ANTI-INFLAMMATORY AND ANALGESIC ACTIONS OF RED AND WHITE LOTUS SEEDS (*Nelumbo nucifera*) IN ALBINO RATS

VIKRAMA CHAKRAVARTHI. P.

Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2006

Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680 651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled "**ANTI-INFLAMMATORY AND ANALGESIC ACTIONS OF RED AND WHITE LOTUS SEEDS *(Nelumbo nucifera)* IN ALBINO RATS**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy

Vikrama Chakravarthi.P

CERTIFICATE

Certified that this thesis, entitled "**ANTI-INFLAMMATORY AND ANALGESIC ACTIONS OF RED AND WHITE LOTUS SEEDS *(Nelumbo nucifera)* IN ALBINO RATS**" is a record of research work done independently by **Dr.Vikrama Chakravarthi.P.,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Dr. N.Gopakumar,
(Chairman, Advisory Committee)
Professor and Head,
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences,
Pookot.

Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr.Vikrama Chakravarthi.P.,** a candidate for the degree of **Master of Veterinary Science in Pharmacology and Toxicology,** agree that this thesis entitled **"ANTI-INFLAMMATORY AND ANALGESIC ACTIONS OF RED AND WHITE LOTUS SEEDS *(Nelumbo nucifera)* IN ALBINO RATS"** may be submitted by **Dr.Vikrama Chakravarthi.P.,** in partial fulfilment of the requirement for the degree.

Dr.N.Gopakumar,
(Chairman, Advisory Committee)
Professor and Head,
Department of Pharmacology and Toxicology,
College of Veterinary and Animal sciences,
Pookot.

Dr.A.D.Joy,
Associate Professor,
Department of Pharmacology &Toxicology,
College of Veterinary and Animal Sciences
Mannuthy.
(Member)

Dr.P.T.Philomina,
Professor and Head,
Department of Physiology,
College of veterinary and
Animal Sciences, Pookot.
(Member)

Dr.K.T.Prasanna Kumari,
Associate Professor,
Department of Plant Breeding and Genetics,
College of Horticulture,
Vellanikkara.
(Member)

External Examiner 04/1/2026

ACKNOWLEDGEMENTS

I may be given credit for having blazed the trail but when I look at the subsequent developments I feel the credit is due to others rather than to myself.

- Alexander Graham Bell

On this day I met the finale of my endeavor I express my deep hearted gratitude and indebtness to my esteemed advisor ***Dr.N.Gopakumar*** *, Professor and Head, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookot, for his support, advice and guidance rendered throughout the course of my post graduate study.*

The search of suitable word to express my thanks is still not over as it is beyond the power of my expression to ***Dr. A.M. Chandrasekharan Nair****, Head and Associate Professor, Department of Pharmacology and Toxicology for making me rethink many of the issues that I thought I had answered and thus enabling me to arrive at a better understanding of them. His critics helped me recognize when I was skirting the hard questions. I am particularly grateful for his emphasis on simplicity and elegance in life.*

It was indeed a fortunate to have ***Dr. A.D. Joy,*** *Associate Professor, Department of Pharmacology and Toxicology as a member of the advisory committee. His valuable guidance, creative suggestions, constructive criticism and constant encouragement during the course are not only praiseworthy but also unforgettable.*

I am very grateful to ***Dr.P.T.Philomina,*** *Professor and Head, Department of Physiology, the other member of advisory committee for pushing me just bit further when I needed it. She has given me a total freedom to carry over my work in the Department of Physiology. I acknowledge her as a person who taught me to think rationally and logically.*

I would like to thank ***Dr.K.T.Prasanna Kumari****, Associate Professor, Department of Plant Breeding and Genetics, a member of advisory committee,*

for the valuable offer to carryingout the project work given by Kerala State Council for Science and Technology and Environment.

My great indebtedness and gratitude are due to ***Dr. K. Venugopalan, Dr. C.M. Aravindakshan, Shri. V.R. Raghunandanan,*** *and* ***Dr. P.T.A. Usha*** *for their pleasant co-operation, warm friendship and affectionate encouragement during the course of study.*

I thank ***Dr.Krishnan Nair,*** *Associate Professor and Head, Department of Microbiology, and* ***Dr.k..karthikyani,*** *Assistant professor, Department of Physiology, for the help of providing the facilities to carryout the analysis.*

The help rendered by ***Smt. Sujatha,*** *Assistant Professor and Head, Department of Statistics in the statistical interpretation of the results is gratefully acknowledged.*

I like to thank ***Shri. P.R. Chandrasekhar,*** *Instrumentation Engineer and Head, Central Instrumentation Lab for his expert technical assistance and rendering of facilities during the course of my study.*

I take great pleasure in thanking ***Dr. E. Nanu,*** *Dean i/c, Faculty of Veterinary and Animal science for providing me the facilities for my research.*

I gratefully acknowledge the advice, support and help rendered by ***Dr. Jeyamurugan*** *in promptly assisting me during my research work.*

The invaluable help rendered by my beloved seniors ***Drs.Jerald, Senthil, Anu ,Sangeetha*** *and* ***Mr.Jogesh*** *are duly acknowledged. My days in COVAS were made much more enjoyable by the companionship of my colleagues,* ***Drs.Deepa, Bibu and Saritha.*** *I feel lucky to share my postgraduate studies with them.*

My friends have served as a support network in many ways over these years and this journey was no different. I wish to thank my friends ***Drs.Lu, Shanmugam, Lonkar, Jega, Prejit, Rishi, Babu, Ariprasath, Sivanasen, Balaji, Muthu, Mohan, Tamil, Sudha, Eswar, Vijay, Majo, Vidya, Reji, Geetha, Ranjith, Raja gopal, Acty, Niteen and Dinkar.*** *A wholehearted bouquet of thanks to all of them.*

I was particularly fortunate to have had respectful seniors ***Drs.Sasi (mams), Kowsig, Senthil (kondi), Sekar, Arun, Raja, Murugan and Rajaganapathy,*** *in the PG hostel, with whom I have shared many hours of interesting discussions. They helped my stay in hostel as an enjoyable and a memorable one.*

I sincerely thank to ***Kerala State Council for Science, Technology and Environment*** *for providing financial assistance to the project entitled* ***"Comparative evaluation of red and white flowered ecotypes of sacred lotus (Nelumbo nucifera Gaertn)".***

No words of thanks could be enough to those ***mute creatures,*** *which have laid down their invaluable life for my work. I am deeply indepted from bottom of my heart to those creatures.*

A very big thanks goes of course to my ***loving parents*** *for instilling the love of learning in me and to* ***my sister*** *for accompanying me through the ups and downs of my life. I would not be where I am if not for their steadfast encouragement. If life is a journey, they helped me not only in charting a great course but also in providing me with the skills I need to conquer the road ahead.*

Without the help of ***God*** *nothing would have been possible. I bow before the* ***almighty,*** *for the interminable blessings that have helped me in every stage of my life.*

Vikrama Chakravarthi.P

CONTENTS

LIST OF TABLES

LIST OF FIGURES.

Introduction

1. INTRODUCTION

Inflammation is a biological response to a series of chemical reactions whose major functions are protection from infection and resolution of tissue damage caused by injury. During these reactions, there will be increased capillary permeability along with the migrating leukocytes into the injured area and removal of toxic materials as well as cellular debris. As a result of cell injury, an intricate system is activated causing the release of numerous inflammatory mediators such as histamine, serotonin, bradykinin, hageman factor, lysosomal enzymes, prostaglandins and leukotrienes. These mediators initiate a three phase process consisting of vasodilatation, increased vascular permeability and leucocytic exudation all of which occur simultaneously in a multiple interaction process resulting in characteristic clinical signs of heat, redness, swelling, pain, and diminished functions.

On the whole, inflammation is a beneficial process, however, it has been realized that inflammation like other vital processes, may at times wander away from its beneficial path and become considerably more harmful to the organism than the noxious stimulus that initiated the reaction. Diseases, which are thought to be immunological origin such as rheumatoid arthritis, glomerulonephritis are associated with inflammatory reactions, which provide no obvious benefit but rather inflict harm upon the host.

In the process of inflammation, lysosomes present in the cells are damaged resulting in the release of hydrolytic enzymes, which bring about extensive damage to the surrounding tissues (Higgs *et al* ., 1975). Therefore it becomes important to find ways for arresting such harmful inflammatory processes.

A variety of drugs have been used to minimize the discomfort arising due to the inflammatory process. In the past, chiefly the natural and synthetic steroids were employed in the anti-inflammatory therapy. Because of the occurrence of undesirable side effects with steroids, adrenal insuffiency, fluid and electrolyte abnormalities, attention was directed towards the currently available non-steroidal anti-inflammatory drugs (NSAID) agents. The anti-inflammatory action of NSAID rest in their ability to inhibit the activity of cyclooxygenase (Vane, 1971). Most of the currently available drugs inhibit both cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX –2) activities and thereby the production of prostaglandins and thromboxane .The inhibition of COX-2 is thought to mediate atleast in part through antipyretic, analgesic and anti-inflammatory action of NSAID. The simultaneous inhibition of COX-1 results in unwanted side effects would lead to gastric ulcers, while COX-2 selective inhibition should specifically alleviate pain and inflammation. The COX-2 selective drugs do lower gastro toxicity than non-selective NSAID but the findings of elevated incidence of myocardial infarction raises the question whether all of the side effects that may be associated with these drugs are known.

Another avenue for the use of NSAID is the alleviation of pain. Pain is a subjective affair though it may be accompanied by measurable physiological responses such as reflex withdrawal movements, changes in vasomotor tone, blood pressure, heart rate, breathing and sweating. Pain can be elicited by noxious stimulus in normal persons and it is also the outstanding symptom of many diseases.

Pain can be considered in two categories like nociceptive and neuropathic pain. Nociceptive pain is defined as the pain in response to an obvious stimulus, while neuropathic is defined as pain in the absence of such stimulus. Neuropathic pain results from current or past damage to the peripheral or central nervous system, and is due to aberrant processing of information in the peripheral or central nervous system. Neuropathic pain can be described in terms of characteristics

of the pain, the site of injury, or the presumed site of aberrant neural activity that is producing the pain. Peripheral neuropathic pain is caused by injury to a peripheral nerve. After tissue damage the healing process accompanied by a period of hyperalgesia (increased sensivity to pain) and allodynia (nociceptive reaction to normally non noxious stimulus). This hyperalgesia is due to the proliferation of regenerating nerve fibres.

The pain receptors get sensitized because of the presence of autacoids. These receptors are more prone to mechanical and other stimulus. The NSAIDs arrest the sensitization of nociceptors to such stimulation by preventing the production of arachidonic acid cascade products and useful for treating the conditions associated with mild to moderate pain. Their effects appear to be mediated through central and peripheral mechanisms.

Diclofenac has been found to be effective both as an anti-inflammatory and analgesic agent but the number of drawbacks of long term use of NSAIDs such as gastrointestinal tract ulceration, disturbances in platelet function, changes in renal function etc. has led to the search for better alternative especially herbal drugs.

The herbal medicine consists of natural plant substances, are used for prevention and treatment of ailments. This practice has existed since prehistoric times and flourishes today as the primary form of medicine. Over 80,000 species of plants are in use throughout the world. The use of herbal drugs remains a good alternative to allopathic agents, with fewer side effects.

Varieties of plants have been used for the purpose of anti-inflammatory and analgesic activity in herbal medicine. *Nelumbo nucifera* (lotus) is one of the plants that have been used for its medicinal properties since ancient times. Almost all parts of the plants are used for the treatment of conditions associated with health abnormalities. Hence, the present study is aimed to prove scientifically the anti-inflammatory and analgesic property of lotus seeds as well as to compare the activity of red and white lotus seeds.

Review of Literature

REVIEW OF LITERATURE

2.1. *Nelumbo nucifera*

Nelumbo nucifera (lotus) commonly known as kamala, thamara or padma is an aquatic herb found everywhere in the country. This plant is often cultivated in India, for their elegant flower, which is the national flower of India.

A formulation of medicines comprising lotus seed is used in ayurvedic practice to treat the kidney inflammation and reproductive tract disorders. The rhizomes, flowers, stalk and leaves of lotus are used in the form of infusion in fever as refrigerant and diuretic (Mitra *et al.*, 1973). They detected alkaloids, steroids and reducing sugars in various extracts of rhizomes of *N.nucifera.*

Almost all parts of this plant are used in traditional medicinal practice to treat various diseases. The rhizomes are used as nutritive, mucilaginous agent, demulcent, diuretic, cholagogue and are effective in piles, dyspepsia and diarrheoa (Kritikar and Basu, 1975).

2.2. ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF *N.nucifera*

Mukherjee *et al* .(1996) reported that the methanolic extract of rhizomes of *Nelumbo nucifera* in doses of 200mg/kg, 300mg/kg and 400mg/kg, i.p. had shown reduction in spontaneous activity, decrease in exploratory behaviour pattern by the head dip and Y–maze test, reduction in muscle relaxant activity and depression of pain response in mice. It also potentiated the pentobarbitone induced sleeping time in mice.

The methanolic extract of *Nelumbo nucifera* rhizome as well as the steroidal triterpenoid (betulinic acid) isolated from it, possessed significant anti-

inflammatory activity in carrageenin and 5-hydroxytryptamine induced rat paw oedema models. The effects produced were comparable to that of phenylbutazone and dexamethasone (Mukherjee *et al* ., 1997).

Jiri (2003) reported that the betulin, a triterpene, present in *Nelumbo nucifera* has shown anti-inflammatory activity against carrageenin and serotonin induced paw oedema in rats.

The extracts from *Nelumbo nucifera* Gaertn, were used in the treatment of tissue inflammation in traditional Chinese medicine. It inhibited peripheral blood mononuclear cells proliferation (PBMC) activated with phytohemagglutinin (PHA). By a bioassay-guided fractionation procedure, NN-B-4 (*Nelumbo nucifera* B-4) identified from *N. nucifera* ethanolic extracts significantly suppressed activated PBMC proliferation. The inhibitory action of NN-B-4 did not involve direct cytotoxicity. In an attempt to further localize the point of arrest in the peripheral blood mononuclear cells proliferation, a set of key regulatory events leading to the cell proliferation, including cell cycle progression, production and gene expression of interleukin-2 (IL-2), IL-4, IL-10, and interferon-gamma (IFN-gamma) were examined. Cell cycle analysis indicated that NN-B-4 arrested the cell cycle progression of activated PBMC from the G1 transition to S phase. The suppressant effects of NN-B-4 on the proliferation of PBMC activated by PHA mediated through inhibition of early transcripts of PBMC, like IL-2, IFN-gamma, and cdk4 and arrest of cell cycle progression (Liu *et al.*, 2004).

Xiao *et al.* (2005) reported anti-inflammatory and the anti-oxidant activity of isoliensinine, a bisbenzyl alkaloid extracted from the seed embryo of lotus, on bleomycin induced pulmonary fibrosis. They observed a significant inhibitory effect on bleomycin induced pulmonary fibrosis, which could be attributed to its former properties.

2.3. PHARMACOLOGICAL PROPERTIES OF *Nelumbo nucifera*

The *Nelumbo nucifera* plant apart from the anti-inflammatory and analgesic properties showed other properties also.

Mazumder *et al.* (1992) reported that the petroleum ether extracts of seeds of *N.nucifera* administered to mice at a dose rate of 3mg/kg body weight i.p. has shown significant contraceptive, antiestrogenic, antiprogestonal activities. These findings indicated that the extract of *N.nucifera* seed could affect the estrus cycle by blocking the biogenesis of ovarian steroids at any intermediate stage and thereby antifertility activity.

Cour *et al.* (1995) reported that in Chinese traditional medicine *Nelumbo nucifera* (rhizomes and seeds) was one of the medicinal plants used for the treatment of hyperlipedimia .The decoction of *N.nucifera* had a significant reducing effect on the serum triglyceride and cholesterol level.

Mukherjee *et al.* (1995) observed that the methanolic extract of *N.nucifera* at the dose rate of 100-600mg/kg, p.o. inhibits the castor oil induced diarrhoea .It reduced the gastrointestinal motility after administration of charcoal meal and had significant inhibition of PGE_2 induced enteropooling in rats. The results established the efficacy of *N.nucifera* rhizome as an antidiarrhoeal agent.

In vitro antifungal and antiyeast activity of methanolic extract of rhizome of *N.nucifera* in doses of 250-350µg/ml was investigated against five different types of fungi and yeast by sensitivity test, disc diffusion test, spore germination technique and turbidity technique. The effect produced by the extract was comparable with the standard antifungal agent griseofulvin and found to be active against all organisms tested (Mukherjee *et al.*, 1995a).

Gupta *et al.* (1996) proved the antisteroidogenic effect of seeds of *N.nucifera* (2.5-7.5 mg/kg, p.o.) in sexually immature female and male rats. The seeds of *N. nucifera* showed remarkable delay in the sexual maturation in the prepubertal female rats and significant reduction in sperm count and motility in male rats. An accumulation of cholesterol and ascorbic acid were also noted. These results indicated the suppression of steroidogenesis in both testes.

Mukherjee *et al.* (1996a) investigated the antipyretic activity of methanolic extract of rhizome of *N.nucifera* on normal body temperature and yeast induced pyrexia in rats. The extract in doses of 200,300 or 400 mg /kg (po) produced significant dose dependant lowering of normal temperature and yeast induced elevation of body temperature. The effect was comparable with the standard antipyretic drug, paracetamol (150mg/kg, i.p.). Sinha *et al.* (2000) described the antipyretic potential of *N. nucifera* stalk extract on normal body temperature and yeast induced pyrexia in rats at a dose level of 200 and 400 mg/kg. It caused a significant lowering of body temperature and yeast induced elevation of temperature.

Mukherjee *et al.* (1997a) reported that the ethanolic extract of *N.nucifera* when administered orally had marked reduction in the blood sugar level of normal glucose fed hyperglycemic and streptozotocin induced diabetic rats. The extract improved glucose tolerance and potentiated the action of exogenously administered injected insulin in normal rats.

The ethanolic extract of *Nelumbo nucifera* seeds exhibited antioxidant and hepatoprotective effects by inhibiting the production of serum enzymes and cytotoxicity induced by intraperitoneal injection of carbon tetra chloride and oral administration of aflatoxin B_1 (Sohn *et al* ., 2003).

Wang *et al.* (2003) found that the methanolic extract of lotus plumule and blossom had possessed strong reducing powers and free radicals scavenging abilities. However only the methanolic extract of lotus plumule exhibited ion-chelating properties, which might contribute to its higher anti-oxidant activities.

Kuoy *et al.* (2005) reported the inhibitory effects of ethanolic extracts from the seeds of *N. nucifera* on herpes simplex virus type 1 (HSV-1) replication. By a bioassay-guided fractionation procedure, NN-B-5 (*Nelumbo nucifera*- B-5) was identified from seeds of *N. nucifera*. NN-B-5 significantly blocked HSV-1 multiplication in HeLa cells without apparent cytotoxicity. A set of key regulatory events leading to the viral multiplication was examined, including HSV-1 DNA synthesis and viral immediate early gene expressions to elucidate the point in HSV-1 replication where arrest occurred. It indicated that the mechanism of action NN-B-5 seemed to be mediated through, inhibition of early transcripts such as ICP0 and ICP4 (ICP-infected cell protein) and blockage of viral products accumulation and progeny production.

Ling *et al.* (2005) isolated procyanidins from lotus seedpod, which exhibited strong anti-oxidant activity and inhibited the lipooxygenase activity by more than 90% at a concentration of 62.5 μg/ml.

Rai *et al.* (2006) investigated the anti-oxidant activity of hydro-alcoholic extract of *Nelumbo nucifera* seeds (HANN) *in vitro* and *in vivo* models. The extract administered to rats at 100 and 200 mg/kg body weight orally for 4 days prior to carbon tetrachloride (CCl_4) treatment. It showed a significant dose dependent increase in the level of superoxide dismutase and catalase and a significant decrease in the level of thiobarbituric acid reactive substances, when compared to CCl_4 treated control in both liver and kidney. These changes observed at 100 mg/kg body weight treatment were comparable to standard Vitamin E at 50 mg/kg treatment. The results

supported significant antioxidant nature of HANN. They evaluated the acute toxicity of HANN in mice in which there was no signs of toxicity observed up to the oral dose of 1,000 mg/kg body weight. They also reported that the *Nelumbo nucifera* seeds contained alkaloids, saponins, phenolics and carbohydrates.

