh Kumar, Prabhjot Singh, Hanumantharao GR, Ashok KP, Virinder SP. *Piper longum* Linn. Extract inhibits TNF-α-induced expression of cell adhesion molecules by inhibiting NF-kB activation and microsomal lipid peroxidation. Phytomedicine. 2008 ; 15 (4) : 284-91.

37. Ojewole JAO. Evaluation of the analgesic, anti-inflammatory and anti-diabetic properties of *Sclerocarya birrea* stem-bark aqueous extract in mice and rats. Phytother Res. 2004 ; 18 : 601-8.

38. Osadebe PO, Okoye FBC. Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. J Ethnopharmacol. 2003 ; 89 (1) : 19-24.

39. Perez RM, Perez S, Zavala MA, Salazar M. Anti-inflammatory activity of the bark of *Hippocratea excels*. J Ethnopharmacol. 1995 ; 47 : 85-90.

40. Perumal Samy R, Ponnampalam G, Peter H, Maung MT, Savarimuthu I. Effect of aqueous extract of *Tragia involucrata* Linn. On acute and subacute inflammation. Phytother Res. 2006 ; 20 (4) : 310-2.

41. Puntero BF, Peinado II, Fresno AMVD. Anti-inflammatory and anti-ulcer activity of *Teucrium buxifolium.* J Ethnopharmacol. 1997 ; 55 (2) : 93-8.

42. Raju I, Moni M, Subramanian V. Anti-inflammatory and free radical scavenging activity of *Ricinus communis* root extract. J Ethnopharmacol. 2006 ; 103 (3) : 478-80.

43. Rasool M, Sabina EP. Anti-inflammatory effect of the Indian Ayurvedic herbal formulation Triphala on adjuvant-induced arthritis in mice. *Phytother Res*. 2007 ; 21 (9) : 889-94.

44. Rasool M, Varalakshmi P. Protective effect of *Withania somnifera* root powder in relation to lipid peroxidation, anti-oxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats. Fundam Clin Pharmacol. 2007 ; 21 (2) : 157-64.

45. Sabino KCC, Castro FA, Oliveira JCR, Dalmau SRA, Coelho MGP. Sucessful treatment of collagen-induced arthritis in mice with a hydro alcohol extract of seeds of *Pterodon pubescens*. Phytother Res. 1999 ; 13 (7) : 613-5.

46. Schmid B, Ludtke R, Selbmann HK, Kotter I, Tschirdewahn B, Schaffner W. Efficacy and tolerability of a standardized willow bark extract in patients with osteoarthritis: randomized placebo-controlled, double blind clinical trial. Phytother Res. 2001 ; 15 (4) : 344-50.

47. Shahavi VM, Desai SK. Anti-inflammatory activity of *Butea monosperma* flowers. Fitoterapia. 2008 ; 79 (2) : 82-5.

48. Sharma ML, Kaul A, Khajuria A, Singh S, Singh GB. Immunomodulatory activity of boswellic acids (Pentacyclic triterpene acids) from *Boswellia serrata*. Phytother Res. 1996 ; 10 (2) : 107-12.

49. Singh S, Khajuria A, Taneja SC, Johri RK, Singh J, Qazi GN. Boswellic acids : A leukotriene inhibitor also effective through topical application in inflammatory disorders. Phytomedicine. 2008 ; 15 (6) : 400-7.

50. Singh S, Khajuria A, Taneja SC, Khajuria RK, Singh J, Johri RK. The gastric ulcer protective effect of boswellic acids, a leukotriene inhibitor from *Boswellia serrata*, in rats. Phytomedicine. 2008 ; 15 (6) : 408-15.

51. Singh B, Sahu PM, Sharma MK. Anti-inflammatory and anti-microbial activities of triterpenoids from *Strobilanthes callosus* nees. Phytomedicine. 2002 ; 9 (4) : 355-9.

52. Venil NS, Rucha C, Asavari KJ, Sanjay B, Bhushan P, Arvind C. The relationship between chondroprotective and anti-inflammatory effects of *Withania somnifera* root and glucosamine sulphate on human osteoarthritic cartilage *in vitro.* Phytother Res. 2008 ; 22 (10) :1342-8.

53. Vignon E, Mathieu P, Louisot P, Richard M. *In vitro* effect of non-steroidal anti-inflammatory drugs on proteoglycanase and collagenase activity in human osteoarthritic cartilage. Arthritis Rheum. 1993 ; 34 (10) : 1332-5.

