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COMPARATIVE STUDY OF ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE EFFECT OF *Tinospora cordifolia* (Chittamruthu) AND *Vitex negundo* Linn. (Karinochi) IN RATS

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

Master of Veterinary Science

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DECLARATION

I hereby declare that this thesis entitled **"COMPARATIVE STUDY OF ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE EFFECT OF *Tinospora cordifolia* (Chittamruthu) AND *Vitex negundo* Linn. (Karinochi) IN 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.

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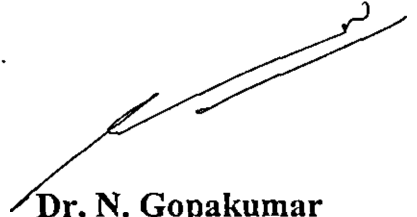

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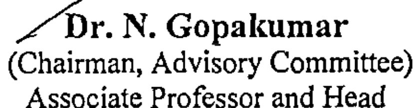

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CONTENTS

Sl. No.	*Title*	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	5
3	MATERIALS AND METHODS	18
4	RESULTS	32
5	DISCUSSION	67
6	SUMMARY	77
	REFERENCES	80
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1.	Effect of treatments on increase in paw volume after 1 hour of carrageenin induced paw oedema in rats, ml	33
2.	Effect of treatments on increase in paw volume after 2 hour of carrageenin induced paw oedema in rats, ml	34
3.	Effect of treatments on increase in paw volume after 3 hour of carrageenin induced paw oedema in rats, ml	34
4.	Percentage inhibition of paw oedema by treatments in carrageenin induced inflammation in rats, %	36
5.	Anti-nociceptive effect of different treatments on reaction time by tail flick test in rats	36
6.	Effect of treatments on adrenal gland wet weight in carrageenin induced inflammation in rats, g/100 g body weight	39
7.	Effect of treatments on adrenal gland wet weight in tail flick model of nociception in rats, g/100 g body weight	39
8.	Effect of treatments on adrenal ascorbic acid level in carrageenin induced inflammation in rats, mg/100 g of gland	40
9.	Effect of treatments on adrenal ascorbic acid level in tail flick method of nociception in rats, mg/100 g of gland	40
10.	Effect of treatments on adrenal cholesterol level in carrageenin induced inflammation in rats, g/100 g of gland	43
11.	Effect of treatments on adrenal cholesterol level in tail flick method of nociception in rats, g/100 g of gland	43
12.	Effect of treatments on serum cholesterol level in carrageenin induced inflammation in rats, mg/dl	46
13.	Effect of treatments on serum cholesterol level in tail flick method of nociception in rats, mg/dl	46

Table No.	Title	Page No.
14.	Effect of treatments on plasma thiobarbituric acid reacting substance in carrageenin induced inflammation in rats,nM/dl	48
15.	Effect of treatments on aspartate amino transferase level in carrageenin induced inflammation in rats, IU/L	50
16.	Effect of treatments on aspartate amino transferase level in tail flick method of nociception in rats, IU/L	50
17.	Effect of treatments on alanine amino transferase level in carrageenin induced inflammation in rats, IU/L	52
18.	Effect of treatments on alanine amino transferase level in tail flick method of nociception in rats, IU/Ll	52
19.	Effect of treatments on total leukocyte count in carrageenin induced inflammation in rats, /µl of blood	55
20.	Effect of treatments on total leukocyte count in tail flick method of nociception in rats, /µl of blood	55
21.	Effect of treatments on total erythrocyte count in carrageenin induced inflammation in rats, millions/µl of blood	57
22.	Effect of treatments on total erythrocyte count in tail flick model of nociception in rats, million/µl of blood	57
23.	Effect of treatments on haemoglobin concentration in carrageenin induced inflammation in rats, g/dl	60
24.	Effect of treatments on haemoglobin concentration in tail flick method of nociception in rats, g/dl	60
25.	Effect of treatments on differential leukocyte count in carrageenin induced inflammation in rats, %	62
26.	Effect of treatments on differential leukocyte count in tail flick method of nociception in rats, %	64