2.4. ACUTE INFLAMMATORY MODEL -CARRAGEENIN INDUCED PAW OEDEMA

In the present study on the anti-inflammatory effect of *N.nucifera* seeds, the carrageenin induced paw oedema was taken as a prototype of acute inflammatory models.

The carrageenin was a mixture of polysaccharides containing sulfated galactose units, derived from the Irish Sea moss *Chondrus crispus* (Smith *et al.*, 1955).

Among the many methods used for screening and evaluation of anti-inflammatory drugs, the most commonly employed technique was based upon the ability of such agents to inhibit the oedema produced in the hind paw of the rat by injection of a phlogistic agent. Carrageenin is one of the phlogistic agents possessing distinct advantages over others (Winter *et al.*, 1962). They also measured the amount of swelling in carrageenin injection by determining the amount of mercury it displaced.

Carrageenin induced oedema was a biphasic response. The first phase was mediated through the release of histamine, serotonin, and kinins, whereas the second phase was related to the release of prostaglandin and slow reacting substances which peaked at 3 hours (Vinegar *et al.*, 1969).

Carrageenin caused oedema, an increase in paw volume, and an exacerbated sensitivity to thermal and mechanical stimuli, which was known as hyperalgesia. Conventional non-steroidal anti- inflammatory drugs, cyclooxygenase - 2 inhibitors and prostaglandin monoclonal antibodies were effective as anti-inflammatory agents in this model (Chan *et al* .,1999).

2.5. PLANTS EXHIBITING ANTI- INFLAMMATORY EFFECT IN ACUTE INFLAMMATORY MODEL

Different plant extracts have been screened for anti- inflammatory effects in acute inflammatory model especially on carrageenin induced paw oedema.

Pendse *et al.* (1977) reported the anti-inflammatory effect of aqueous extract from *Tinospora cordifolia* (Neem Giloe) stem .The aqueous extract at the dose rate of 60 mg/100g administered orally and intra peritoneally showed significant reduction in carrageenin induced inflammation in rats.

Trivedi *et al.* (1986) observed the anti-inflammatory property of glycosidal fraction from *Acacia farnesiana* ethanolic extract, at the dose rate of 100µg/kg.i.p. The extract revealed a promising anti-inflammatory effect in carrageenin oedema model in rats.

The water soluble fraction of alcoholic extract prepared from rhizome of *Picrorhiza kurroa* at the dose of 100mg /kg inhibited the carrageenin induced paw oedema in rats (Pandey and Das, 1989).

Asmawi *et al.* (1993) observed the anti-inflammatory activity in the methanolic extract of *Emblica officinalis* leaves at the dose of 2g/kg on carrageenin and dextran induced rat hind paw oedema.

The ethanolic extract of *Vitex leucoxylon* leaf at the dose level of 200mg/kg and 400mg/kg i.p. showed significant inhibition of carrageenin induced paw oedema in rats (Makwana *et al.*, 1994).

The alcoholic extract of the roots of *Ageratum conyzoids* at 100 mg/kg and 300mg/kg p.o.showed significant anti -inflammatory activity in a dose dependant manner on carrageenin induced paw oedema .The effect shown by 300mg/kg dose of alcoholic extract was comparable to the effect shown by 100mg/kg dose of aspirin (Tandan *et al.*, 1994).

Lupeol, a triterpene isolated from the petroleum ether fraction of ethanolic extract of *Ixora coccinea* leaves in doses of 100 mg/kg and 200mg/kg, p.o. had shown anti-inflammatory activity in carrageenin induced paw oedema in albino rats (Zachariah *et al* ., 1994).

Sandhika is an ayurvedic drug used in the treatment of rheumatoid arthritis. It consists of aqueous extract of plants namely *Commiphora mukul*, *Boswellia serrata*, *Strychnus nuxvomica*, and *Semecarpus anacardium* and showed significant anti- inflammatory property with no detectable adverse effect . at the dose of 0.25g/kg body weight p.o. against carrageenin paw oedema model in albino rats (Chaurasia *et al.*, 1995).

The anti-inflammatory effects of aqueous suspension of total xanthones from *Swertia chirata* were investigated in albino rats at a dose level of 50mg/kg, p.o. against carrageenin, serotonin, bradykinin, dextran and PGE_1 induced oedema. The results revealed the significant anti-inflammatory activity against carrageenin, serotonin and bradykinin induced paw oedema (Islam *et al.*, 1995).

The triglyceride fraction of oil from *Ocimum sanctum* (3ml/kg, i.p.) offered higher protection against carrageenin induced paw oedema in rats as

compared to the fixed oil. The fixed oil of *O. sanctum* also exhibited significant anti-inflammatory activity against carrageenin, serotonin, histamine and PGE_2 induced inflammation in rats. The *O.sanctum* fixed oil contains linoleic acid, which could contribute to dual inhibition of the arachidonic acid metabolism resulting in anti-inflammatory activity (Singh *et al.*, 1996).

The petroleum ether extract and chloroform extract of the seeds of *Pongamia pinnata* at the dose rate of 50-100mg/kg, i.p. showed potent acute anti-inflammatory effect. The maximum anti-inflammatory effect was shown by ethanolic extract in bradykinin induced oedema model. The possible mechanism of action could be inhibition of prostaglandin synthesis and decreased capillary permeability. Petroleum ether extract inhibited histamine and 5- hydroxytryptamine induced inflammation probably by their lipophilic constituents preventing the early stages of inflammation (Singh *et al.*, 1996a).

Gmelina asiatica root powder was effective at the dose rate of 50-200mg/kg, p.o. in reducing the carrageenin induced paw oedema during various phases of acute inflammation in rats. The probable mechanism of its anti-inflammatory effect may be its anti-proliferative, anti-oxidative and lysosomal membrane stabilizing effects (Ismail *et al* ., 1997).

Jigrine, another polyherbal formulation, exhibited anti-inflammatory activity against carrageenin induced acute inflammation but not against cotton pellet granuloma model (subacute inflammation). The effect on biochemical parameters suggested that the mechanism of its anti-inflammatory effect could be in its antioxidant and membrane stabilising effect (Karunakar *et al* ., 1997).

Rao and Mishra (1997) reported that the methanolic extract of the aerial part of *Sida rhombifolia* (Atibala) at the dose rate of 100mg/kg possessed significant oedema suppressant activity in the carrageenin induced paw oedema model in rats.

The probable mechanism of action may be due to its inhibitory effects on release of mediators of inflammation such as histamine, 5- hydroxytryptamine, bradykinin etc.

Tandan *et al.* (1997) found that the alcoholic extract of rhizome from *Hedichium spicatum* at the dose rate of 300mg/kg p.o. had shown significant anti-inflammatory activity in carrageenin induced paw oedema.

Oil of *Psidium guajava leaves* showed anti–inflammatory activity at the dose rate of 0.8 ml /kg body weight orally in carrageenin induced paw oedema. The result was comparable to that of ketorolac tromethamine (Kavimani *et al* ., 1997).

Oil of *Cymbopogan martini* leaves produced dose dependent inhibition of carrageenin induced paw oedema at 0.8 ml/kg dose.The oil produced inhibition of oedema comparable to that of diclofenac sodium (Krishnamoorthy *et al* ., 1998).

The alcoholic extract of *Justica procumbens* has been screened for *in vivo* anti-inflammatory activity against formalin induced paw oedema in albino rats. It revealed promising anti–inflammatory activity at a dose of 100mg/kg body weight orally (Mruthyunjayaswamy *et al.*, 1998).

The flavonoid isolated from *Caralluma attenuata* at the dose rate of 2-4 mg/kg has shown significant anti-inflammatory activity against carrageenin induced paw oedema in rats (Ramesh *et al.*, 1998).

Jana *et al.* (1999) reported that the dried rhizome extract of *Zingiber officinale* (100mg/kg, p.o.), dried leaves extract of *Vitex negundo* (100 mg/kg, p.o.) and dried stem extract of *Tinospora cordifolia* (50 mg/kg. p.o.) had shown significant anti-inflammatory effect on both carrageenin induced paw oedema and cotton pellet granuloma models in rats.

Singh (1999) reported that the fixed oil of *Ocimum basilicum* has produced significant anti-inflammatory activity against carrageenin, histamine, serotonin, PGE_2, bradykinin and hyaluronidase induced paw oedema in rats at the dose rate of 3 mg/kg. It also inhibited the arachidonic acid and leucotrine induced oedema .On the basis of these findings, it was reported to block both cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism.

Telang *et al.* (1999) demonstrated the anti-inflammatory activity of hydroalcoholic extracts of *Vitex negundo* leaves at the dose rate of 500mg/kg and 1000mg/kg p.o. It was more pronounced on sub acute (carrageenin induced granuloma pouch model) rather than acute inflammation (carrageenin induced paw oedema) in rats.

Mujumdar *et al.* (2000) conducted studies on the rhizome extract of *Curcumba amada* and concluded that the extract had significant anti-inflammatory activity at the dose rate of 200mg/kg, p.o. in carrageenin induced paw oedema and cotton pellet granuloma models in rats.

An herbal formulation named JCB containing *Alpinia galanga, Commiphora wightii, Boswellia serrata, Foenicutum vulgare, Glycyrrhiza glabra, Vitex negundo* and *Anethum graveolens* had shown anti-inflammatory effect at the dose rate of 350mg/kg and 700mg/kg body weight, p.o. against carrageenin induced paw oedema in rats (Venkataranganna *et al.*, 2000).

Vetriselvan *et al.* (2000) made investigations on the anti-inflammatory activity of alcoholic extracts of *Aerva lanata* at the dose rate 800mg/kg in carrageenin induced paw oedema in rats and stated that the extract had significant anti-inflammatory activity.

The ethanol extract and butanol fraction of *Pergularia extensa* leaves in doses of 100mg/kg, i.p. exhibited significant anti-inflammatory activity against carrageenin induced rat paw oedema .The activity was the highest in early phases of carrageenin induced inflammation and results were comparable to that of aspirin (Hukkeri *et al* ., 2001).

Sanjay *et al.* (2001) revealed the anti-inflammatory activity of *Azadirachta indica* (neem) seed oil in albino rats against carrageenin-induced oedema. Neem seed oil at doses of 1, 2, 4 and 8 ml/kg body weight was injected intraperitoneally to rats one hour prior to carrageenin injection. It showed significant effect on 3 –6 hours at 2ml/kg body weight and 2-24 hours, in case of 4ml/kg and 8ml/kg dose after carrageenin injection.

Sankar *et al.* (2001) observed that hydroalcoholic extract of *Elephantopus scaber* at the dose rate of 60mg/kg, p.o. had possessed significant anti-inflammatory activity in the carrageenin pedal oedema in rats.

The methanolic extract of *Citrus sinensis* peel at the dose of 150mg/kg and 300mg/kg, p.o. exhibited effective anti-inflammatory activity on carrageenin induced paw oedema model in rats (Ramachandran *et al* ., 2002).

Vetrichelvan and Jegadeesan (2002) studied the anti-inflammatory activity of alcoholic extract of *Achyranthes bidentata* on carrageenin induced oedema model and in cotton pellet granuloma in rats. The result showed that the alcoholic extract (375mg/kg and 500mg/kg, p.o.) has shown maximum inhibition of oedema by 63.52 per cent and 79.73 per cent respectively.

The lupeol and 19 α-H lupeol isolated from the roots of *Strobilanthus callosus* and *S.ixiocephala* respectively, had shown significant anti-inflammatory activity against carrageenin induced paw oedema. Both compounds in doses of

200,400 and 800 mg/kg produced a dose dependent inhibition of paw oedema (Agarwal and Rangari, 2003)

The methanolic extract of *Caesalpinia bonducella* leaves was investigated for anti-inflammatory activity at the doses of 50, 100 and 200 mg/kg, body weight. A maximum inhibition of 50.6%, 51.1% and 52.3% was noted at the dose of 200 mg/kg after 3 hour of treatment in carrageenin, dextran, and histamine induced pedal edema respectively (Gupta *et al.*, 2003).

The ethyl acetate extract of *Sarcostemma brevistigma* in doses of 650mg/kg, p.o. produced significant inhibition of carrageenin induced oedema in rats (Lalitha *et al.*, 2003).

Shirwaikar and Somashekar (2003) reported the anti-inflammatory effect of ethanolic extract prepared with leaves of *Aristolochia bracteolata* at the dose rate of 400mg/kg, p.o. in carrageenin induced inflammation in rats .The alcoholic extract and ethyl acetate fraction of *A.bracteolata* also showed promising antioxidant activity through its free radical scavenging ability thereby supporting its anti-inflammatory property.

Singh and Jaggi (2003) studied the effects of chloroform and ethanolic extracts of stem, leaf and stem calli of *Ocimum sanctum* against carrageenin induced rat paw oedema. The ethanol extract of callus tissue in doses of 100-800mg/kg, p.o. exhibited maximum significant anti-inflammatory activity followed by ethanol extracts of leaves of *O. sanctum* at the doses of 400-800mg/kg, administered orally.

The biflavonoid fraction isolated from the ethyl acetate extract of seeds from *Semecarpus anacardium* in doses of 75mg/kg and 150mg/kg had shown significant anti-inflammatory activity against carrageenin induced oedema .It was also reported as an effective cyclooxygenase- 2 enzyme inhibitor in *in vitro*

cyclooxygenase-2 catalyzed prostaglandin biosynthesis assay (Selvam and Jachak, 2004).

Ahamed *et al.* (2005) found that the oral administration of the alcoholic extracts of *Araucaria bidwillii* Hook. significantly inhibited the carrageenin (18.61%, 32.12% and 45.64%) and serotonin (32.81%, 38.68% and 40.75%) induced hind paw oedema in rats at 100, 200 and 300 mg/kg respectively which was comparable to standard drug indomethacin at the dose rate of 5 mg/kg (68.51% and 63.28%).

Arulmozhi *et al.* (2005) studied the effect of lyophilized extract of *Saphindus trifoliatus* in various *in vitro* and *in vivo* models .It showed *in vitro* inhibitory activity against the major inflammatory mediators like 5-lipooxygenase, cyclooxygenase, leucotriene B_4 and nitric oxide synthase. It possessed inhibitory activity in *in vivo* at the dose rate of 20 and 100mg/kg, i.p against acute inflammation induced by carrageenin, serotonin and zymosan in rats and mice.

The leaves of *Garcinia xanthochymus* (Guttiferae) was investigated for anti-inflammatory activity using carrageenin induced rat paw edema method. The results demonstrated that the percentage inhibition by petroleum ether extracts and methanolic extract was 86.4 and 80.7 per cent respectively compared to standard drug ibuprofen (Pal *et al.,* 2005).

Sheeja *et al.* (2005) studied the anti-inflammatory activity of ethanol and petroleum ether extract from *Nothapodytes foetida* leaves in carrageenin induced oedema on 3 dose level of 50mg/kg, 100mg/kg, and 200mg/kg, p.o. The results revealed that the ethanolic extract significantly reduced the inflammation which was comparable with standard drug ibuprofen.

The chloroform extract of dried leaves of *Trichilia connaroides*, was screened for anti-inflammatory activity, using formalin-induced inflammation in Swiss mice and Wistar rats. The extract in the doses 60 and 90 mg/kg, significantly suppressed formalin-induced paw edema. The effect of 90 mg/kg extract was comparable to that of standard drug (Purnima *et al* ., 2006).

2.6. ACUTE PAIN MODEL-TAIL FLICK METHOD:

In the present study on analgesic effect of *N.nucifera* seeds, the tail flick method was taken as a prototype of acute pain models.

Amour and Smith (1941) reported that the tail flick method was a simple, rapid technique for the determination of pain threshold in rats and the individual variation under a variety of conditions was found to be small.

Velankar *et al.* (1998) made investigations on the comparative analgesic activity of individual and combinations of certain non-steroidal anti-inflammatory drugs like diclofenac sodium, aspirin, flubifropen, nimusulide in rats. The reaction time was recorded by tail flick method in all the treatment groups, administered with different combinations. The result indicated that analgesic activity was time dependent and highest at 90 minutes post drug administration.

2.7. PLANTS EXHIBITING ANALGESIC EFFECT IN ACUTE PAIN MODEL

Many studies on different plant extracts have been analyzed for analgesic effects in acute pain model especially on tail flick method.

The aqueous extract *Azadirachta indica* leaves was tested for analgesic potency at the dose rate of 10, 30,100mg/kg, i.p. in mice. It enhanced tail withdrawal latencies in tail flick model of nociception. Khanna *et al.* (1995) also observed that

the pre-treatment with the opioid antagonist, naloxone (1mg/kg, i.p.) and central nor adrenaline depleter DSP-4 (N-2-Chloroethyl-N ethyl-2-bromobenzylamine-50mg/kg, i.p.) attenuated the analgesia. The serotonin synthesis inhibitor, parachlorophenylalanine methylester hydrochloride (300mg/kg, i.p.) also potentiated the analgesic effect. These results suggested that both the central and peripheral mechanisms and complex neural pathways might be involved in this effect.

Singh *et al.* (1996a) evaluated the analgesic activity of petroleum ether extract and direct ethanolic extract from *Pongamia pinnata* seeds by using the technique of tail flick response in albino rats. The findings revealed that extracts had significant analgesic activity at doses higher than 100 mg/kg.

Krishnaveni *et al.* (1997) found that the 4', 5, 6-Trihydroxy-3'7-Dimethoxy flavone from *Vicoa indica* had a potent analgesic effect at a dose rate of 50mg/kg and 100mg/kg, i.p. in tail flick and tail clip methods in rats

The petroleum ether extract, benzene extract and ethanolic extract of *Pongamia pinnata* roots showed significant analgesic effect in the tail flick test in rats (Singh *et al.*, 1997).

The petroleum ether extract, benzene extract, acetone extract and ether extract of *Abies pindrow* Royle leaf showed significant analgesic effect in the hot wire induced tail flick response in rats. Singh *et al.* (1998) suggested that the phyto constituents such as flavonoids and terpenoids of *A. pindrow* Royle were responsible for the inhibition of prostaglandins.

The hydroalcoholic extract of *Vitex negundo* leaves showed analgesic activity at the dose of 500mg/kg and 1000mg/kg orally against tail immersion test and acetic acid induced writhing in mice. It significantly increased the reaction time

in tail immersion model and reduced the writhing movement in acetic acid induced writhing in mice (Telang *et al* ., 1999).

Chandra (2001) elucidated the analgesic effect of aqueous and alcoholic extracts of *Madhuka longifolia* in the graded doses of 4 to 64 mg/kg, administered intra muscularly for 3 days. Both extracts produced dose dependent inhibition in tail flick method, hot plate and chemical writhing methods in rats/mice.

The ethanolic extract and petroleum ether extract of dried leaves of *Pergularia extensa* (200 mg/kg. i.p.) have shown significant analgesic effect in tail flick method (Jalanpure *et al* ., 2002).

Biswal *et al.*(2003) made investigations on the ethanolic extract of seeds from *Trigonella foenum graceum* .The extract at the dose rate of 5. 10 and 20 mg/kg body weight administered intra peritoneally showed analgesic activity against tail flick method in rats .

Chattpalliwar *et al.* (2003) observed that the ethanolic extract of *Martynia diandra* had produced analgesic effect in formalin induced pain. The extract also revealed dose dependent anti-nociception in neurogenic pain.

Dharmasiri *et al.* (2003) suggested that the analgesic activity of aqueous extract of *Vitex negundo* leaves might be mediated via PG synthesis inhibition, antihistamine, membrane stabilizing and anti-oxidant activities. The extract exhibited dose dependant analgesic activity at one hour of treatment in the hot plate test. But it did not show the analgesic activity in tail flick test in rats at the same dose rate of 2.5 and 5 g/kg.