54. Vongtau HO, Abbah J, Mosugu O, Chindo BA, Ngazal IE, Salawu AO, et al. Anti-nociceptive profile of the methanolic extract of *Neorautanenia mitis* root in rats and mice. J Ethnopharmacol. 2004 ; 92 : 317-24.

55. Wittenberg RH, Willburger RE, Kleemeyer KS, Peskar BA. *In vitro* release of prostaglandins and leukotrienes from synovial tissue, cartilage and bone in degenerative joint diseases. Arthritis Rheum. 1993 ; 36 (10) : 1444-50.

56. Venu P. Strobilanthes Blume (Acanthaceae) in peninsular India. 1st Ed. Kolkata: Botancial Survey of India; 2006. p. 89-93, 129-32.

57. Mukherjee PK. Quality control of herbal drugs, an approach to evaluation of Botanicals. 1st Ed. New Delhi: Business horizons; 2002. p. 379-401.

58. Raaman N. Phytochemical techniques. 1st ed. New Delhi: New India publishing agency; 2006. p. 19-24.

59. Divakar MC, Jayaprakasam R. Anti-inflammatory and free radical scavenging activities of sea cucumber and cuttle fish glandular extracts. Indian Drugs. 2005 ; 43 (6) : 471-5.

60. Cheol Shin H, Hwang HJ, Kang KJ, Lee BH. An antioxidative and anti-inflammatory agent for potential treatment of osteoarthritis from *Ecklonia cava*. Arch Pharm Res. 2006 ; 29 (2) : 165-71.

61. OECD Guidelines for the testing of chemicals, No.425. Acute oral toxicity-modified up and down procedure. Paris, France : 2001.

62. Jha PK, Mazumdar B, Bhatt JD. Analgesic activity of venlafaxine and its interactions with tramadol, celecoxib and amlodipine in mice. Indian J Pharmacol. 2006 ; 38 (3) : 181-4.

63. Archana P, Tandan SK, Chandra S, Lal S. Anti-pyretic and analgesic activities of *Caesalpinia bonducella* seed kernel extract. Phytother Res. 2005 ; 19 : 376-81.

64. Nirmal SA, Jawale PP, Atpade SS, Pal SC, Chavan MJ. Anti-inflammatory and analgesic activity of *Achras sapota* bark. Indian J Nat Prod. 2005 ; 21(3):19-22.

65. Victor BO, Yusuf YO, Elizabeth AB, Ayodele OS. Analgesic and anti-inflammatory properties of *Nelsonia canescens* leaf extract. J Ethnopharmacol. 2005 ; 99 : 153-6.

66. Janusz MJ, Hookfin EB, Heitmeyer SA, Woessner JF, Freemont AJ, Hoyland JA, et al. Moderation of iodoacetate – induced experimental osteoarthritis in rats by matrix metalloproteinase inhibitors. Osteoarthritis Cartilage. 2001 ; 9 : 751-60.

67. Toledo MI, Siqueira JM, Araujo LCL, Oga S. Acute and sub acute toxicity of *Cochlospermum regium* piiger. Phytother Res. 2000 ; 14 : 359-61.

68. Hess SM, Milonig RC. Assay for anti-inflammatory drugs. In: Lepow, IH, Ward PA (eds.), Inflammation, mechanism and control. New York: Academic press; 1972. p.1-12.

69. Dingle TJ, Lucy JA, Fell B. Studies on the mode of action of Vitamin A III. The release of bound protease by the action of Vitamin A. Biochem J. 1961; 79 : 505-13.

70. Shen TY, In: Rabinowitz, JL, Myerson RM, (Eds). Topics in medicinal chemistry. New York; Interscience Publishers; 1967. p.29.

71. Weissmann G, Spilberg I, Krakauer K. Arthritis induced in rabbits by lysates of granulocyte lysosomes. Arthritis Rheum. 1969 ; 12 : 103-9.

72. Sadique J, Al-Rqobah WA, Bughaith MF, El-Gindy AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia. 1989 ; 60 : 525-32.

73. Oyedapo OO, Famurewa AJ. Antiprotease and membrane stabilizing activities of extracts of *Fagara zanthoxyloides*, *Olax subscorpioides* and *Tetrapleura tetraptera.* Int J Pharmacogn. 1995 ; 33 : 65-9.