The methanolic, chloroform and petroleum ether extracts of *Cleome rutidosperma* were investigated for analgesic (narcotic, and non-narcotic) activity in tail flick method and acetic acid induced writhing in mice at a dose of 100mg/kg

administered orally. All these extracts showed significant analgesic activity compared to control and standard drug treatment with aspirin and morphine sulphate (Bose *et al*., 2004).

Gupta and Tandon (2004) reported the anti nociceptive activity of *Vitex negundo* leaf extracts at the dose rate of 100,250 and 500mg/kg on tail flick test and acetic acid induced writhing in rats.

The aqueous extracts of *Pongamia pinnata* seeds at the dose rate of 300mg/kg body weight orally showed potent analgesic activity similar to aspirin in a tail flick method in rats (Bhoite *et al.*, 2005).

The chloroform extract of dried leaves from *Trichilia connaroides* was screened for analgesic activity using thermal agents induced inflammation in Swiss mice and Wistar rats. The extract treated animals (60 and 90 mg/kg) exhibited statistically significant elevation in mean basal reaction time in thermally induced nociception and the effect of 90 mg/kg extract was almost comparable to that of standard drug (Purnima *et al.*, 2006).

The aqueous extract of *Smilax china* at the dose rate of 1000mg/kg, administered orally had a significant anti nociceptive activity against hot plate test in mice. Further studies were done to understand the molecular basis of mechanism of action of aqueous extract on cyclooxygenase -2. It revealed that the extract inhibited the prostaglandin production by suppression of cyclooxygenase-2 protein synthesis in whole blood assay (Shu *et al*., 2006).

2.8. CHRONIC PAIN MODEL- EXPERIMENTAL NEUROPATHY:

Bennett and Xie (1988) demonstrated a peripheral mononeuropathy in adult rats by placing constrictive ligatures around the common sciatic nerve. The postoperative behaviour of these rats indicated that hyperalgesia: allodynia and

possibly spontaneous pain (or dysthesia). The hyperalgesic responses and allodynia were evident on 2nd postoperative day and lasted for over 2 months. They were inferred from the mechanical allodynia.

Chapman *et al.* (1998) found that ligation of two (L5-L6) of the three spinal nerves that form the sciatic nerve produced a partial denervation of the hindlimb. Following ligation rats exhibited withdrawal responses to normally innocuous punctuate, mechanical and cooling stimuli (induced by acetone) applied to the leshioned hind paw. Such mechanical and cooling allodynia was not observed in sham- operated rats. They later observed that a significantly greater proportion of spinal neurons of ligated rats exhibited spontaneous activity at post operative days 7-10 and 14-17 days compared with controls.

Plaza *et al* .(2004) reported that in mononeuropathy models, the mechanical allodynia observed since the second day after surgery, and the mechanical sensitivity was significantly higher for 14 days of the study. as compared to sham-operated animals. The chronic administration of topiramate, at the dose of 50 mg/kg/day, significantly diminished the mechanical sensitivity and shortened the period of allodynia in rats.

Bingham *et al.* (2005) proved the analgesic effect of dual acting cyclooxygenase-2 inhibitor GW406381X (2-(4-ethoxyphenyl)-3-(4-(methylsulfonyl) phenyl)-pyrazolo(1,5-b) pyridazine ,where X denotes the free base) in chronic constriction nerve injury in rats and mouse partial nerve ligation, both models of neuropathic pain. The agent administered chronically for 9 days in doses of 5mg/kg, p.o. had fully reversed the mechanical allodynia and hyperalgesia, the main signs of neuropathic pain.

2.9.DICLOFENAC:

Diclofenac is a non-steroidal anti-inflammatory compound possessing the anti-inflammatory, analgesic and antipyretic activities. The anti-inflammatory activity shown by diclofenac is equal to that of indomethacin in the carrageenin oedema assay as well as in the adjuvant arthritis model. It also possessed antibradykinin activity, which is three times higher than that of indomethacin and 25 times higher than that of phenylbutazone (Scherrer and Whitehouse, 1974).

Unlike the other non-steroidal anti-inflammatory drugs diclofenac had a dual mode of action via cyclooxygenase as well as lipooxygenase pathways. Dicofenac interacted with the arachidonic acid cascade at the level of cyclooxygenase and inhibited its activity. Inhibition of this key enzyme was also found in *invitro* studies which might consequently prevent the formation of thromboxanes, prostaglandin and prostacyclins (Menasse *et al.*, 1978).

Diclofenac is mainly used as the sodium salt for the relief of pain and inflammation in conditions like rheumatoid arthritis, ankylosing spondylitis, renal colic, acute gout and following surgical procedures. Diclofenac might also be given as the potassium salt (Todd and Sorkin, 1988).

The percentage inhibition of paw oedema observed in the diclofenac treated group in a study conducted by Bothara *et al* .(1998) was 67.24 per cent.

Reversible elevation of serum aminotransferase occurred in patients treated with diclofenac sodium (Tripathi, 1999).

Diclofenac was rapidly and completely absorbed after oral administration, then a peak plasma concentration was reached within 2 to 3 hours and its plasma half-life was one to 2 hours. It is metabolized in the liver by cytochrome P450 isoenzyme of the CYP2C family to 4-hydroxy diclofenac and other hydroxylated

undergo glucuronidation and sulfation .The metabolite are excreted in urine and bile (Roberts and Morrow 2001).

Diclofenac-potassium was developed as an immediate-release tablet with the aim of providing rapid onset of action after oral administration and also provides rapid pain relief within 60 to 90 minutes (McNeely and Goa, 1999).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Ninety six adult Sprague-Dawley rats of either sex weighing 150-200 g, procured from Small Animals Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for the study. All the rats were maintained under identical feeding and management practices in the laboratory. They were divided into two main groups of 48 animals each for anti-inflammatory and analgesic studies.

3.2 TEST SAMPLES:

3.2.1. Collection of Plant Materials

The plants under study were *Nelumbo nucifera seeds*. Two types of lotus seeds namely red flowered lotus (red lotus seed) figure 1, white flowered lotus (white lotus seed) figure 2, were used in the present study. The red lotus seeds were collected from the Nagercoil district of Tamil Nadu .The white lotus seeds were collected from Pilicode in Kasargode district of Kerala.

3.2.2. Prepartion of extract

The lotus seeds were cleaned, and pulverized to a coarse powder. The powder was extracted with methanol using soxhlet extraction apparatus. The extract was evaporated to dryness with the help of a vacuum evaporator and kept in a airtight container in refrigerator. On an average, 100 g of dried powder of seeds gave 10g of dry extract.

3.2.3. Gum acacia

Five per cent of gum acacia was used as vehicle for experimental drugs.

Fig.1. Lotus (*Nelumbo nucifera*) Red type flower and seed

Fig.2. Lotus (*Nelumbo nucifera*) White type flower and seed

3.2.4. Diclofenac Potassium

Diclofenac potassium was gifted by IND-SWIFT Ltd., Chandigarh. It was used as a standard drug at the dose rate of 3 mg/kg body weight administered orally.

3.2.5. Celecoxib

Celecoxib, the selective COX-2 inhibitor was used as a standard drug for COX-2 inhibition assays @ 10mg/kg body weight instead of diclofenac potassium.

3.3. SCREENING OF METHANOLIC EXTRACTS OF RED AND WHITE LOTUS SEEDS FOR ACTIVE PRINCIPLES

The methanolic extracts of red and white lotus seeds were tested for the presence of various active principles namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

3.3.1. *Tests for Detection of Steroids*

Salkowski test

The extract weighing 5 mg was dissolved in 3 ml of chloroform and then shaken with 3 ml concentrated sulphuric acid. The development of red colour indicates the presence of steroids.

Liberman Burchardt test

The 5 mg of extract was dissolved in 3 ml of chloroform. Then five drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added to it

through the sides. Development of a reddish ring at the junction of two layers indicates the presence of steroids.

3.3.2. *Tests for Detection of Alkaloids*

The extract weighing 500 mg was dissolved in 5 ml of ammonia and then extracted with equal volume of chloroform. To this, 5 ml dilute hydrochloric acid was added. The acid layer obtained was used for chemical tests for the alkaloids.

Mayer's test

To 1 ml of acid extract, three to five drops of Mayer's reagent were added (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g of potassium iodide in 10 ml of water and then made the volume to 100 ml with distilled water). The development of a creamy white precipitate indicates the presence of alkaloids.

Wagner's test

Three to five drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide dissolved in 100 ml of water) were added to 1 ml of the acid extract. The development of reddish brown precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of the acid extract, three to five drops of Hager's reagent (1 g of picric acid dissolved in 100 ml of water) were mixed. The development of yellow precipitate indicates the presence of alkaloids.

Dragendroff's test

Three to five drops of Dragendroff's reagent (Stock solution (1) 0.6 grams of bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid then 10 ml of water was added. Stock solution (2) six grams of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400 ml) was mixed with 1 ml of acid extract. The development of a reddish brown precipitate indicates the presence of alkaloids.

3.3.3. *Test for Detection of Phenolic compounds*

Five milligram of the extract was mixed with 1 ml of water and five drops of ten per cent ferric chloride was added to it. The development of dark blue colour indicates the presence of phenolic compounds.

3.3.4. *Tests for Detection of Tannins*

Ferric chloride test

Two milligram of the extract was mixed with 3 ml of one per cent ferric chloride solution. The development of a blue, green or brownish colour indicates the presence of tannins.

Gelatin test

The extract weighing 0.5 g was mixed with few drops of one per cent solution of gelatin containing ten per cent sodium chloride. The development of a white precipitate indicates the presence of tannins.

3.3.5. *Tests for Detection of Flavonoids*

Ferric chloride test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), few drops of neutral ferric chloride solution was mixed. Development of green colour indicates the presence of flavonoids.

Lead acetate test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), few drops of neutral ten per cent lead acetate was mixed. Development of a yellow precipitate indicates the presence of flavonoids.

3.3.6. *Tests for Detection of Glycosides*

Sodium hydroxide test

A small amount of the extract (5 mg) was mixed with 1 ml water and added 5-6 drops of sodium hydroxide solution (10 %). The development of a yellow colour indicates the presence of glycosides.

Benedict's test

To 1 ml of the extract (0.5 g extract in 1 ml of water), 5 ml of Benedict's reagent was added. The mixture was boiled for two minutes. The development of brown to red colour indicates the presence of glycosides.

3.3.7. *Test for Detection of Diterpenes*

Five milligram of the extract was mixed with 3 ml of copper acetate solution (5 %). The development of green colour indicates the presence of diterpenes.

3.3.8. *Tests for Detection of Triterpenes*

Salkowski test

Five mg of the extract was mixed with 3 ml of chloroform and then it was shaken with 3 ml of concentrated sulphuric acid. The development of yellow colour in lower layer on standing indicates the presence of triterpenes.

Lieberman Burchardt test

Three to five drops of acetic acid and 1 ml concentrated sulphuric acid were added to 3 ml of chloroform solution of the extract (3 mg extract in 3 ml chloroform). The development of deep red ring at the junction of two layers indicates the presence of triterpenes.

3.3.9. *Test for the Detection of Saponins*

Foam test

Small amount of the extract (about 5 mg) was shaken with 3 ml of water. The development of the foam that persists for ten minutes indicates the presence of saponins.

3.4 EXPERIMENTAL DESIGN

3.4.1 Anti-inflammatory Screening

The carrageenin induced rat paw oedema method was followed as prescribed by Winter *et al.* (1962). Forty eight adult rats were divided into 6 groups of eight each and treated as follows.

Group I - Vehicle alone (5 per cent gum acacia @ 1 ml /kg body weight) administered per os for 7 days

Group II - Vehicle alone for 7 days + diclofenac potassium (3 mg/kg body weight) administered per os on 7^{th} day.

Group III - Methanolic extract of red lotus seeds (400 mg/kg body weight) administered per os for 7 days

Group IV - Methanolic extract of red lotus seeds (600 mg/kg body weight) administered per os for 7 days

Group V - Methanolic extract of white lotus seeds (400 mg/kg body weight) administered per os for 7 days.

Group VI - Methanolic extract of white lotus seeds (600 mg/kg body weight) administered per os for 7 days.

The paw oedema was induced after half an hour of drug administration by injection of 0.05 ml of 2 per cent w/v carrageenin suspension in normal saline into plantar aponeurosis of the left hind paw of rat.

The hind paw volume was measured by the method of Chattopadhyay *et al.* (1986) at 0 and 3 hours after carrageenin injection using a Plethysmometer (figure 3).

The percentage inhibition of paw oedema was calculated by using the following formula.

$$(1 - \frac{V_t}{V_c}) \times 100$$

Fig. 3. Measurement of paw volume in Plethysmometer

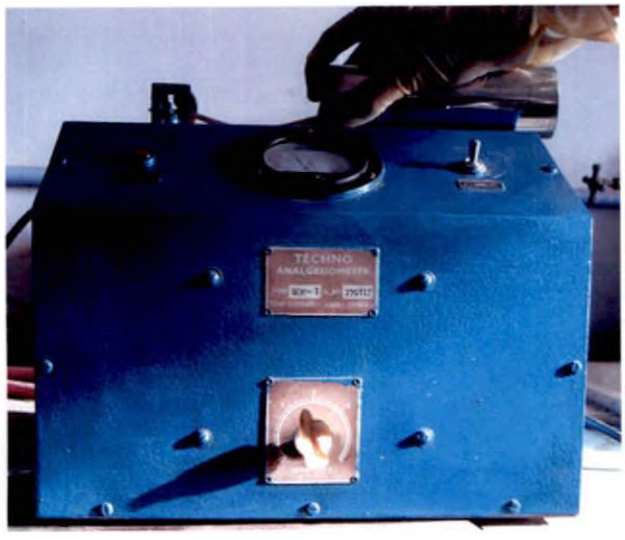


Fig. 4. Measurement of reaction time (tail flick) in Analgesiometer

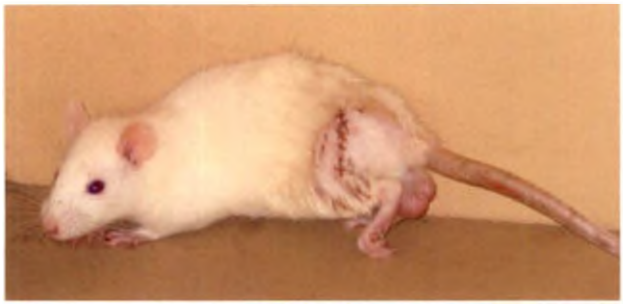


Fig. 5. Postural abnormality in experimental neuropathy model

Where, V_t is the mean increase in paw volume of the treated group and V_c is the mean increase in paw volume of the control group.

After measuring the paw volume, blood was collected from retro orbital plexus by puncturing with heparinised capillary tubes into sterile vials containing disodium salt of ethylene diamine tetra acetic acid (EDTA sodium) at the rate of 1 mg/ml for estimation of haematological parameters. For the estimation of cholesterol, blood was collected in sterile centrifuge tubes without anticoagulant for serum.

The rats were sacrificed after blood collection .The tissue was collected from inflammed hind paw and stored in –20° C for the extraction of cyclooxygenase -2 enzyme.

3.4.1.1. *Estimation of Cyclooxygenase 2 (COX-2) Enzyme Inhibition:*

The COX-2 protein extraction was done based on the method described by Anderson *et al.* (1996).

Tissue extracts preparation:

The tissue collected from inflammed paw (0.3–1 g) was added to 2 ml of phosphate buffer saline containing 1% CHAPS (3-(3-cholamidopropyl) Dimethylammonio-1-propane sulphonate). The tissue was kept at 4 -8° C for 24 hour. Then tissue was homogenized using glass homogenizer and then centrifuged at 2000 RPM for 15 min. The supernatant was collected and analysed for COX-2 enzyme estimation.

a) Spectrophotometric determination of COX-2

Reagents:

1. Arachidonic acid

2. Gelatin

3. Hematin

4. N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD)

5. Tris –Hcl

6. Tween-20

Procedure:

Cyclooxygenase is a bifunctional enzyme exhibiting both the cyclooxygenase and peroxidase activities. The cyclooxygenase component converted arachidonic acid to its hydroperoxy endoperoxide (PGG_2) and the peroxidase component reduced the endoperoxide component to the corresponding substrate (PGH_2), the precursor of prostaglandin, thromboxanes and prostacyclin. The cyclooxygenase–2 inhibition assay measured the peroxidase activity of cyclooxygenase enzyme. It was assayed colorimetrically by monitoring the appearance of oxidized TMPD at 600 nm in spectrophotometer (Copeland *et al* ., 1994).

The tissue extract incubated in enzyme reaction buffer (100 mM Tris-Hcl buffer, pH 6.5, containing 0.1% Tween-20, gelatin at 1 mg/ml, 3 uM hematin, and 100 uM TMPD). One unit of enzyme activity was defined as the amount of enzyme required to cause a change in TMPD absorbance at 600 nm. After the indicated preincubation time (5 minutes), the enzymatic reaction was initiated with 100 uM arachidonic acid, and the initial velocity of the reaction was measured following the oxidation of TMPD at 600 nm. The velocities observed at different inhibitor treated tissue extracts were divided by the velocity observed for enzyme samples preincubated for the same time (without any treatment), and this ratio was multiplied by 100 to yield per cent control activity.

b) Quantification of COX -2 enzyme inhibition by ELISA:

Procedure:

The cyclooxygenase -2 protein was measured using a quantitative ELISA assay employing a rabbit polyclonal antibody raised against purified ovine COX-2 (Cayman chemicals kit). All incubations were conducted at room temperature with agitation. The 96-well plates were coated with 200 µl per well of anti-COX-2 antibody diluted to 3 µg/ml in phosphate buffer saline (PBS). The plates were then incubated for 1 hour, emptied by inversion and washed three times with 200 µl per well of undiluted blocking buffer. Samples (tissue extract-50 µl per well) were then added and the plates incubated for a further 3 hour. They were then washed three times with 200 µl per well of PBS containing 0.05% Tween 20.

The COX-2 standard (3 µg/ ml in PBS, 0.05% Tween 20, 10% Block buffer) was added (100 µl per well) and the plate incubated for 1 hour. The plates were washed again as described above. Both plates were incubated with horseradish peroxidases-linked streptavidin (100 µl of 0.3 µg/ml in PBS. 0.05% Tween 20, 10% Block buffer) for 1 hour. Finally, the plates were washed three times with 250 µl per well of PBS containing 0.05% Tween 20. Peroxidase activity was monitored at 450 nm using 100 µl TMB (tetra methyl benzidine)/Peroxide substrate solutions. The level of COX-2 protein in each sample was estimated by comparison of optical density to that of a COX-2 standard. Total sample protein was determined using the Protein Assay Kit and COX-2 protein levels were expressed as picograms COX-2 per millilitre of protein (Nantel *et al*, 1999).

3.4.1.2. Estimation of serum transaminases.

a. Alanine Amino Transferase (ALT)

The UV-Kinetic test prescribed by Reitman and Frankel (1957) was followed. The Kit from Agappe diagnostics was used.

Reagents

Reagent -1 (R_1)

Tris Buffer (pH 7.5)	100 mmol/L
L-alanine	500 mmol/L
Lactate dehydrogenase	≥ 1200 µ/L

Reagent- 2 (R_2)

2-Oxoglutarate	15 mmol/L
Nicotinamide Adenine-Dinucleotide (NADH)	0.18 mmol/L

Four volumes of Reagent 1 (R_1) was mixed with one volume of Reagent 2 (R_2)

Procedure

1. One ml of the working reagent was taken in test tubes marked sample and reagent.
2. Hundred microlitres of the serum sample was added to the test tubes marked sample.
3. Reagent and serum were mixed well and incubated for one minute at 37°C.
4. The change in optical density per minute, ΔOD/min was measured at 340 nm for 3 minutes.
5. The ALT level in U/L was calculated by the formula

   ALT activity in U/L = OD/min x 1745

b.Aspartate Amino Transferase (AST)

The UV-Kinetic test prescribed by Reitman and Frankel (1957) was followed. The Kit from Agappe diagnostics was used.

Reagents

Reagent 1 (R_1)

Tris Buffer (pH 7.8)	80 mmol/L
L-aspartate	240 mmol/L
Lactate dehydrogenase	≥ 600 μ/L
Malate dehydrogenase	≥ 600 μ/L

Reagent 2 (R_2)

2-Oxoglutarate	12 mmol/L
Nicotinamide Adenine - Dinucleotide (NADH)	0.18 mmol/L

Four volumes of Reagent 1 (R_1) was mixed with one volume of Reagent 2 (R_2)

Procedure

1. One ml of the working reagent was taken in test tubes marked blank and sample.
2. Hundred microlitres of serum was taken and mixed well in the test tube marked sample.
3. After one minute of incubation at 37°C, the change of optical density per minute, ΔOD/min was measured at 340 nm for 3 minutes at 37°C.
4. The AST level in U/L was calculated by the formula

   AST activity in U/L = ΔOD/min x 1745

3.4.1.3. Serum Cholesterol

The total cholesterol level in serum was estimated by enzymatic CHOD-PAP Method (Allain *et al.*, 1974) using kit from Agappe Diagnostics.

Reagents

Piper buffer pH 6.7	50 mmol/L
Phenol	24 mmol/L
Sodium cholate	0.5 mmol/L
4-amino antipyrene	0.5 mmol/L
Cholesterol esterase	≥ 180 μ/L
Cholesterol oxidase	≥ 200 μ/L
Peroxide	≥ 1000 μ/L
Cholesterol standard	200 mg/dl

Procedure

1. One ml of the working reagent was taken in test tubes marked standard, blank and sample.
2. Ten micro litres of serum and standard were taken in respective test tubes.
3. The contents were mixed well, incubated at 37°C for five minutes and read the optical density at 505 nm.
4. The cholesterol concentration in mg per cent was calculated by the formula.

   Cholesterol concentration = O.D. of sample/O.D. of standard x 200, mg/dl

3.4.1.4 Estimation of Haematological Parameters

3.4.1.4.1. *Total Leukocyte Count*

The leukocytes were counted by standard dilution technique using Thomas fluid diluent. Counting of leukocytes was done in the zone of leukocytes

in the haemocytometer focused under low power of the microscope (Schalm, 1986).

3.4.1.4.2. *Differential Leukocyte Count*

Blood smears were prepared from the freshly drawn blood using slide technique. Smear was stained with Leishman's stain and cells were counted under oil immersion (Schalm *et al.*, 1986).

3.4.2. Analgesic Screening

a. Tail flick method

Analgesic effect in rats was assessed by tail flick method (Dandiya and Menon, 1963) in analgesiometer. This instrument had a nichrome wire, which would be heated to the required temperature and maintained by means of heat regulators .The current passing through the nichrome wire will be indicated by an ammeter, which indirectly gave the temperature of wire. A jacket surrounded the nichrome wire through which water was circulated that prevented the platform from getting heated up. The upper surface of the jacket served as a platform on which the tail of the rat could be placed. This ensured that only that portion of the tail, which laid on the wire was heated. The ammeter was set to 4 amperes. So that the heat produced in the nichrome was same throughout the experiment .The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting.

Experimental design:

The experimental design was followed as indicated in the anti-inflammatory screening.

On the 7th day after administration of drugs, reaction time for each group was measured at 30, 60, 90 and 120 minutes using Techno-analgesiometer figure 4 (Davies *et al.*, 1946). Animals with the reaction time of more than 10 seconds were discarded to avoid tissue injury. The blood was collected after 120 minutes and the serum and haematological parameters were assessed as described in the anti-inflammatory study.

b.Experimental neuropathy model

Procedure:

Forty eight rats were divided into 6 groups of eight each. Then surgery was done in all animals under anasthesia. Anesthesia was induced in Sprague-Dawley rats by the injection of thiopentone sodium (40mg/kg, i.p.) and maintained with subsequent injection if needed. After a local incision, the biceps femoralis of each leg was bluntly dissected at midthigh to expose the sciatic nerve. Each nerve was then mobilized with care taken to avoid undue stretching. Four 4-0 chromic gut sutures were tied loosely with a square knot around the sciatic nerve. Both incisions were closed layer to layer with silk sutures and the rats allowed to recover. During the next days, the animals showed a mild eversion of the affected paw, mild-to-moderate degree of foot drop and postural abnormalities (Figure 5). The rat were allowed a period of atleast 7 days to

recover from surgery before behavioral testing began. The dosing was started on 8^{th} day after the stable reductions in the paw withdrawal tendency. The experimental design was followed as indicated earlier.

On the last day after dosing the sensitivity of the ipsilateral and contralateral hindpaw to cooling was assessed by the application of a drop of acetone onto the plantar region of the foot (Kim and Chung 1997). Although this stimulus may include both chemical and tactile components, it was used to ensure that these original behavioural reports could be replicated. In this case each trial consisted of five applications of acetone, separated by a period of 5 min. The response frequency to acetone was calculated as the (no. of foot withdrawals/5 (no. of applications)) × 100

3.7. STATISTICAL ANALYSIS OF DATA

Results were analyzed by using one-way ANOVA test for comparison between control groups and treatment groups II, III, IV, V and VI as described by Snedecor and Cochran (1985). Significance in the difference of the means was tested using Least Significant Difference (LSD). Results were expressed as mean ± standard error.

Results

4. RESULTS

4.1. PHYTOCHEMICAL ANALYSES (Table 1):

4.1.1 Steroids

As per the Salkowski test, red colour was obtained and Liberman Burchardt test also gave a reddish ring at the junction in both the extracts. Thus it could be concluded that the detectable level of steroids was present in methanolic extract of red and white lotus seeds.

4.1.2 Alkaloids

A creamy white precipitate as per Mayer's test and a reddish brown coloured precipitate as per Wagner's test were obtained and Dragendroff's test yielded reddish brown precipitate. Hager's test produced yellow precipitate in both extracts. Thus, the tests revealed the presence of detectable level of alkaloids in both lotus seed extracts.

4.1.3 Phenolic Compounds

A dark blue colour was produced, when lotus seed extract was mixed with ten per cent of ferric chloride indicating the presence of phenolic compounds.

4.1.4 Tannins

Intense blue colour was not obtained in ferric chloride test and a white precipitate was not obtained in gelatin test in both lotus seed extracts. These results indicated the absence of tannins in the lotus seeds.

4.1.5 Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in lead acetate test were obtained and it indicated the presence of flavonoids in methanolic extract of red and white lotus seeds.

4.1.6 Glycosides

In the Benedict's test, brown colour was obtained indicating the presence of glycosides. Yellow colour was obtained by mixing the extract with sodium hydroxide, which also indicated the presence of glycosides in methanolic extract of red and white lotus seed.

4.1.7 Diterpenes

Diterpene was detected in the extract of both red and white lotus seed as indicated by the green colour, when it was mixed with copper acetate solution.

4.1.8 Triterpenes

As per Liberman Burchardt test, a deep red ring appeared at the junction of two layers in methanolic extract of red and white lotus seed. These results indicated the presence of triterpenes in methanolic extract of red and white lotus seed.

4.1.9 Saponins

In the foam test, foam was persisted for 10 minutes in the white and red lotus seeds extracts, which indicated the presence of saponins.

Table 1. Screening of methanolic extract of red and white lotus (*Nelumbo nucifera)* seeds for active principles

Sl.no	Active Principles	Red lotus seed	White lotus seed
1	Steroids	Detected	Detected
2	Alkaloids	Detected	Detected
3	Tannins	Not detected	Not detected
4	Flavonoids	Detected	Present
5	Glycosides	Detected	Present
6	Phenolic compounds	Detected	Present
7	Diterpenes	Present	Detected
8	Triterpenes	Present	Detected
9	Saponins	Detected	Present

4.2. INVESTIGATION OF ANTI-INFLAMMATORY PROPERTY OF METHANOLIC EXTRACT OF RED AND WHITE LOTUS (*Nelumbo nucifera)* SEEDS

4.2.1. CARRAGEENIN INDUCED PAW OEDEMA

The change in foot pad volume in rats administered with the alcoholic extract of seeds from *Nelumbo nucifera* (lotus) red and white types after one hour, two hour and third hour of carrageenin administration are presented in Tables 2, 3 and 4, respectively and Fig.6. Percentage inhibition of paw oedema, exhibited by the treatment groups, is presented in Table 5 and Fig.7. The rats fed with methanolic extract of red lotus seeds, lower dose (Group III-400mg/kg) exhibited 36.92, 38.35 and 44.22 per cent inhibition of oedema induced by carrageenin at first, second and third hour respectively. The higher dose (Group IV-600mg/kg) also exhibited anti-inflammatory effect where the percentage of inhibition of oedema was 41.12, 41.24 and 44.76 per cent at first, second and third hour respectively, after carrageenin injection. The methanolic extract of white lotus seeds, lower dose (Group V-400mg/kg) showed inhibition of paw oedema in all phases of acute inflammation. It exhibited 40.42, 45.59 and 45.44 per cent of inhibition of edema at first, second and third hour respectively after carrageenin injection. The methanolic extract of white lotus seeds, higher dose (Group VI-600mg/kg) inhibited the inflammation and the percentage of inhibition is 50.47, 47.76 and 47.21 per cent at first, second and third hour respectively, after carrageenin injection. The standard drug diclofenac (Group II) showed 60.75, 62.67 and 61.09 per cent inhibition after first hour, second hour and third hour respectively after carrageenin injection.

Table 2. Effect of treatments on paw volume (ml) one hour after carrageenin injection in rats

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	0.4750	0.1200	0.3430	0.3000	0.2800	0.2400
2	0.4438	0.2300	0.2812	0.3300	0.2500	0.1800
3	0.3912	0.1700	0.1875	0.2700	0.2600	0.1400
4	0.3712	0.1600	0.2813	0.2700	0.3000	0.1600
5	0.4700	0.1650	0.2187	0.2400	0.2350	0.2200
6	0.3500	0.1200	0.2812	0.2400	0.2350	0.2900
7	0.3900	0.1800	0.1787	0.2700	0.2600	0.2900
8	0.5300	0.1950	0.2425	0.2400	0.2900	0.1800
Mean ± SE	0.4280 ± 0.02[a]	0.1680 ± 0.01[d]	0.2700 ± 0.01[b]	0.2520 ± 0.02[b]	0.2550 ± 0.01[b]	0.2120 ± 0.02[cd]

Means bearing same superscript do not differ significantly at $P<0.05$

Group I - Control 5% gum acacia (@ 1ml/kg) alone per os for 7 days.

Group II - Vehicle alone for 7 days + diclofenac potassium (3 mg/kg body weight) administered per os on 7th day.

Group III - Methanolic extract of red lotus seeds (400 mg/kg body weight) administered per os for 7 days

Group IV - Methanolic extract of red lotus seeds (600 mg/kg body weight) administered per os for 7 days

Group V - Methanolic extract of white lotus seeds (400 mg/kg body weight) administered per os for 7 days.

Group VI - Methanolic extract of white lotus seeds (600 mg/kg body weight) administered per os for 7 days.

Table 3. Effect of treatments on paw volume (ml) two hours after carrageenin injection in rats

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	0.6900	0.2187	0.4800	0.4300	0.3800	0.3700
2	0.6700	0.2500	0.4200	0.4200	0.3400	0.3550
3	0.6450	0.2863	0.4300	0.3700	0.3800	0.3550
4	0.7200	0.2870	0.4100	0.4025	0.4500	0.3600
5	0.7200	0.2488	0.4000	0.4175	0.3900	0.3900
6	0.7000	0.2470	0.4475	0.4400	0.3400	0.3700
7	0.6900	0.2863	0.4425	0.3700	0.3600	0.300
8	0.6850	0.2400	0.3800	0.4000	0.3700	0.3900
Mean ± SE	0.6910 ± 0.01[a]	0.2580 ± 0.01[f]	0.4260 ± 0.01[b]	0.4060 ± 0.01[c]	0.3760 ± 0.01[d]	0.3610 ± 0.01[e]

Means bearing same superscript do not differ significantly at P<0.05

Table 4. Effect of treatments on paw volume (ml) three hours after carrageenin injection in rats

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	0.7400	0.2987	0.4100	0.4600	0.4000	0.4600
2	0.7400	0.2900	0.4600	0.3800	0.4400	0.3400
3	0.7800	0.2760	0.3800	0.4400	0.4300	0.4000
4	0.7100	0.3350	0.4600	0.4600	0.4200	0.3600
5	0.7400	0.3000	0.3100	0.3700	0.3200	0.3400
6	0.7700	0.2450	0.4100	0.4000	0.4200	0.3600
7	0.7100	0.2500	0.4600	0.3400	0.4200	0.4200
8	0.6900	0.2900	0.3600	0.4300	0.3600	0.4200
Mean ± SE	0.7350 ± 0.01[a]	0.2860 ± 0.01[c]	0.4100 ± 0.01[b]	0.4060 ± 0.02[b]	0.4010 ± 0.01[b]	0.3880 ± 0.03[b]

Means bearing same superscript do not differ significantly at P<0.05

Figure 6. Effect of treatments on inhibition of carrageenin induced paw oedema in rats.

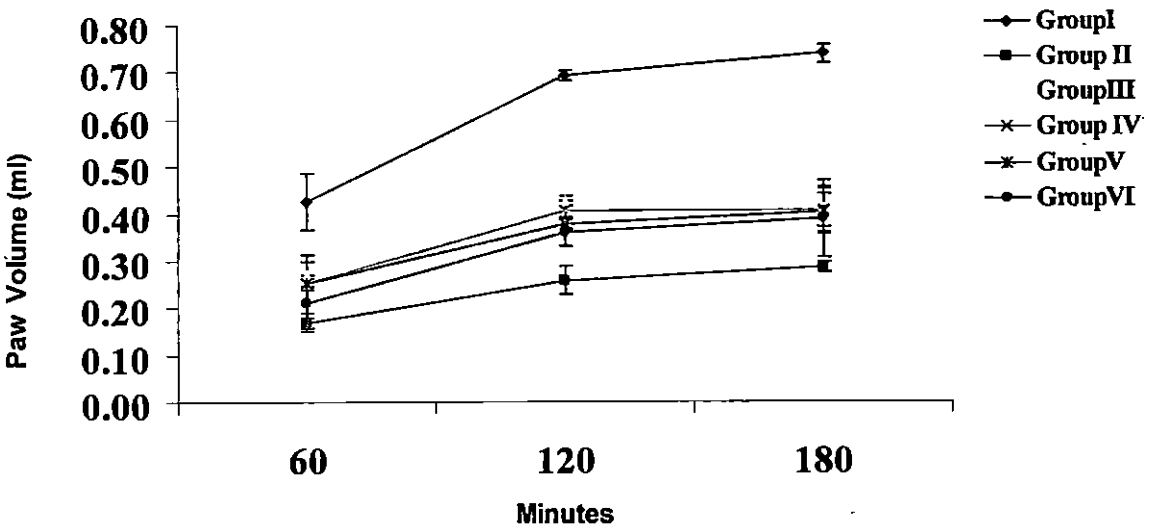


Figure 7. Effect of treatments on percentage inhibition of carrageenin induced oedema in rats.

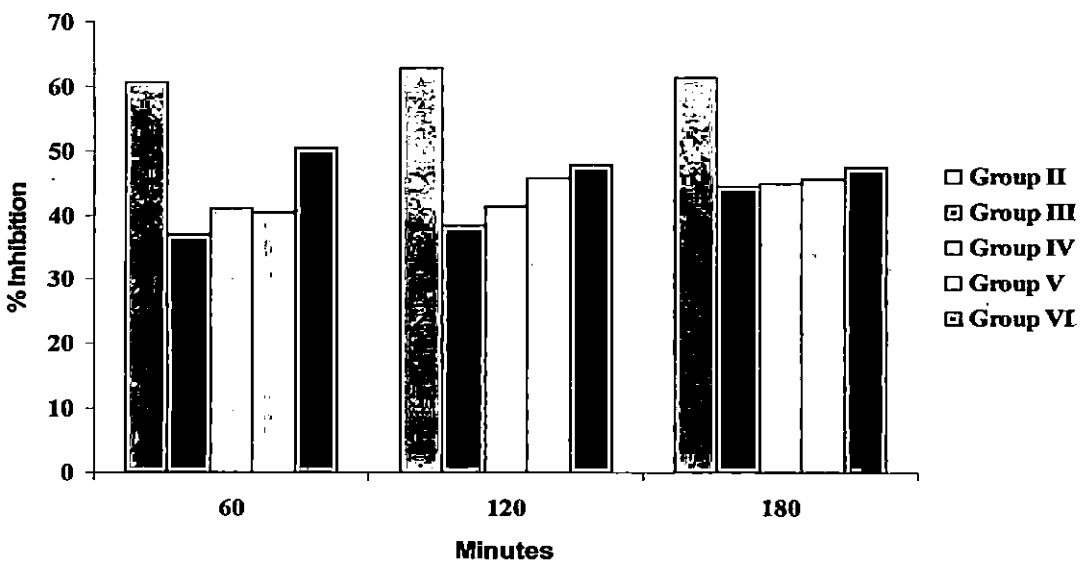


Table 5.Percentage inhibition of paw oedema by different treatments in carrageenin induced paw oedema in rats.

Time Interval (min.)	Group II	Group III	Group IV	Group V	Group VI
60	60.75	36.92	41.12	40.42	50.47
120	62.67	38.35	41.24	45.59	47.76
180	61.09	44.22	44.76	45.44	47.21

Table 6.Effect of treatments on inhibition of cyclooxygenase-2 enzyme by ELISA (Pg/ml)

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	82.11	33.50	45.54	41.12	40.50	38.25
2	80.00	36.20	49.27	44.58	39.50	37.43
3	79.94	34.40	48.74	46.44	39.50	39.64
4	79.28	37.20	46.32	42.56	40.50	38.21
5	83.55	35.10	47.27	41.24	41.50	37.27
6	84.37	32.50	46.29	43.34	41.00	39.63
7	83.13	34.00	49.53	46.51	40.50	38.27
8	82.29	37.40	49.28	40.37	40.60	37.49
Mean ± S.E	**81.83 ± 0.67**	**35.04** ** **± 0.63**	**47.78** ** **± 0.57**	**43.27** ** **± 0.85**	**40.45** ** **± 0.24**	**38.27** ** **± 0.33**

**- Significant at P<0.01

Table 7. Percentage inhibition of cyclooxygenase-2 in treatment groups by ELISA.

Group	Group II	Group III	Group IV	Group V	Group VI
% Inhibition	57.18	41.61	47.12	50.57	53.23

4.2.2. CYCLOOXYGENASE-2 ENZYME INHIBITION

The results obtained for cyclooxygenase- 2 enzyme by ELISA method are presented in the tables 6 and 7 and figure 8. The standard COX-2 inhibitor celecoxib showed 57.18 per cent inhibition. The rats fed with methanolic extract of red lotus seed showed 41.61 and 47.12 per cent inhibition at the dose rate of 400 mg/kg and 600mg/kg respectively. While the white lotus seed administered group exhibited 50.57 and 53.23 per cent inhibition at the dose rate of 400 mg/kg and 600mg/kg respectively.