74. Mathy Hartert M, Derby Dupont GP, Reginster JYL, Ayache N, Pujol JP, Henrotin YE. Regulation by reactive oxygen species of interleukin-1, nitric oxide and prostaglandin E2 production by human chondrocytes. Osteoarthritis Cartilage. 2002 ; 10 : 547-55.

75. Taskiran D, Racic SM, Georgescu H, Evans C. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun. 2000 ; 200 : 142-8.

76. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol.1968 ; 32 : 295-310.

77. Bentley GA, Newton SH, Starr J. Studies on the anti-nociceptive action of agonist drugs and the interaction with opoid mechanisms. Br J Pharmacol. 1983 ; 79 : 125-34.

78. Derardt R, Jougney S, Delevalacee C, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol. 1980 ; 51 : 17-24.

79. Levini JD, Lau W, Kwait G, Goetzl EJ. Leukotriene B4 produces hyperalgesia that is dependent on the polymorphonuclear leucocytes. Science. 1984 ; 225 : 743-5.

80. Dhara AK, Suba V, Sen T, Pal S, Nag Chaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of *Tragia involucrate*. J Ethnopharmacol. 2000 ; 72 : 265-8.

81. Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn. 1957 ; 111 : 409-19.

82. Venegar R, Scheiber W, Hugo R. Biphasic development of carrageenan edema in rats. J Pharmacol Exp Ther. 1969 ; 166 : 96-103.

83. Crunkhon P, Meacock SER. Medicators of inflammation induced in the rat paw by carrageenan. Br J Pharmacol. 1971 ; 42 : 392-402.

84. Katzung BG. Basic and Clinical Pharmacology. 7th ed Stanford : Connecticut ; 1998. p. 578-9.

85. Dirosa M. Biological properties of carrageenan. J Pharm Pharmacol. 1972 ; 24 : 89-102.

86. Dirosa M, Giroud JP, Willoughby DA. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol. 1971 ; 104 : 15-29.

87. Kavimani S, Vetrichelvan T, Ilango R, Jaykar B. Anti-inflammatory activity of the volatile oil of *Toddalia asiatica*. Indian J Pharm Sci. 1996 ; 58 (2) : 67-70.

88. Kalbhen DA, Buchmann U. Die Tierexperimentelle gonarthrose der ratte und ihre therapie mit glykosamino glykanpolysulfat (GAGPS). Z Rheumatol. 1985 ; 44 : 100-7.

89. Guingamp C, Gegout Pottie P, Philippe L, Terlain B, Netter P, Gillet P. Mono-iodoacetate – induced experimental osteoarthritis. A dose-response study of loss of mobility, morphology and biochemistry. Arthritis Rheum. 1997 ; 40 : 1670-9.

90. Gabayy O, Sanchezyz C, Salvaty C, Chevyx F, Bretonx M, Nourissatyk, et al. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. Osteoarthritis Cartilage. 2010 ; 18 : 106-16.

Lawley Road P.O., Coimbatore - 641 003

No. BSI/SC/5/23/06-07/Tech. 1566 21.12.2006

To

Mr. Desu Brahma Srinivasarao
Ph.D. Scholar
J.S.S. College of Pharmacy
Rocklands, Post Box 20,
Ootacamund- 643 001.

Sir,

The plant specimen brought for identification has been confirmed as *Phlebophyllum kunthianum* Nees (= *Strobilanthes kunthianus* (Nees) T. And. ex Benth.) - Acanthaceae.

Yours sincerely,

21/12/06

(G.V.S. Murthy)
Joint Director

Joint Director,
Botanical Survey of India
Southern Circle, Coimbatore-3

No. BSI/SC/5/23/06-07/Tech. 1567 21.12.2006

To

Mr. Desu Brahma Srinivasarao
Ph.D. Scholar
J.S.S. College of Pharmacy
Rocklands, Post Box 20,
Ootacamund- 643 001.

Sir,

The plant specimen brought for identification has been confirmed as *Phlebophyllum versicolor* (Wight) Bremek. (= *Strobilanthes cuspidatus* (Benth.) T. And.) - Acanthaceae.

Yours sincerely,

21/12/06

(G.V.S. Murthy)
Joint Director

Joint Director
Botanical Survey of India
Southern Circle, COIMBATORE