The data obtained for COX-2 enzyme inhibition by spectrophotometric assay are presented in the tables 8 and 9 and figure 9. The standard drug COX-2 specific inhibitor celecoxib showed 69.28 per cent inhibition. The methanolic extract of red lotus seed, lower dose (400 mg/kg) revealed 37.68 per cent inhibition and higher dose (600mg/kg) had showed 42.31 per cent inhibition. The methanolic extract of white lotus seed lower dose (400 mg/kg) exhibited 42.02 per cent inhibition whereas the higher dose (600mg/kg) had possessed 44.63 per cent inhibition

4.2.3. BIOCHEMICAL PARAMETERS

4.2.3.1. Alanine Amino Transferase (ALT)

The ALT values are depicted in the table 10. The mean ALT levels of control and diclofenac treated group were 31.38 ± 0.73 and 32.13 ± 0.95 U/L, respectively. The rats fed with methanolic extract of red lotus seed, lower dose (Group III) and higher dose (Group IV) showed the mean value of 29.50±1.18, 27.13±0.44 U/L, respectively. The methanolic extract of white lotus seed, lower dose

Table 8. Effect of treatments on inhibition of cyclooxygenase-2 enzyme by Spectrophotometer (U/ml)

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	0.036	0.017	0.030	0.027	0.027	0.030
2	0.045	0.013	0.025	0.018	0.029	0.026
3	0.034	0.015	0.046	0.036	0.036	0.024
4	0.059	0.014	0.032	0.031	0.016	0.035
5	0.026	0.014	0.017	0.019	0.037	0.014
6	0.075	0.013	0.050	0.039	0.017	0.035
7	0.047	0.014	0.017	0.022	0.017	0.014
8	0.046	0.013	0.014	0.021	0.037	0.025
Mean ± SE	0.0460 ±0.01 [a]	0.0142 ±0.01 [d]	0.0289 ±0.01 [c]	0.0267 ±0.02 [c]	0.0270 ±0.02 [c]	0.0254 ±0.02 [b]

Means bearing same superscript do not differ significantly at $P<0.05$

Table 9. Percentage inhibition of cyclooxygenase-2 in treatment groups by Spectrophotometer

Group	Group II	Group III	Group IV	Group V	Group VI
% Inhibition	69.28	37.68	42.31	42.02	44.63

Figure 8. Effect of treatments on percentage inhibition of cyclooxygenase -2 by ELISA method

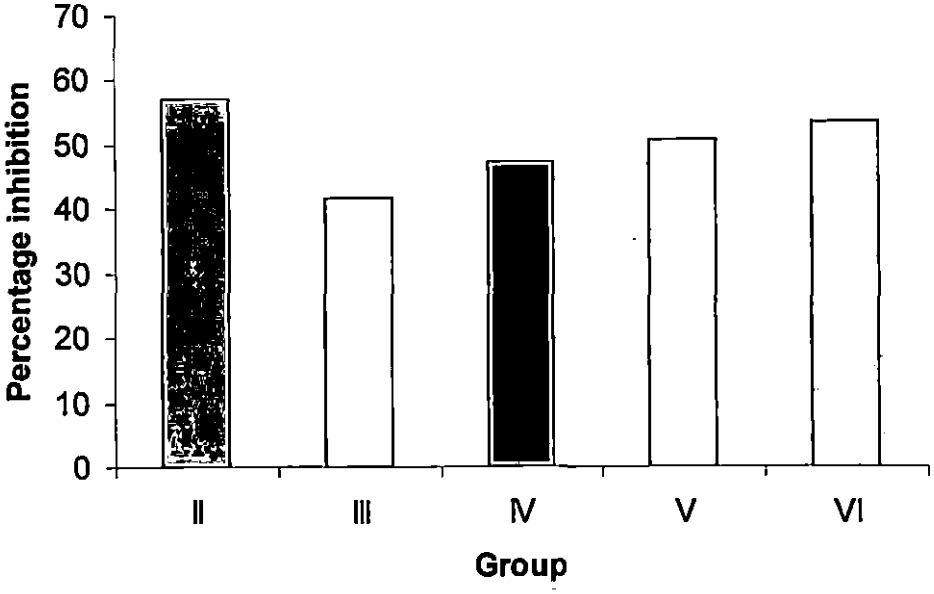
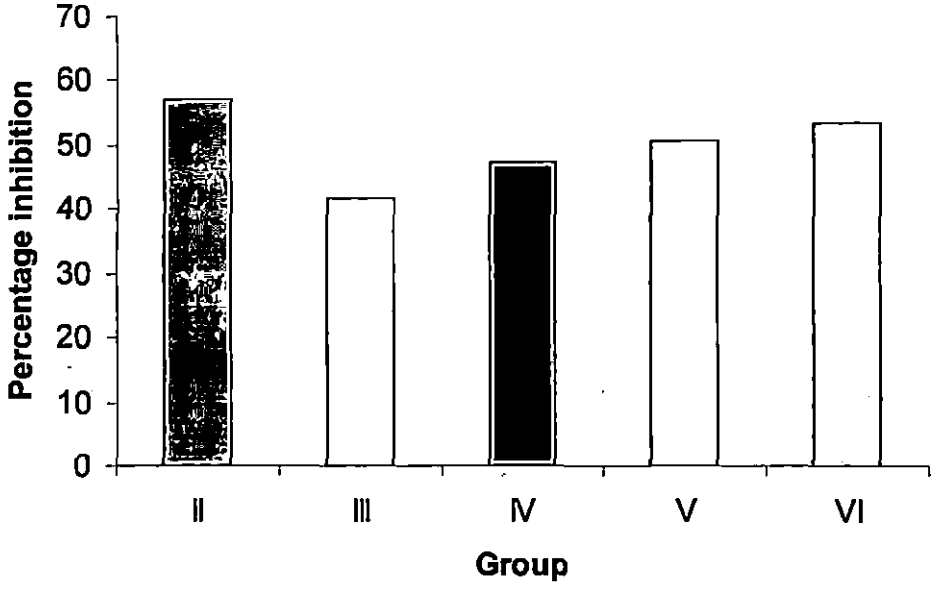


Figure 9. Effect of treatments on percentage inhibition of cyclooxygenase-2 by spectrophotometeric method.



(Group V) and higher dose (Group VI) showed the mean value of 32.75 ± 1.04, 30.38 ± 0.77 U/L, respectively.

4.2.3.2. Aspartate Amino Transferase (AST)

The AST values are presented in table 11.The mean values for the control and diclofenac treated group were 118.50 ± 0.65, 120.75 ± 2.03 U/L, respectively. The mean value for the rats fed with lower dose (Group III) and higher dose (Group IV) showed 127.75 ± 2.93 and 103.75 ± 1.60 U/L, respectively. The rats administered with lower and higher dose (Group V and VI) of white lotus seed extract exhibited the value of 130.25 ± 2.18 and 106.50 ± 1.39 U/L, respectively.

4.2.3.3 Serum cholesterol

The total cholesterol level in serum are presented in the table 12. The mean serum cholesterol for control and diclofenac treated groups (I and II) were 54.00 ±1.18, 57.50±1.50 mg/dl, respectively. The rats fed with red lotus seed, lower and higher dose (Group III and Group IV) values were 48.00 ± 0.70, 46.88 ± 0.81 mg/dl, respectively. The white lotus seed treated groups showed more reduction of cholesterol when compared to red lotus seed groups. The values were 46.00 ± 0.86, 37.75 ± 0.79 mg/dl, for white lotus seed, lower dose and higher dose (Group V and VI) respectively.

4.2.4. HAEMATOLOGICAL PARAMETERS:

4.2.4.1. Total Leukocyte Count (TLC)

The results of the total leukocyte count are presented in the table 13.The values were with in the normal range. The control and diclofenac treated groups (I and II) had a value of 11210 ± 815.00, 9912 ± 281.06 /µl of blood respectively. The rats fed with methanolic extract of red lotus seed, lower and higher dose (Group III

Table 10. Effect of treatments on alanine amino transferase (ALT) level in carrageenin induced paw oedema in rats, U/L

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	29	29	34	27	34	28
2	33	36	29	28	36	34
3	31	34	31	27	28	33
4	28	28	26	29	29	34
5	34	34	25	25	32	28
6	31	32	28	28	34	30
7	33	31	34	26	33	29
8	32	33	29	27	36	30
Mean ± SE	31.38 ± 0.73^{ab}	32.13 ± 0.95^{ab}	29.50 ± 1.18^{bc}	27.13 ± 0.44^{a}	32.75 ± 1.04^{a}	30.38 ± 0.77^{ab}

Means bearing same superscript do not differ significantly at P<0.05

Table 11.Effect of treatments on aspartate amino transferase (AST) level in carrageenin induced paw oedema in rats, U/L

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	119	121	140	101	137	110
2	120	119	128	99	117	107
3	118	112	131	107	128	114
4	121	127	110	104	132	102
5	120	126	127	98	136	104
6	116	114	130	112	131	103
7	116	127	129	105	132	107
8	118	120	127	104	129	105
Mean ± SE	118.50 ± 0.65^{b}	120.75 ± 2.03^{b}	127.75 ± 2.93^{a}	103.75 ± 1.60^{c}	130.25 ± 2.18^{a}	106.50 ± 1.39^{c}

Means bearing same superscript do not differ significantly at P<0.05

Table 12.Effect of treatments on serum cholesterol level in carrageenin induced paw oedema in rats, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	58	55	49	47	46	40
2	54	58	47	49	44	34
3	53	55	46	44	45	40
4	53	54	49	44	48	36
5	60	55	52	50	50	38
6	52	63	48	46	43	38
7	52	54	47	46	44	40
8	50	56	46	49	48	36
Mean ± SE	54.00 ± 1.18[a]	57.50 ± 1.50[a]	48.00 ± 0.70[b]	46.88 ± 0.81[b]	46.00 ± 0.86[b]	37.75 ± 0.79[c]

Means bearing same superscript do not differ significantly at P<0.05

Table 13. Effect of treatments total leucocyte count in carrageenin induced paw oedema in rats, per microlitre of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	7500	10200	6500	13800	7400	6500
2	8100	10000	7100	9400	7800	4700
3	12800	9000	7200	8600	7300	6800
4	10500	10000	6800	8200	8100	7400
5	12800	11000	7000	860	7500	7200
6	12000	9000	7000	8200	8100	6200
7	12200	9200	7100	9600	8700	6600
8	13800	10900	7300	9500	8000	6400
Mean ± SE	11210 ± 815.00[ab]	9912 ±281.06[bc]	7000 ± 134.26[c]	9487 ± 647.56[bc]	7900 ± 155.75[c]	6600 ± 302.42[d]

Means bearing same superscript do not differ significantly at P<0.05

and IV) showed the value of 7000 ± 134.26 and 9487 ± 647.56 /µl of blood respectively. The methanolic extract of white lotus seed, lower dose (Group V) showed the value of 7900 ± 155.75 /µl of blood and the value for the higher dose (Group VI) were 6600 ± 302.42 /µl of blood.

4.2.4.2. Differential Leukocyte Count

4.2.4.2.1. Neutrophil:

The results of neutrophil count are presented in Table 14. The control and diclofenac treated groups (I and II) the counts were 52.00 ± 1.20, 48.25 ± 1.83 per cent, respectively. The rats administered with red lotus seed extract, lower dose and higher dose (Group III and IV) showed a neutrophil count of 50.63 ± 0.77 and 49.75 ± 1.00 per cent, respectively. The white lotus seed extract lower and higher dose (Group V and VI) had shown the counts of 50.38 ± 0.73, 51.63 ± 0.75 per cent, respectively.

4.2.4.2.2. Lymphocytes

The results are presented in Table 15. All the groups showed a decrease in lymphocyte count than the normal values. The counts were 45.50 ± 1.18, 50.00 ± 1.64, 46.75 ± 0.59, 47.63 ± 0.97, 47.38 ± 0.76 and 46.25 ± 0.62 per cent for group I to VI, respectively.

4.2.4.2.3. Monocyte

Monocyte count is presented in Table 16. The monocyte counts were1.13 ± 0.22, 1.00 ± 0.32, 1.38 ± 0.18, 1.50 ± 0.38, 1.38 ± 0.18 and 1.63 ± 0.18 per cent for group I to VI, respectively.

4.2.4.2.4. Eosinophils

The data are presented in Table 17. The eosinophil counts were 0.88 ± 0.12, 0.50 ± 0.20, 1.50 ± 0.27, 1.13 ± 0.18, 0.88 ± 0.22 and 0.50 ± 0.20 per cent for group I to VI, respectively.

Table 14. Effect of treatments on neutrophil count in carrageenin induced paw oedema in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	55	58	52	49	50	53
2	52	50	47	45	49	53
3	45	48	48	49	51	54
4	56	42	50	53	47	48
5	52	44	51	47	53	49
6	50	48	53	51	53	52
7	52	44	51	51	51	51
8	54	52	53	53	49	53
Mean ± SE	52.00 ± 1.20^{a}	48.25 ± 1.83^{b}	50.63 ± 0.77^{ab}	49.75 ± 1.00^{ab}	50.38 ± 0.73^{ab}	51.63 ± 0.75^{ab}

Means bearing same superscript do not differ significantly at P<0.05

Table15.Effect of treatments on lymphocyte count in carrageenin induced paw oedema in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	44	42	45	45	46	46
2	46	48	49	52	49	45
3	52	50	49	49	47	45
4	42	56	47	45	51	49
5	46	54	47	51	45	48
6	48	50	47	47	45	45
7	42	54	45	47	47	47
8	44	46	45	45	49	45
Mean ± SE	45.50 ± 1.18^{b}	50.00 ± 1.64^{a}	46.75 ± 0.59^{b}	47.63 ± 0.97^{ab}	47.38 ± 0.76^{ab}	46.25 ± 0.62^{b}

Means bearing same superscript do not differ significantly at P<0.05

Table 16. Effect of treatments on monocyte count in carrageenin induced paw oedema in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	0	0	1	4	2	1
2	2	2	1	2	2	1
3	2	1	2	1	1	1
4	1	2	2	1	1	2
5	1	0	1	1	1	2
6	1	0	1	1	2	2
7	1	2	2	1	1	2
8	1	1	1	1	1	2
Mean ± SE	1.13 ± 0.22[a]	1.00 ± 0.32[a]	1.38 ± 0.18[a]	1.50 ± 0.38[a]	1.38 ± 0.18[a]	1.63 ± 0.18[a]

Means bearing same superscript do not differ significantly at P<0.05

Table 17. Effect of treatments on eosinophils count in carrageenin induced paw oedema in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	1	0	2	2	2	0
2	0	0	3	1	0	1
3	1	1	1	1	1	0
4	1	0	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	0	1
7	1	0	2	1	1	0
8	1	1	1	1	1	0
Mean ± SE	0.88 ± 0.12[ab]	0.50 ± 0.20[ab]	1.50 ± 0.27[a]	1.13 ± 0.18[a]	0.88 ± 0.22[ab]	0.50 ± 0.20[b]

Means bearing same superscript do not differ significantly at P<0.05

4.3. INVESTIGATION OF ANALGESIC PROPERTY OF METHANOLIC EXTRACT OF RED AND WHITE LOTUS (*Nelumbo nucifera*) SEEDS

4.3.1. ACUTE PAIN MODEL -TAIL FLICK METHOD:

The reaction time measurement (in seconds) was considered as an index of nociception. The reaction time for every treatment groups at 30 minutes interval up to two hours are recorded and presented in table 18 and figure 10.

The diclofenac and the methanolic extract of red and white lotus seed treated groups exhibited significant analgesic activity compared to controls. The increase in reaction time was gradual for all the treatment groups and it was peak at 90 minutes for all groups except group II that reached its peak at 120 minutes.

The reaction time for groups I, II, III, IV, V and VI before the starting of experiment (0 minute) was 3.00 ± 0.15, 3.24 ± 0.04, 3.13 ± 0.07, 3.04 ± 0.19, 3.10 ± 0.12 and 3.20 ± 0.09 seconds, respectively. The reaction time for groups I, II, III, IV, V and VI at 30 minutes was 3.66 ± 0.07, 3.68 ± 0.07, 3.49 ± 0.08, 3.71 ± 0.05, 3.71 ± 0.07 and 3.70 ± 0.07 seconds, respectively. All groups showed significant increase in reaction time compared to control. The reaction time for groups I, II, III, IV, V and VI at 60 minutes were 3.76 ± 0.11, 5.03 ± 0.20, 3.88 ± 0.20, 4.19 ± 0.20, 4.18 ± 0.05 and 4.21 ± 0.03 seconds, respectively.

The reaction time at 90 minutes for groups I, II, III, IV, V and VI was 4.05 ± 0.15, 5.95 ± 0.08, 6.14 ± 0.03, 6.25 ± 0.03, 5.60 ± 0.29, and 6.39 ± 0.12, seconds respectively. At 120 minutes the reaction time for the treatment groups I, II III, IV, V and VI were 3.75 ± 0.12, 6.31 ± 0.08, 5.16 ± 0.05, 5.26 ± 0.07, 5.30 ± 0.07, and 5.39 ± 0.11 seconds, respectively.

Table18.Effect of treatments on analgesic activity by tail flick model in rats.

Time interval	Reaction time in seconds (mean ± SE)					
	Group I	Group II	Group III	Group IV	Group V	Group VI
0 min.	3.00±0.15	3.24±0.04	3.13±0.07	3.04± 0.19	3.10±0.12	3.20±0.09
30 min.	3.66±0.07	3.68±0.07*	3.49±0.08*	3.71±0.05*	3.71±0.07*	3.70±0.07*
60 min.	3.76±0.11	5.03±0.20*	3.88±0.20*	4.19±0.20*	4.18±0.05*	4.21±0.03*
90 min.	4.05±0.15	5.95±0.08*	6.14±0.03*	6.25±0.03*	5.60±0.29*	6.39±0.12*
120 min.	3.75±0.12	6.31±0.08*	5.16±0.05*	5.26±0.07*	5.30±0.07*	5.39±0.11*

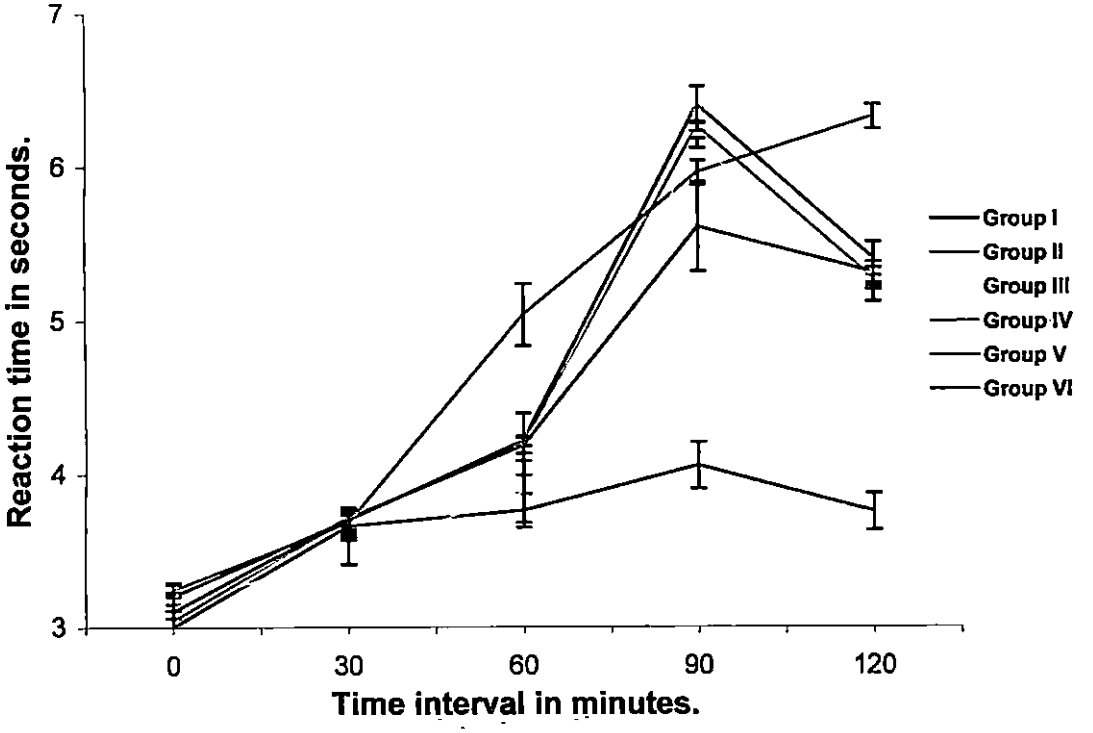
*- Significant at P<0.01

Table 19.Effect of treatments on alanine amino transferase (ALT) level in tail flick analgesic model in rats, U/L

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	28	33	41	26	40	27
2	34	31	36	27	31	26
3	33	33	34	29	40	21
4	31	34	38	34	34	20
5	29	36	36	45	36	19
6	31	27	39	27	38	24
7	31	32	35	17	38	26
8	30	33	34	24	40	23
Mean ± SE	30.88 ± 0.70^{b}	32.38 ± 0.93^{b}	36.63 ± 0.89^{a}	28.63 ± 2.89^{b}	37.13 ± 1.15^{a}	23.25 ± 1.06^{c}

Means bearing same superscript do not differ significantly at P<0.05

Figure 10. Effect of treatments on tail flick method of analgesic model in rats.



4.3.2. BIOCHEMICAL PARAMETERS

4.3.2.1. Alanine Amino Transferase (ALT)

The data of ALT are given in table 19. The mean value of ALT for control and diclofenac treated groups (I and II) were 30.88 ± 0.70 and 32.38 ± 0.93 U/L, respectively. The rats fed with red lotus seed extract lower and higher dose groups (III and IV) mean values were 36.63 ± 0.89 and 28.63 ± 2.89 U/L, respectively. The rats fed with methanolic extract of white lotus seed, lower and higher dose (Group V and VI) mean values were 37.13 ± 1.15 and 23.25 ± 1.06 U/L, respectively.

4.3.2.2. Aspartate Amino Transferase (AST)

The data of AST are presented in table 20. The values for control and diclofenac treated groups (I and II) were 115.75 ± 1.73 and 116.63 ± 2.07 U/L, respectively. The mean values for red lotus group lower and higher dose groups (III and IV) were 126.25 ± 1.81 and 100.63 ± 1.89 U/L, respectively. The rats fed with white lotus seed extracts, lower and higher dose (Group V and VI) has shown the mean value of 124.00 ± 0.65 and 106.63 ± 1.50 U/L, respectively.

4.3.2.3 Serum Cholesterol

The total cholesterol values in serum are presented in table 21.The mean serum cholesterol level for the groups I and II were 65.00 ± 0.68, 69.75 ± 0.72 mg/dl, respectively. The rats fed with methanolic extract of red lotus seed lower and higher dose (Group III and IV) mean values were 52.13 ± 2.17 and 47.63 ± 0.88 mg/dl, respectively. The rats administered with methanolic extract of white lotus seed, lower and higher dose (Group V and VI) showed the mean values of 49.50 ± 0.65 and 42.25 ± 1.14 mg/dl, respectively.

Table 20.Effect of treatments on aspartate amino transferase (AST) level in tail flick analgesic model in rats, U/L

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	114	116	136	103	124	104
2	113	124	124	99	123	102
3	114	113	126	94	124	114
4	111	122	127	95	121	112
5	116	116	131	104	123	107
6	114	107	121	105	124	106
7	117	121	124	103	126	104
8	127	114	121	102	127	104
Mean ± SE	115.75 ± 1.73[b]	116.63 ± 2.07[b]	126.25 ± 1.81[a]	100.63 ± 1.89[d]	124.00 ± 0.65[a]	106.63 ± 1.50[c]

Means bearing same superscript do not differ significantly at P<0.05

Table 21.Effect of treatments on serum cholesterol level in tail flick method in rats, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	66	68	55	49	51	42
2	64	69	48	47	52	46
3	64	71	57	45	47	47
4	62	72	56	48	49	44
5	67	70	59	47	50	38
6	68	70	52	43	51	40
7	64	72	50	41	47	42
8	65	66	40	48	49	39
Mean ± SE	65.00 ± 0.68[a]	69.75 ± 0.72[a]	52.13 ± 2.17[b]	47.63 ± 0.88[c]	49.50 ± 0.65[b]	42.25 ± 1.14[c]

Means bearing same superscript do not differ significantly at P<0.05

4.3.3. HAEMATOLOGICAL PARAMETERS

4.3.3.1 Total Leukocyte Count (TLC)

The results are presented in the table 22.The values were with in the normal range. Control and diclofenac group (I and II) had the mean value of 10112 ± 582.9, 9762 ± 1854.0 /µl of blood, respectively. The rats fed with methanolic extract of red lotus seed, lower and the higher dose (Group III and IV) showed the mean values of 6762 ± 200.2 and 8925 ± 457.4 /µl of blood, respectively. The methanolic extract of white lotus seed, lower and the higher dose treated groups (V and VI) showed the mean values of 7125 ± 521.9 and 6988 ± 134.4 /µl of blood, respectively.

4.3.3.2. Differential Leukocyte Count

4.3.3.2.1. Neutrophils

The data are presented in Table 23. The neutrophil counts for the group I to VI were 47.00 ± 1.21, 48.25 ± 1.67, 45.63 ± 0.78, 48.00 ± 0.85, 47.50 ± 0.80 and 48.88 ± 0.55 per cent, respectively.

4.3.3.2.2. Lymphocytes

The results of lymphocyte count are presented in Table 24. The counts for groups I to VI were 51.00 ± 1.07, 50.25 ± 1.95, 51.75 ± 0.60, 49.25 ± 0.94, 50.25 ± 0.76 and 48.88 ± 0.51 per cent, respectively.

4.3.3.2.3. Monocyte

The results are given in Table 25. The monocyte counts for the group I to VI were 1.13 ± 0.22, 1.00 ± 0.33, 1.38 ± 0.26, 1.50 ± 0.38, 1.38 ± 0.18 and 1.63 ± 0.18 per cent, respectively.

4.3.3.2.4.Eosinophils

The values are presented in Table 26. The eosinophil counts for the group I to VI were 0.88 ± 0.12, 0.50 ± 0.33, 1.50 ± 0.26, 1.13 ± 0.13, 0.88 ± 0.23 and 0.50 ± 0.19 per cent, respectively.

4.3.4. EXPERIMENTAL NEUROPATHY:

In neuropathy pain models the mechanical and thermal stimulus studies are considered as an index of pain reflex measurement. Based on the number of withdrawal responses exhibited, the analgesic property of drug is assessed.

In the present study the cold stimulus induced by application of acetone on the plantar region of operated paw was taken as pain reflex measurement. The paw withdrawal reflex exhibited by the animal after cold stimulus is considered as an index of nociception. The data for experimental neuropathy was given in the table 27 and figure 11. The mean number of foot withdrawal reflexes for the groups II, III, IV, V and VI were 2.13 ± 0.36, 3.75 ± 0.46, 3.63 ± 0.50, 3.75 ± 0.52 and 3.50 ± 0.33, respectively.

Table 22.Effect of treatments level total leucocyte count in tail flick analgesic model in rats, per microlitre of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	12400	10200	6000	8100	10700	7600
2	10700	10400	6200	9200	6800	6500
3	9400	9800	6400	8600	7100	6800
4	8900	9400	6400	8200	6400	7400
5	9400	9600	7400	8500	6600	7000
6	8400	9200	7200	8600	6400	7100
7	9000	10400	7300	12000	6200	7200
8	12700	9100	7200	8200	6800	6500
Mean ± SE	10112 ± 582.9^{ab}	9762 ± 1854.0^{bc}	6762 ± 200.2^{d}	8925 ± 457.4^{bc}	7125 ± 521.9^{c}	6988 ± 134.4^{d}

Means bearing same superscript do not differ significantly at P<0.05

Table 23.Effect of treatments on neutrophil count in tail flick analgesic model in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	50	42	47	49	45	47
2	47	48	42	45	49	51
3	40	50	43	49	49	49
4	51	42	45	45	47	48
5	47	54	46	47	45	49
6	45	50	48	51	45	47
7	47	54	46	51	51	51
8	49	46	48	47	49	49
Mean ± SE	47.00 ± 1.21^{a}	48.25 ± 1.67^{a}	45.63 ± 0.78^{ab}	48.00 ± 0.85^{a}	47.50 ± 0.83^{a}	48.88 ± 0.55^{ab}

Means bearing same superscript do not differ significantly at P<0.05

Table 24.Effect of treatments on lymphocyte count in tail flick analgesic model in rats

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	49	58	50	45	51	51
2	51	50	54	52	49	47
3	57	48	54	49	49	50
4	47	58	52	52	51	49
5	51	44	52	51	53	48
6	53	48	52	47	53	50
7	51	44	50	47	47	47
8	49	52	50	51	49	49
Mean ± SE	51.00 ± 1.07[a]	50.25 ± 1.95[a]	51.75 ± 0.60[a]	49.25 ± 0.94[ab]	50.25 ± 0.76[a]	48.88 ± 0.51[ab]

Means bearing same superscript do not differ significantly at P<0.05

Table 25.Effect of treatments on monocyte count in analgesic model in rats

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	0	0	1	4	2	1
2	2	2	1	2	2	1
3	2	1	2	1	1	1
4	1	2	2	1	1	2
5	1	0	1	1	1	2
6	1	0	1	1	2	2
7	1	2	2	1	1	2
8	1	1	1	1	1	2
Mean ± SE	1.13 ± 0.22[a]	1.00 ± 0.33[a]	1.38 ± 0.26[a]	1.50 ± 0.38[a]	1.38 ± 0.18[a]	1.63 ± 0.18[a]

Means bearing same superscript do not differ significantly at P<0.05

Table 26.Effect of treatments on eosinophil count in tail flick analgesic model in rats

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	1	0	2	2	2	0
2	0	0	3	1	0	1
3	1	1	1	1	1	0
4	1	0	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	0	1
7	1	0	2	1	1	0
8	1	1	1	1	1	0
Mean ± SE	0.88 ± 0.12[a]	0.50 ± 0.33[ab]	1.50 ± 0.26[a]	1.13 ± 0.13[ab]	0.88 ± 0.23[ab]	0.50 ± 0.19[b]

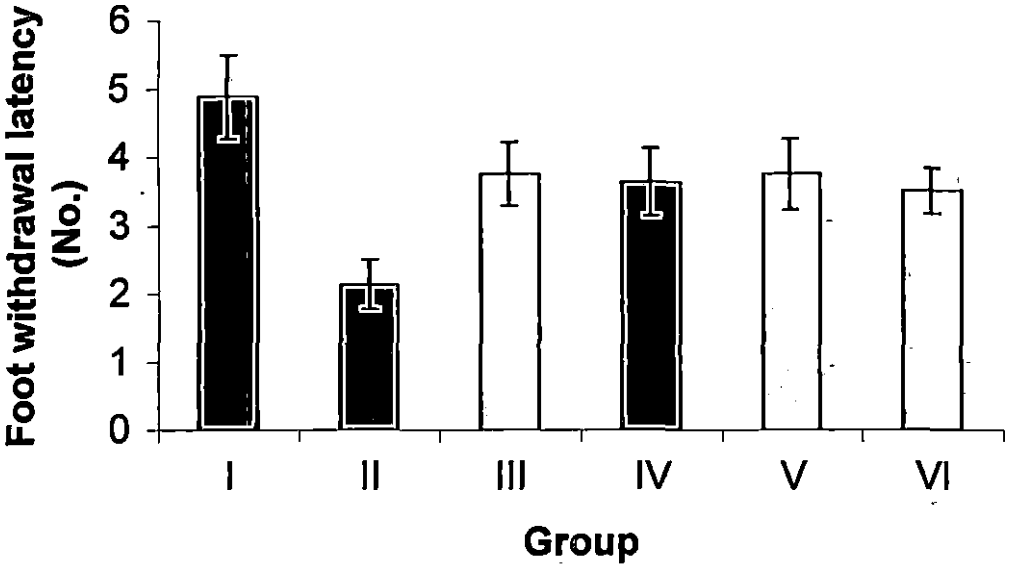
Means bearing same superscript do not differ significantly at P<0.05

Table 27. Effect of treatments on foot withdrawal latency (No.) in experimental neuropathy model in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	7	3	5	3	5	4
2	6	2	6	2	6	4
3	4	2	2	2	4	6
4	2	1	4	3	3	3
5	4	4	3	2	4	5
6	7	2	4	4	3	4
7	4	1	3	6	2	2
8	5	2	3	3	2	3
Mean ± SE	4.88 ± 0.61[a]	2.13 ± 0.36[c]	3.75 ± 0.46[ab]	3.63 ± 0.50[ab]	3.75 ± 0.52[ab]	3.50 ± 0.33[b]

Means bearing same superscript do not differ significantly at P<0.05

Figure 11. Effect of treatments on experimental neuropathy model of analgesic activity in rats



Discussion

5. DISCUSSION

It is well known that the traditional herbal remedies exist before the introduction of modern medicine and even now a good percentage of world population depend on herbal practices for their health care. Herbal drugs are widely used in recent years for preventive and therapeutic purposes. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases. Lotus is one of the principal ingredients in various ayurvedic preparations used as health restorative agents in the ancient time and almost all parts of this plant are used. Also now, there is a growing interest in identifying and characterizing the natural compounds with anti-inflammatory and analgesic activity, ever since they have been suggested in modern medicine. With these objectives in mind, the present study was aimed to determine the active principles of lotus seeds and their anti-inflammatory as well as analgesic properties in rats.

5.1. ANALYSIS OF LOTUS (*Nelumbo nucifera)* SEED EXTRACT FOR ACTIVE PRINCIPLES.

The phytochemical analysis of lotus seeds revealed the presence of alkaloids, flavonoids, glycosides, steroids, phenolic compounds, diterpenes and triterpenes in them. Wu *et al.*(2004) isolated alkaloids liensinine,and its analogues,isoliensinine and neferine from embryo of the seed of lotus employing preoperative counter current chromatography. Ling *et al.* (2005) isolated procyanidins from lotus seedpod, which exhibited strong antioxidant activity. Rai *et al.* (2006) investigated the active principles of *N.nucifera* seeds and find the seeds contain alkaloids, saponins, phenolics and carbohydrates.

Phenolic compounds comprise a wide range of plant substances ranging from simple monocyclic compound to complex polyphenolic compounds. Among the naturally occurring phenolic compounds, flavonoids form the largest group. Flavonoids are used as anti-inflammatory agents, antioxidants, stress modifiers, antiviral, anticarcinogenic and antiallergic agents. Some flavonoids may be found in association with sugars and are called flavonoid glycosides. These are demonstrated to have anti-inflammatory, antiallergic, antithrombotic and vasoprotective properties.

Flavonoids are known to inhibit prostaglandins, which are involved in inflammation and pain perception (Rajnarayana *et al.*, 2001). The anti-inflammatory property of *N.nucifera* may be due to the presence of flavonoids.

Saponins are glycoside compounds used as antioxidants, anti-inflammatory agents, regulators of cell proliferation and in the treatment of cytotoxicity of cancer cells.

Terpenoids form the largest group of plant principles, which are derived biosynthetically from the molecule of isoprene. They are categorized as monoterpenoids, diterpenoids, triterpenoids, and sesquiterpenoids and are used as antioxidants and anti-inflammatory agents.

5.2. EVALUATION OF THE ANTI-INFLAMMATORY EFFECT OF METHANOLIC EXTRACT OF LOTUS (*Nelumbo nucifera)* SEEDS

5.2.1. Effect on carrageenin induced paw volume

Carrageenin induced paw oedema was taken as a prototype for acute anti-inflammatory model. The present study proved that the methanolic extract of white and red lotus seeds have significant anti-inflammatory effect in carrageenin induced acute inflammation. Both types showed significant reduction of paw volume on the second and third hour after carrageenin injection .The higher dose groups (Group IV

and VI-600mg/kg) showed more activity than corresponding lower doses (Group III and V-400mg/kg) in both phases of inflammation.

The anti-inflammatory activity of *N.nucifera* rhizome was reported by Mukherjee *et al.* 1997. They observed that the methanolic extract of *Nelumbo nucifera* rhizome as well as the steroidal triterpenoid (betulinic acid) isolated from it, possessed significant anti-inflammatory activity in carrageenin and 5-hydroxytryptamine induced rat paw oedema models. The effects produced were comparable to that of phenylbutazone and dexamethasone.

Xiao *et al* .(2005) reported the antioxidant and anti-inflammatory activity of isoliensinine, a bisbenzyl alkaloid extracted from the seed embryo of lotus, on bleomycin induced pulmonary fibrosis. They observed a significant inhibitory effect on bleomycin induced pulmonary fibrosis, which could be attributed to its former properties.

The percentage inhibition of oedema by methanolic extract of red lotus seed at both the doses was increased upto 3 hours after carrageenin injection. However, the methanolic extract of white lotus seed at both the doses has not shown time dependant inhibition of oedema. However they showed higher inhibition of oedema from the 60 minutes itself.

The diclofenac, standard drug (Group II) has shown more significant anti-inflammatory activity in both the phases of inflammation induced by carrageenin than red lotus seed and white lotus seed extracts. The percentage inhibition of paw oedema observed in the diclofenac treated group was around 62 per cent. The highest inhibition noticed is 62.67per cent at two hours after carrageenin injection. This observation in the present study is in agreement with the observation obtained by Bothara *et al* . (1998), who got 67 .24 per cent inhibition of oedema with diclofenac sodium.

While comparing all lotus seed treatment groups, the white lotus seed @ 600 mg/kg (Group VI) had possessed more pronounced effects than others in both phases of carrageenin induced oedema. It exhibited 50.47, 47.76 and 47.21 per cent inhibition of oedema at first, second and third hour after carrageenin injection. It indicated that the higher dose group of white lotus seed extract is having more anti-inflammatory effect than others according to present study.

A significant reduction in percentage of oedema from first to three hour after carrageenin injection in the present study revealed that both phases of inflammatory reactions were affected by methanolic extract of lotus seed.

The carrageenin induced oedema is a biphasic response. The initial phase is attributable to the release of histamine, serotonin and kinin in the first hour after carrageenin injection a more pronounced second phase is attributable to the release of prostaglandin like substance in second to third hour after carrageenin injection (Vinegar *et al.*, 1969). Complement activity is also involved throughout the carrageenin induced inflammation (Giroud and Willoughby, 1970). So the anti-inflammatory effect of red and white lotus seed may be due to its anti-complement activity, inhibition of the synthesis, release or action of inflammatory mediators viz., histamine, serotonin and prostaglandin.

The inhibition of inflammatory mediators by flavonoids and glycocides were revealed by Singh and Pandey (1996b) when they studied the anti-inflammatory activity of *Pongamia pinnata* seed extract in rats. In lotus seed extract also the flavonoids and glycocides were detected which may contribute to the anti-inflammatory activity.

5.2.2. Effect on cyclooxygenase -2 enzyme inhibition:

In order to assess the anti-inflammatory property of lotus seeds, another more advanced assay based on cyclooxygenase-2 (COX-2) enzyme inhibition was done in spectrophotometer and ELISA. The cyclooxygenase -2 enzyme is believed to play a role in inflammation by the production of prostaglandin from arachidonic acid.

The recent finding of a second COX isoform (COX-2) provided the basis for the discovery of anti-inflammatory drugs with improved safety. COX-1 is expressed in most tissues and cells and is abundant in the GI tract, kidney, and platelets. Prostaglandins formed by this enzyme are important for normal physiological function in these tissues. The second isoform, COX-2, is prominently expressed in inflamed tissues, where it produces proinflammatory prostaglandins (Masferrer et al., 1994; Seibert et al., 1994) and to a lesser extent constitutively expressed in brain and kidney. This suggested that COX-2 could provide a well defined molecular target for rational drug development, with the hypothesis that specific inhibitors of this enzyme may achieve anti-inflammatory and analgesic efficacy without affecting production of physiological PGs (Needleman and Isakson, 1997).

Chan *et al.* (1999) reported that the carrageenin induced paw oedema was accompanied by the induction of COX-2 enzyme. The conventional non-steroidal anti-inflammatory drug, cyclooxygenase -2 inhibitors and prostaglandin monoclonal antibodies has shown anti-inflammatory activity in carrageenin induced paw oedema model.

Zhang *et al.* (1997) observed an elevation of COX-2 enzyme in carrageenin induced paw oedema model, with the concomitant increase in the prostaglandin production.

In the present study, for assay of COX-2 enzyme activities the carrageenin paw oedema model was followed. The results revealed the inhibition of cyclooxygense-2 enzyme by methanolic extracts of lotus seeds in a significant manner.

The higher dose groups showed more inhibition of paw oedema than its corresponding lower dose group. Both exhibited a dose dependant inhibition of paw oedema .The celecoxib, the specific COX-2 inhibitor showed more significant effect than others.

5.2.3. Effect on the serum transaminases (AST and ALT)

Both transaminases are widely distributed. The serum transaminases activity increases during damage to organs.

In the present study, a mild decrease in AST and ALT level was noticed in groups treated with higher dose (Group IV and VI) of red and white lotus seed, conversely a mild increase in level of both enzymes was observed in the groups treated with lower dose.(Group III and IV) of extracts. However the values were within the normal range.

The diclofenac treated group (Group II) showed a mild elevation of AST and ALT level in the present study. It may be reversible elevation of enzyme as observed by Tripathi (1999). He recorded a reversible elevation of serum aminotransferase activity occurred in patients treated with diclofenac sodium.

5.2.4.Effect of treatments on serum cholesterol

The serum cholesterol values in anti-inflammatory screening are presented in table 12. In the groups treated with lotus seed extract (Groups III, IV, V and VI) there was a significant decrease in the level of serum cholesterol than control as well

as well as diclofenac treated (Group I, II) suggesting hypolipedemic properties of lotus seed extracts.

In Chinese traditional medicine *Nelumbo nucifera* (rhizomes and seeds) was used as one of the medicinal plants for the treatment of hyperlipedemia (Cour *et al.*, 1995). It was noticed that the decoction of *N.nucifera* had a significant reducing effect on the serum triglyceride and cholesterol level.

5.2.5. Effect of treatments on haematological parameters.

The present study showed an insignificant decrease in total WBC count in all the groups, but the values were within the normal range. The results also revealed no significant variation in differential leukocyte count.

The normal range of leukocyte count in rats is 6000 to 18000 leukocytes per cu.mm. and the percentage of various cells as follows, neutrophils -24 to 47 per cent, lymphocytes- 47 to 73 per cent, monocytes -0 to 3 per cent and eosinophils- 0 to 7 per cent (Farris and Griffith, 1963).

Ramesh *et al.* (2001) also recorded similar observation when they studied the effect of diclofenac on haematological parameters in dogs which revealed that the diclofenac did not produce any significant variation in both total leukocyte count and differential leukocyte count.

5.3. EVALUATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF LOTUS (*Nelumbo nucifera)* SEEDS

The analgesic activity of *N.nucifera* was tested in the present study by two centrally mediated analgesic models like tail flick reaction and experimental neuropathy.

5.3.1. Effect on acute pain model-tail flick method:

The tail flick test is a complex response to an acute, non-inflammatory, nociceptive input and is considered as a good model for studying central anti-nociceptive activity.

A significant increase in the reaction time for tail flick method indicated the analgesic effect by red and white lotus seed and also elucidates the involvement of central mechanism in analgesic action. Analgesic effect mediated through central mechanism indicates the involvement of endogenous opioid peptides and biogenic amines like 5HT (Bensemana and Gascon, 1978; Glazer *et al.*, 1981).

In the present study diclofenac treated group (Group-II) showed a gradual increases in reaction time till the end of the observation, and it attained peak at 120 minutes. The increase in reaction time was gradual for all the lotus seed treated groups and it attained the peak at 90 minutes.

Velankar *et al.* (1998) also got peak analgesic action at 90 minutes post administration of NSAIDs like diclofenac, flubiprofen and nimusulide, when they administered different combinations of above drugs in tail flick analgesic model in rats.The analgesic activity of diclofenac has been traditionally related to the inhibition of prostaglandin synthesis (Menasse *et al.*, 1978), direct blockade of inflammatory sensitization by activation of NO-cGMP pathways (Tonussi and Ferreira, 1994) and involvement of opioid pathways in the central analgesic effect (Bjorkman, 1995).

In the present study, the diclofenac (Group-II) and red and white lotus seed extract treated groups (Groups III, IV, V and VI) showed a significant analgesic effect compared to that of control group (Group I), but the activity shown by the red and white lotus seed extracts were less than to that of diclofenac treated group. The

The methanolic extract of red and white lotus seed at both dose levels exerts a similar reaction time suggesting an increase in the dose from 400mg/kg to 600mg/kg body weight will not have significant influence in the analgesic activity.

Mukherjee *et al.* (1996) also reported the depression of pain response when methanolic extract of rhizomes from *Nelumbo nucifera* in doses of 200mg/kg, 300mg/kg and 400mg/kg, i.p. was administered in mice.

The flavonoids were reported to have analgesic activity (Hossinzadeh *et al.*, 2002) by reduced availability of prostaglandins. Hence the presence of flavonoids in the methanolic extract of red and white lotus seed might probably also contribute for the analgesic activity.

5.3.2. Effect on the serum transaminases (AST and ALT)

In the present study, serum AST and AST level showed a relative and insignificant reduction in higher dose group (IV and VI) treated with methanolic extract of red and white lotus seed. Whereas, the lower dose groups (III and V) showed an increase in the enzyme level. However, the values were within the normal range. The diclofenac treated group (Group-II) showed relative and insignificant elevation of AST and ALT level in the present study as in anti-inflammatory analysis also This may be a reversible elevation of serum enzyme activity

5.3.3. Effect of treatments on serum cholesterol

The value of serum cholesterol for analgesic screening is presented in table 21. There was a significant decrease in the level of serum cholesterol towards normal level in all lotus seed extract treated groups (Groups III, IV, V and VI).

Cour *et al.* (1995) also noticed a reduction in serum triglyceride and cholesterol level when they administered decoction of *N.nucifera* in rats.

5.3.4. Effect of treatments on haematological parameters.

The total leukocyte count and differential leukocyte counts were found within the normal range in all the groups including diclofenac treated groups.

5.3.5. Effect on chronic pain model –Experimental neuropathy model:

Partial injury to somatosensory nerves sometimes causes causalgia in humans. Causalgia is characterized by spontaneous burning pain combined with hyperalgesia and allodynia and usually follows an incomplete peripheral nerve injury. Allodynia, a pain sensation due to normally innocuous stimulation, is a particularly troublesome symptom in patients with peripheral nerve injury. Bennett and Xie (1988) described a peripheral neuropathy due to nerve constriction in the rat that produces disorders of pain sensation like those seen in man.

In the present study by neuropathy model, a non-significant reduction in foot withdrawal reflex was noticed in all the lotus seed treated groups except group VI, which showed a significant reduction, indicating the analgesic activity of white lotus seeds at a dose above 600mg/kg even though it was not comparable to diclofenac (Group II).

In inflammatory and neuropathic pain models the involvement of centrally mediated nociception and upregulation of COX-2 enzyme were noted. The administration of ketorolac a mixed COX-1 and COX-2 inhibitor reduces the allodynia induced in neuropathic pain models (Ma and Eisenach, 2003). Also, the prostaglandins play a significant role in different phases of inflammatory pain reactions. Prostaglandins elicit pain by direct stimulation of sensory nerves and also sensitize sensory nerves to other pain provoking stimuli (Campbell, 1991). Hence the analgesic effect of lotus seed extracts may be due to prevention of prostaglandin production through cyclooxygenase enzyme inhibition.

From the results of the present study it can be inferred that methanolic extract of red and white lotus seeds is an effective anti-inflammatory and analgesic agents. While comparing the lotus seed extracts, the white lotus seed @ 600 mg/kg body weight revealed higher effect than others.

Summary

6. SUMMARY

The present study was undertaken to assess the anti-inflammatory and analgesic effect of red and white lotus (*Nelumbo nucifera)* seeds in rats. Diclofenac potassium was used as a standard drug for both anti-inflammatory and analgesic screening. The celecoxib was taken as a standard drug for the cyclooxygenase –2 inhibition assays.

Anti-inflammatory effect was studied in carrageenin induced hind paw oedema model of acute inflammation. The analgesic action in acute and chronic pain models was studied by tail flick and experimental neuropathy model respectively, were used to assess the analgesic effect of the treatment groups

Forty eight adult Sprague Dawley rats of 150-200 g body weight were divided into six groups of eight each and maintained for the anti-inflammatory study. All groups were fed with normal feed ad libitum. Five per cent gum acacia were fed to Group I and II in which the Group II received the diclofenac potassium @ 3mg/kg on the 7^{th} day before carrageenin administration .The methanolic extract of *N.nucifera* seeds of red and white varieties @ 400mg/kg and 600mg/kg were fed to group III, IV, V and VI respectively, for 7 days.

Similarly six other groups were maintained for analgesic screening in both tail flick and experimental neuropathy model. A dose of 400mg/kg and 600mg/kg body weight of red and white lotus seed was administered to the groups III, IV, V and VI respectively, for 7 days in tail flick method. In experimental neuropathy model, the dose schedule started on day 8^{th} and has given upto 14^{th} day.

In anti-inflammatory screening, both red and white lotus seeds showed significant inhibition of carrageenin induced oedema at the first and second phase. The white lotus seed treated with higher dose (Group VI-600mg/kg) exhibited more activity than others.

In case of analgesic screening, all the treated groups showed significant effect in tail flick method. A lower effect in experimental neuropathy model

indicating that the lotus seed is more effective in acute pain model rather than chronic pain model.

Different parameters like cyclooxygenase –2 enzyme inhibition assays, serum level of enzymes like ALT, AST and total cholesterol and haematological parameters like total and differential leukocyte count were recorded in anti-inflammatory screening on 7th day of the experiment. In analgesic study, the tail flick method was used. Biochemical parameters like serum ALT, AST, total cholesterol and haematological parameters total and differential leukocyte count were noted in analgesic study also.

The cyclooxygenase-2 (COX-2) enzyme inhibition assay was carried out by ELISA and spectrophotometer in order to find the more specific anti-inflammatory action of lotus seed. In this assay the celecoxib, COX-2 inhibitor was used as a standard drug. The results showed that the red and white lotus seed methanolic extracts possessed significant COX-2 enzyme inhibition .The effect were lesser than the celecoxib, but significantly higher than the control. The rats fed with white lotus seed extract higher dose (Group VI-600mg/kg) have shown maximum inhibition of cyclooxygenase -2 enzyme over others. The red lotus seed, lower dose (Group III-400mg/kg) showed lesser effect than the corresponding higher dose group (Group IV-600mg/kg). The white lotus seed, higher dose (Group VI–600mg/kg) group had exhibited more potent inhibition on COX-2 enzyme than its lower dose (Group V–400mg/kg). The results indicated that the probable anti-inflammatory action of these lotus seed extracts might be due to COX-2 enzyme inhibition, which is essential for prostaglandin production.

While analysing the serum biochemical parameters, the methanolic extracts of lotus seeds showed reduction of serum cholesterol. It was believed that this effect might be due to its hypolipedemic action.

The serum transaminase enzymes like AST and ALT level were within the normal level in both anti-inflammatory and analgesic studies. The methanolic extract

of lotus seed, lower dose (Group III and V-400mg/kg) showed increase in the enzyme activity whereas the higher dose (Group IV and VI-600mg/kg) exhibited reduction in activity.

Even though there was a slight variation in neutrophil count, all the values were within the normal range. The total leukocyte counts showed reduction in both anti-inflammatory and analgesic studies, however, the value were within the normal range.

From the present study it can be concluded that the methanolic extract of lotus seeds have anti-inflammatory and analgesic effect in rats. While further analysing the probable mechanism of anti-inflammatory action, it is revealed that it inhibits cyclooxygenase-2 enzyme.

References

REFERENCES

Agarwal, R.B. and Rangari, V.D. 2003. Anti-inflammatory and antiarthritic activities of lupeol and 19α-H- lupeol isolated from *Strobilanthus callosus* and *Strobilanthus ixiocphala* roots. *Indian J. Pharmacol.* 35:384 - 387

Ahamed, K.N., Kumar, V., Raja, S., Mukherjee, K. and Mukherjee, P.K. 2005. Anti-nociceptive and anti-inflammatory activity of *Araucaria bidwillii* hook. *Iranian J.Pharmacol.Toxicol.* 4:105-109

Allain, C.C., Poon, L.S. and Chan, C.S.G. 1974. Enzymatic determination of serum cholesterol. *Cli. Chem.* 20: 470-475

Amour, D.F.E. and Smith, D.L. 1941. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* 72:74-79

Anderson,G.D., Hauser, S.D., McGarity, L.K., Bremer, M.E., Isakson, P.C. and Gregory, A.S. 1996. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. *J. Cli.Invest.* 97(11): 2672–2679

Arulmozhi, D.K., Veeranjaneyalu, A., Bodhankar, S.L. and Aruva, S.K. 2005.Pharmacological investigations of *Sapindus trifoliatus* in various *invitro* and *invivo* models of inflammation. *Indian J.Pharmacol.* 37(2): 96-102

Asmawi, M.Z., Ankanranta, H., Moilanen, E. and Vapaatalo, H. 1993. Anti-inflammatory activities of *Emblica officinalis* Gaertn leaf extract. *J.Pharma. Pharmacol.* 45:581-584.

Bennett, G.J. and Xie, Y.K. 1988. A peripheral neuropathy in the rat that produces disorders of pain sensation like those seen in man. *Pain* 33:87-108

Bensemana, D. and Gascon, A.L. 1978. Relationship between analgesia and turnover of brain biogenic amines. *Can. J. Physiol. Pharmacol.* 56: 721-730

Bhoite, P.Y., Somkumar, A.P., Pawar, S.D. and Gatne, M. 2005. Comparative assessment of analgesic activity of *Pongamia pinnata* and aspirin in rats. *Vet. J.Pharmacol.Toxicol.*4(1): 36-38

*Bjorkman, R. 1995. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. *Acta Anaesthesiol. Scand.* 39: 1-44

Bingham, S., Berwick, P.J., Bountra, C., Brown, T., Campbell, I.B., Clayton, N., Collins, S.D., Davey, P.T., Goodland, H.Gray, N., Haslam, C., Hatcher, J.P., Hunter, J.A.J., Lucas, F., Murkitt, G., Naylor, A., Pickup, E., Sergeant, B., Summerfield, S.G., Stevans, A., Strattn, S.C. and Wiseman, J. 2005. The cyclooxygenase-2 inhibitor GW406381X (2-(4-ethoxyphenyl)-3-(4-(methylsulfonyl) phenyl)-pyrazolo (1,5-b) pyridazine where X denotes the free base) is effective in animal models of neuropathic pain and central sensitization. *J.Pharm.Exp.Ther.* 312: 1162-1169

Biswal, S., Das, M.C. and Nayak, P. 2003. Anti-nociceptive activity of seeds of *Trigonella foenum graceum* in rats. *Indian J.Physiol. Pharmacol.* 47(4): 479-480

Bose, A., Saravanan, V.S., Karunanidhi ,N. and Gupta, J.K.2004. Analgesic and locomotor activity of extracts of *Cleome rutidosperma. Indian J.Pharm.Sci.* 66(6): 795-797

Bothara, K.G., Kadan, S.S. and Shivram, V.S. 1998. Synthesis and pharmacological screening of novel anti-inflammatory agents. *Indian Drugs.* 35:372-376

Campbell, W.B. 1991. Lipid-derived autacoids: Eicosanoids and platelet activating factor. Goodman and Gilman's *The Pharmacological Basis of Therapeutics*.(Eds. Gilman, A.G., Rall, T.W., Neis, A.S. and Taylor, P.). Eighth edition.Pergamon Press, New York, pp. 607-608

Chan, C.C., Boyce, S., Brideau, C., Charleson. S., Cromlish, W., Ethier, D., Evans. J., Hutchinson, F.A.W., Forrest, M.J. and Gauthier, J.Y. 1999. Rofecoxib [Vioxx, MK-0966; 4-(4_-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor: Pharmacological and biochemical profiles. *J. Pharmacol. Exp. Ther.* 290: 551–560.

Chandra, D.2001. Analgesic effect of aqueous and alcoholic extracts of *Madhuka longifolia*. *Indian J.Pharmacol.* 33: 108-111

Chapman, V., Suzuki, R. and Dickson, A.H. 1998. Electrophysiological characterization of spinal neuronal response properties in anesthetized rats after ligation of spinal nerves L5-L6.*J.Physiology.* 507 (3): 881-894

Chatpalliwar, V.A., Joharapurkar, A.A., Wanjari, M.M., Chakraborty, R.R. and Kharkar, V.T.2003.Antinociceptive activity of *Martynia diandra* Glox. *Indian J. Pharmacol.* 35: 320-321

Chattopadhyay, R.N., Chattopadhyay, R., Roy, S. and Moitra, S.K. 1986. A simple method for plethysmometric measurement of paw volume of small laboratory animals in the evaluation of anti-inflammatory effect. *Bull. Calcutta School Trop. Med.* 34: 5-8

Chaurasia, S., Tripathi, P. and Tripathi, Y.B.1995. Anti-oxidant and anti-inflammatory property of Sandhika: A compound herbal drug. *Indian J. Exp. Biol.* 33: 428-432.

Copeland, A., Williams, J.M. and John, G.1994. Mechanism of selective inhibition of the inducible isoform of PGGH synthase. *Proc.Natl.Acad.Sci.* 91:11202-11206

Cour, L.B., Molgaard, P. and Yi, Z. 1995. Traditional Chinese medicine in treatment of hyperlipidaemia. *J.Ethnopharm.*46 (2): 125-129

*Dandiya, P.C. and Menon, M.K. 1963. Studies on central nervous system depressants (iii). *Arch. Intern. Pharmacodynamic.* 141: 223-227

Davies,O.L., Raventos, J. and Walpole, A.L.1946. A method for evaluation of analgesic activity using rats. *Br. J. Pharmacol.* 1: 255-260

Dharamsiri, M.G., Jayagody, J.R.A.C., Galhena, G., Liyanage, S.S.P. and Ratnasooriya, W.D. 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo. J. Ethnopharmacol.* 87:199-206

Farris, J.E and Griffith, J.Q. 1963. The rat in laboratory investigation. Hafner Publisher, New york.2 nd Edition.p.411

Giroud, J.P. and Willoughby, D.A. 1970. The interrelations of complement and a prostaglandin-like substance in acute inflammation. *J. Path.* 101: 241-245

Glazer, E.J., Steinbush, H., Verhofstad, A. and Basbaum, A. 1981. Serotonin neurons in nucleus raphe dorsalis and paragigantocellularis of the cat contain enkephalin. *J. Physiol.* 77: 241-245

Gupta, M., Mazumdar, U.K., Mukhopadhyay, R.K. and Sarkar, S. 1996 Antisteroidogenic effect of seed extract of *N.nucifera* in the testis and ovary of the rat. *Indian J. Pharm. Sci.*58 (6): 236-242

Gupta, M., Mazumder, U.K., Kumar, R.S. and Siva Kumar, T. 2003. Studies on Anti-inflammatory, analgesic and antipyretic properties of methanol extract of *Caesalpinia bonducella* leave in experimental animal models. *Iranian J. Pharmacol. Toxicol.* 2:30-34

Gupta, R.K. and Tandon, V.R.2004. Anti-nociceptive effect of *Vitex negundo* Linn leaf extract. *Indian J.Pharmacol.*36 (1): 54.

Harborne, J.B.1991. Phytochemical methods. Guide to modern techniques of plant analysis. Second edition. Chapman and Hall, India. 653 p

*Hossinzadeh, H., Ramezani, M., Fadishei, M. and Mahmoudi, M. 2002. Anti-inflammatory and acute toxicity effects of *Zhumeria majdae* extracts in mice and rats. *Phyto Med.* 9: 135-141

Higgs, G.A., Mccall, E. and Youlten, L.J.F. 1975. A Chemostatic role for prostaglandins from polymorphonuclear leukocytes during phagocytosis. *Br. J. Pharmacol.* 53: 539-546

Hukkeri, I., Patil, B.M., Jalalpure, S.S. and Ali, A., 2001. Anti-inflammatory activity of various extracts of *Pergularia Extensa. Indian J.Pharm. Sci.* 63 (5): 429-431

Islam, C.N., Bandyopadhyay, S.K., Banerjee, S.N., Dutta .K., Das. P.C. 1995. Preliminary studies on the anti-inflammatory effects of *Swertia chirata* in albino rats. *Indian J. Pharmacol* .27:37-39

Ismail, T.S., Gopalakrishnan, S. and Begum, V.H. 1997. Biochemical modes of action of *Gmelina asiatica* in inflammation. *Indian J. Pharmacol.* 29: 306-309

Jana, U., Chattopadhyay, R.N. and Shaw, B.P. 1999. Preliminary studies on anti-inflammatory activity of *Zingiber officinale* Rosc, *Vitex negundo* Linn, *and Tinospora cordifolia* (Willid) miers in albino rats. *Indian J. Pharmacol.* 31: 232-233

Jalalpure, S.S., Habbu, P.V., Patil, M.B., Kulkarni, R.V., Simpi, C.C., Patil, C.C., 2002. Analgesic and antipyretic activity of *Pergularia extensa* in rats. *Indian J. Pharm. Sci.* 64(5): 493-495

Jiri, P.2003. Biologically active pentacyclic triterpenes and their current medicine significance. *J. Appl. Biomed.*1 : 7-12

Karunakar, N., Pillai, K.K., Husain, S.Z. and Rao, M. 1997. Investigations of anti-inflammatory activity of Jigrine. *Indian J. Physiol. Pharmacol.* 41: 134-138

Kavimani, S., Karpagam, R.I. and Jaykar, B.1997. Anti-inflammatory activity of volatile oil of *Psidium guajava. Indian J.Pharm.Sci.*59(3): 142-144

Khanna, N., Goswami, M., Sen, P. and Ray, A. 1995. Anti-nociceptive action of *Azadirachta indica* (neem) in mice: Possible mechanisms involved. *Indian J. Exp. Biol.* 33: 848-850

Kim, K.J. and Chung, J.M. 1997.Comparison of three rodents neuropathic pain models. *Exp. Brain Res.*113(2):200-206

Kirtikar, K.R. and Basu, B.D.1975. Indian Medicinal Plants. Second edition. Periodical Experts, Delhi, pp116-120.

Krishnamoorthy, G., Kavimani, S. and Loganathan.C.1998. Anti-inflammatory effect of the essential oil of *Cymbopogon martini. Indian J. Pharm. Sci.* 60(2): 114-115

Krishnaveni, M., Suja, V., Vasanth, S. and Shyamaladevi, C.S. 1997. Anti-inflammatory and analgesic action of 4', 5, 6 Trihydroxy 3', 7 – dimethoxy flavone from *Vicoa indica* DC. *Indian J. Pharmacol.* 29: 178-181

Kuoy, C., Lin, Y.L., Liu, P. and Tsai, W.J. 2005. Herpes simplex virus type 1 propagation in HeLa cells interrupted by *Nelumbo nucifera*. *J. Biomed. Sci.* 12:1021–1034

Lalitha, K,G., Sethuraman, M.G. and Rajkapoor, B. 2003. Anti-inflammatory effect of *Sarcostemma brevistgma* in rats. *Indian J.Pharm.Sci.*65(2): 210-212

Ling, Z.Q., Xie, B.J. and Yang, E.L. 2005. Isolation, characterization and determination of antioxidative activity of oligomeric procyanidins from the seedpod of *Nelumbo nucifera* gaertn. *J. Agric. Food Chem.* 53 (7): 2441-2445

Liu, C.P., Tsai, W.J., Lin, Y.L., Liao, J.F., Chen, C.H., Kuo, Y.C., 2004. The extracts from *Nelumbo nucifera* suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. *Life Sci.* 75(6): 699-716.

Ma, W. and Eisenach , J. C. 2003. Cyclooxygenase- 2 in infiltrating inflammatory cells in injured nerve is universally up-regulated following various types of peripheral nerve injury. *Neu. Sci.* 121:691–704

Makwana , H.G., Ravishankar , B., Shukla , V.J., Nair . R.B., Vijayan , N.P., Sasikala,C.K ., Saraswathy , V.N. and Bhatt , S.V. 1994. General pharmacology of *Vitex leucoxylon* Linn leaves. *Indian J. Physiol. Pharmacol.* 38: 95-100

Masferrer, J.L., Zweifel, B.S., Manning, P.T., Hauser, S.D., Leahy, K.M., Smith, W.G., Isakson, P.C. and Seibert, K. 1994 . Selective inhibition of inducible

cyclooxygenase 2 *in vivo* is anti-inflammatory and nonulcerogenic. *Proc. Natl. Acad. Sci.* 91:3228–3232

Mazumder, U.K., Gupta, M., Pramanick, G., Muchopadhyay, R.K. and Sarkar, S. 1992. Antifertility activity of seed of *Nelumbo nucifera* in mice. *Indian J. Exp. Biol.* 30(6): 533-534

McNeely, W. and Goa, K.L. 1999. Diclofenac potassium in migraine *Drugs.* 57(6): 991.

Menasse , R., Hedwell , P., Kraetz , J., Pericin, C., Riesterer, L., Sallman, A., Ziel, R. and Jaques, R. 1978. Pharmacological properties of diclofenac sodium and its metabolites. *Scand. J. Rheumatol.* 22: 5-16

Mitra, R., Mehrotra, S., Kapoor, L.D. 1973. Medicinal Plants. *Indian J.Pharm.* 35:207

Mruthyunjayaswamy, B.H.M., Rudresh, K., Swamy, H.K.S., Badami, S.M., Hiremath, S.P.1998. Anti-inflammatory activity of alcohol extract of *Justica procumbens* . *Indian J. Pharm. Sci.* 60(3). 173-175

Mujamdar, A.M., Naik, D.G., Dandge, C.N. and Puntambekar, H.M. 2000. Anti-inflammatory activity of *Curcuma amada* in albino rats. *Indian J.Pharmacol.*32:375-377

Mukherjee, P.K., Das, J., Bala, R., Saha, K., Pal, M. and Saha, B.P. 1995. Antidiarrhoal evaluation of *N.nucifera* rhizome extract. *Indian J.Pharmacol.* 27(4): 262- 264

Mukherjee, P.K., Giri, S.N., Saha, K., Pal, M., Saha, B.P.1995a. Antifungal screening of *N.nucifera* rhizome extract. *Indian J. Microbio.* 35(4): 327-330

Mukherjee, P.K., Balasubramanian, R., Pal, M. and Saha, B.P. 1996. Studies on psycho-pharmacological effects of *N.nucifera* rhizome extract. *J. Ethnopharmacol.* 54: 63-67

Mukherjee, P.K., Das, J., Saha, K., Giri, S.N., Pal, M. and Saha, B.P.1996a. Antipyretic activity of *N.nucifera* rhizome extract. *Indian J. Exp. Biol.* 34: 275-276

Mukherjee, P.K., Saha, K., Das, J., Pal, M., Saha, B.P. 1997. Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera. Planta Med.* 63(4): 367-369

Mukherjee, P.K., Saha, K., Pal, M., Saha, B.P. 1997a. Effect of *Nelumbo nucifera* rhizome extract on blood sugar level in rats. *J. Ethnopharmacol.* 58(3):207-213

Mutalik, S., Paridhavi, K., Mallikaruna Rao, C. and Udupa, N. 2003. Antipyretic and analgesic effect of leaves of *Solanum melongana* linn. in rodents. *Indian J. Pharmacol*. 35:312- 315

Nantel, E., Denis, D., Gordan, R., Northey, A., Lirino, M., Mettters, K.M. and Chan.C.C.1999.Distribution and regulation of COX-2 in carrageenin induced inflammation . *Br. J. Pharmacol.* 128: 853-859

*Needleman, P. and Isakson, P.C. 1997. The discovery and function of COX-2. *J. Rheumatol.*24: 6–8.

Pal, S.C., Nirmals, A., Borhade, P.S., Pawar, C., Kshirsagar, S. and Atpade, S. 2005. Anti-inflammatory activities of the various extracts of leaves of *Garcinia xanthchymus. Indian. J. Pharm. Sci* .67(3):394-395

Pandey, B.L. and Das, P.K. 1989. Immunopharmacological studies on *Picrorhiza Kurroa* cellular mechanisms of anti-inflammatory action. *Indian J. Physiol. Pharmacol.* 33(1): 28-30

Pendse, V.K., Dadhich, A.P., Mathur, P.N., Bal, M.S. and Madam, B.R. 1977. Anti-inflammatory, immunosuppressive and some related pharmacological actions of the water extract of Neem Giloe (*Tinospora cordifolia)*: A preliminary report. *Indian J. Pharmacol.* 9: 221-224

Plaza, A.M., Plaza, P., Maciejewski, R., Czuczwar, M. and Przesmycki, K. 2004. Effect of topiramate on mechanical allodynia in neuropathic pain model in rats: *Pol. J. Pharmacol.* 56: 275–278.

Purnima, A., Prasanna, G.S. and Mathuram, V. 2006. Analgesic and anti-inflammatory activities of the chloroform extracts of *Trichilia connaroides*. *Indian J. Pharm. Sci.* 68(2): 231-233

Rai, S., Wahile, A., Mukherjee, K., Saha, B.P. and Mukherjee, P.K. 2006. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. *J. Ethnopharmacol.* 104 (3): 322-7

Rajnarayana, K., Reddy, M.S., Chaluvadi, M.R. and Krishna, D.R. 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J. Pharmacol.* 33: 2-16

Ramachandran, S., Anbu, J., Saravanan, M., Gnanasam, K.S. and Sridhar, S.K. 2002. Antioxidant and anti-inflammatory properties of *Citrus cinensis* peel extract. *Indian J. Pharm. Sci.* 64(1): 66-67

Ramesh, M., Roy., Rao, A.A.V.N., Prabhakar, M.C., Rao, C., Muralidhar, N. and Reddy, B.M.1998. Anti-nociceptive and Anti-inflammatory activity of

flavonoid isolated from *Caramulla attenuata. J.Ethnopharmacol.* 62:63-66

Ramesh, N., Jayakumar, K., Hornegowda and Narayana, K. 2001. Effect of diclofenac and nimusulide on hematology in dogs. *Indian J. Ani. Sci.*71:221-223

Rao, K.S. and Mishra, S.H. 1997. Anti-inflammatory and hepatoprotective activities of *Sida rhombifolia* Linn. *Indian J. Pharmacol.* 29: 110-116

Reitman, S. and Frankel, S. 1957. Colorimetric determination of serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activity. *Am. J. Clin. Pathol.* 28: 56-63

Roberts, J. and Marrow, J.D.2001. Analgesic and Antipyretic and Anti-inflammatory agents and drugs employed in the treatment of gout. Goodman and Gilman's. The Pharmacological Basics of Therapeutics (Eds.Hardman, J.G., Limbird, L.E. and Gilman, A.G.) Tenth Edition. McGraw – Hill Medical Publishing Division, New york , pp 687-727

Sanjay, K., Bandana, R. and Shantilata, P.2001. Anti-inflammatory effect of neem seed oil on albino rats. *Indian J. Pharmacol.* 31: 303

Sankar, V., Kalirajan, R., Sales, S.V. and Raghuraman, S. 2001. Anti-inflammatory activity of *Elephantabus scaber* in albino rats. *Indian J. Pharm. Sci.* 63(6): 523-525

Schalm, O.W., Jain, N.C. and Carroll, E.J.1986. Veterinary Hematology. 4th edition. Lea and Febiger, Philadelphia .P: 45-48

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L. and Isakson, P. 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc. Natl. Acad. Sci.* 91:12013–12017.

Selvam, C. and Jashak, S.M. 2004. A cyclooxygenase inhibitory biflavonoid from the seeds of *Semecarpus anacardium*. *J.Ethnopharmacol.* 95:209-212

Shirwaikar, A.and Somashekar, A..P.2003. Anti-inflammatory activity and free radical scavenging studies of *Aristolochia bracteolata* lann. *Indian J.Pharm.Sci.* 65(1): 67-69

Sheeja, E., Edwin, E., Dhanbal, S.P. and Suresh, B.2005. Anti-inflammatory activity of the leaves of *Nothapodytes foetida* in rodents. *Indian J. Pharm. Sci.* 67(2): 251-253

Shu, X., Gao, Z.H. and Yang, X. 2006. Anti-inflammatory and antinociceptive activities of *Smilax china* aqueous extract. *J.Ethnopharmacol.* 103:327-332

Singh, B. and Jaggi, R.K. 2003. Anti-inflammatory activity of *Ocimum sanctum* and its cultures. *Indian J. Pharm. Sci.* 65(4): 425-427

Singh, S., Majumdar, D.K. and Yadav, M.R. 1996. Chemical and pharmacological studies on fixed oil of *Ocimum sanctum*. *Indian J. Exp. Biol.* 34: 1212- 1215

Singh, R.K., Joshi, V.K., Goel, R.K., Gambhir, S.S. and Acharya, S.B. 1996a. Pharmacological actions of *Pongamia pinnata* seeds –A preliminary study. *Indian J. Exp. Biol.* 34: 1204-1207

Singh, S., Pandey, B.L. 1996b. Anti-inflammatory activity of seed extracts of *Pongamia pinnata* in rat. *Indian J. Physiol . Pharmacol.*40: 355-358.

Singh, R.K., Nath, G. and Acharya, S.B. 1997. Pharmacological actions of *Pongamia pinnata* roots in albino rats. *Indian J. Exp. Biol.* 35: 831-836

Singh, R.K., Nath, G., Goel, R.K. and Bhattacharya, S.K. 1998. Pharmacological actions of *Abides pindrow* Royle leaf. *Indian J. Exp. Biol.* 36: 187-191

Singh, S.1999. Mechanism of action of anti-inflammatory effect of fixed oil of *Ocimum basilicum* linn. *Indian J. Exp. Biol.*37: 248-252

Sinha, S., Mukherjee, P.K., Mukherjee, K., Pal, M., Mandal, S.C.and Saha, B.P.2000. Evaluation of antipyretic potencial of *Nelumbo nucifera* stalk extract. *Phytother. Res.* P:424-425

Smith, O.B., Neill, O.A.N. and Perlin, A.S.1955. Discovery of carrageenin. *Can.J.Chem.*33, 1352.

Snedecor, G.W. and Cochran, W.G. 1985. *Statistical Methods.* Eighth edition. Oxford and IBM publishing Company, Calcutta, p. 584

Sohn, D.H., Kim, Y.C., Oh, S.H., Park, E.J., Li, X. and Lee, B.H. 2003. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera.* 2003. *Phytomedicine* .10(2-3): 165-169

Tandan, S.K., Chandra, S., Tripathi, H.C. and Lal, J. 1994. Pharmacological effects of *Ageratum cryzoides* roots. *Indian J.Pharmacol.* 56(5): 182-183

Tandan, S.K., Chandra, S., Gupta, S.and Lal, J.1997. Analgesic and anti-inflammatory effects of *Hedychium spicatum Indian J.Pharm.Sci.* (3): 148-149

Telang, R.S., Chatterjee, S. and Varshneya, C. 1999. Studies on analgesic and anti-inflammatory activities of *Vitex negundo* Linn. *Indian J. Pharmacol.*31: 363-366

Todd, P.A. and Sorkin, E.M. 1988. Diclofenac sodium, a repraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. *Drugs* 35: 244-285

*Tonussi, C.R. and Ferreira, S.H. 1994. Mechanism of diclofenac analgesia: Direct blockade of inflammatory sensitization. *Eur. J. Pharmacol.* 251: 173-179

Tripathi, K.D.1999.Essentials of medical pharmacology 4th edition .Jayvee brother's medical publishers. New Delhi. 935 p

Trivedi, C.P., Mode, N.T., Saran, R.K.and Rao, S.S.1986- Bronchodilator and Anti-inflammatory effect of glycocidal fraction of *Acacia farnesiana* . *Indian J.Physiol. Pharmacol.* 30(3):267-268

Velankar, S.S., Sharma, R.K. and Reddy, A.G. 1998. Comparative studies on analgesic activity of individual and combination of certain non steroidal anti-inflammatory drugs in rats. *Indian Vet. Med. J.* 22: 199-202

Venkataranganna, M.V., Gopumadhavan, S., Mitra, S.K. and Anturlikar, S.D. 2000. Anti-inflammatory activity of JCB, A polyherbal formulation. *Indian Drugs.* 37: 543-546

Vetrichelvan, T., Jegatheesan, M., Palaniappan, M.S., Murali, N.P. and Sasikumar, K. 2000. *Indian J. Pharm. Sci.* Diuretic and anti-inflammatory activities of *Aerva lanata* in rats. 300-302

Vetrichelvan, T. and Jegadeesan, M. 2002. Effect of alcoholic extract of *Acyranthus bidentata* blume on acute and sub acute inflammation. *Indian J.Pharmacol.* 34:115-118

Vinegar, R., Schreiber, W. and Hugo, R. 1969. Biphasic development of carrageenin oedema in rats. *J. Pharmacol. Exp. Ther.* 166: 96- 103

*Wang, L., Yen ,J.H., Liang , H.L. and Wu, M.J. 2003. Antioxidant effect of methanol extracts from lotus lotus plumule and blossom (*Nelumbo nucifera*) *J.Food Drug Ana.* 11(1): 60-66

Winter, C.A., Risley, E.A. and Nuss, G.W. 1962. Carrageenin induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111: 544-547

*Wu, S., Sonic, C.A.X., Shout, H., Hong, Z. and Pan,Y. 2004. Preparative counter current chromatography isolation of liens nine and its analogues from embryo of the seed of *Nelumbo nucifera.* using upright coil planet centrifugė with four multiplayer coils connected in the series. *J.Chromatogr. A.* 2004. 1041(1-2):153-162

Xiao, J.H., Zhang, J.H., Chen, H.A.L., Fang, E.X. and Wang, J.L. 2005. Inhibitory effects of isoliensinine on bleomycin induced pulmonary fibrosis in mice. *Planta medica* .71(3): 225-230

Zachariah, R., Nair, S.C.R. and Panicked, V.P.1994. Anti-inflammatory and antimitotic activities of lupeol isolated from the leaves of *Ixora coccinea*. *Indian J. Pharm. Sci.*13(1): 129-132

Zhang, Y., Shaffer, A., Portanova, J., Seibert.K and. Isakson, P.C. 1997. Inhibition of cyclooxygenase–2 rapidly reverses inflammatory hyperalgesia and PGE_2 production. *J. Pharm. Exp. Ther.* 283(3): 1069-1075

*Originals not consulted.

ANTI-INFLAMMATORY AND ANALGESIC ACTIONS OF RED AND WHITE LOTUS SEEDS (*Nelumbo nucifera*) IN ALBINO RATS

VIKRAMA CHAKRAVARTHI. P.

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Scienees
Kerala Agricultural University, Thrissur

2006

Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

ABSTRACT

Anti-inflammatory and analgesic effect of *Nelumbo nucifera* (lotus) seeds, red and white types were assessed and compared in albino rats. The carrageenin induced paw oedema was one of the acute inflammatory models followed in anti-inflammatory screening. The acute and chronic type pain models like tail flick method and experimental neuropathy model of analgesic activity, respectively, were adopted for analgesic screening. Diclofenac potassium at the rate of 3 mg/kg was used as a standard drug for both the studies. In cyclooxygenase-2 enzyme inhibition assays the celecoxib @ 10mg/kg was used as a standard drug. Both red and white types of lotus seeds at the dose rate of 400 mg/kg and 600 mg/kg were taken for the anti-inflammatory and analgesic studies.

Anti-inflammatory effect of red and white lotus seeds was found effective in all phases of carrageenin induced inflammation. The higher dose groups of lotus seed extracts were revealed more inhibition than their corresponding lower dose. While comparing all groups, the higher dose group of white lotus seed, exhibited more pronounced inhibition of paw oedema than others.

Analgesic effect was found significant in both acute and chronic analgesic models. The analgesic activity was more revealed in tail flick method compared to experimental neuropathy model.

The cyclooxygenase-2 (COX-2) enzyme inhibition assays in both ELISA and spectrophotometer showed significant effect than control. The percentage inhibition of COX-2 was more evidenced in both lotus seed extract of higher dose groups than its lower dose. However, the higher dose groups of white lotus seed exhibited more control over inhibition of COX-2 enzyme than others.

In case of biochemical parameters in both anti-inflammatory and analgesic screening the serum cholesterol level was found to be decreased in treatment groups when compared to control. Even though, there was a reduction of serum cholesterol level, all the values were within the normal range.

The rise in serum level of AST and ALT in both inflammation and analgesic models was noticed in lower dose treated group. Conversely, there was a reduction in level of enzyme was noticed in higher dose treated groups. However, the values were with in the normal level.

Both the studies showed the haematological parameters like total leukocyte and differential counts were within the normal range in all groups. even though a non-significant increase in neutrophil count than lymphocyte was noticed in carrageenin induced inflammation and tail flick method in rats.