LIST OF FIGURES

Figure No.	Title	Page No.
1.	*Tinospora cordifolia*	19
2.	*Vitex negundo*	19
3.	Effect of treatments on mean increase of paw volume in carrageenin induced rat paw oedema	35
4.	Effect of treatments on percentage inhibition of carrageenin induced rat paw oedema	35
5.	Effect of treatments on reaction time by tail flick method in rats	37
6.	Effect of treatments on adrenal ascorbic acid in carrageenin induced inflammation in rats, mg/100 g of gland	41
7.	Effect of treatments on adrenal ascorbic acid in tail flick method of nociception in rats, mg/100 g of gland	41
8.	Effect of treatments on adrenal cholesterol in carrageenin induced inflammation in rats, g/100 g of gland	44
9.	Effect of treatments on adrenal cholesterol in tail flick method of nociception in rats, g/100 g of gland	44
10.	Effect of treatments on serum cholesterol in carrageenin induced inflammation in rats, mg/dl	47
11.	Effect of treatments on serum cholesterol in tail flick method of nociception in rats, mg/dl	47
12.	Effect of treatments on plasma thiobarbituric acid reacting substance in carrageenin induced inflammation in rats, nM/dl	48
13.	Effect of treatments on alanine amino transferase and aspartate amino transferase in carrageenin induced inflammation in rats, IU/L	53

Figure No.	Title	Page No.
14.	Effect of treatments on alanine amino transferase and aspartate amino transferase in tail flick method of nociception in rats, IU/L	53
15.	Effect of treatments on total leukocyte count in carrageenin induced inflammation in rats, /ml of blood	56
16.	Effect of treatments on total leukocyte count in tail flick method of nociception in rats, /µl of blood	56
17.	Effect of treatments on total erythrocyte count in carrageenin induced inflammation in rats, millions/µl of blood	58
18.	Effect of treatments on total erythrocyte count in tail flick method of nociception in rats, million/µl of blood	58
19.	Effect of treatments on haemoglobin concentration in carrageenin induced inflammation in rats, g/dl	61
20.	Effect of treatments on haemoglobin concentration in tail flick method of nociception in rats, g/dl	61
21.	Effect of treatments on differential leukocyte count in carrageenin induced inflammation in rats, %	65
22.	Effect of treatments on differential leukocyte count in tail flick method of nociception in rats, %	65

Introduction

1. INTRODUCTION

Inflammation is a defensive life preserving mechanism against noxious stimuli that ensure the survival of the individual.

When tissue injury is caused by a single finite event, such as mechanical trauma, chemical injury, burn or a single exposure to a non-replicating antigen the inflammatory and reparative processes progress smoothly from injury to healing. Thus inflammatory process as a whole is beneficial to restore the homeostatic mechanism of the affected tissue to its former normal and healthy state.

In contrast, when the injurious agent is self replicating like parasite neoplasm, rheumatism and many other, the ensuing inflammatory response becomes much more complex and lead to a crippling inflammatory disease.

Inflammation is manifested by rubor, calor, dolor, tumor and functiolaesia. This is brought about by a complex drama in the stage of damaged/injured tissue with some known and conjectured mediators of inflammation. It is the game of the research scientist to moderate/select suitable anti-inflammatory agents to demolish the inflammatory mediators and not the constitutive one.

The main mediators of inflammation are local hormones, macromolecules (proteolytic enzymes) and cellular (leukocyte) mediators. Local hormones which include the prostaglandins, adenylates (cyclic AMP, ADP), histamine, serotonin and kinins are the main targets of certain anti-inflammatory agents.

The anti-inflammatory action of non steroidal anti-inflammatory drugs (NSAIDs) rests in their ability to inhibit the activity of cyclooxygenase (Vane, 1971). The enzymatic activity of cyclooxygenase (COX) involves bis-

oxygenation of arachidonic acid to PGG_2, which results in prostanoid biosynthesis.

There are two forms of COX enzymes, COX -1 is the constitutive form expressed in platelets, kidney and gastro-intestinal tract, COX -2 on the other hand is generally found in inflammatory exudates and is also involved in nociception (Santos *et al.*, 1998).

Pain is a desirable sensation of noxious stimuli. It is accompanied by measurable, physiological responses such as reflex withdrawal movements, changes in vasomotor tone, blood pressure, heart rate, breathing and sweating. Pain depends on activation of discrete set of receptors and neuronal pathways. Nociceptive component of pain is mediated through receptors by pain producing substance, like Interleukin-2, prostaglandin and substance P.

NASIDs include aspirin, which irreversibly acetylates cyclooxygenase and several other classes of organic acids including propionic acid, acetic acid, oxicam and phenyl acetic acid (diclofenac) derivatives which compete with arachidonic acid at the active site of cyclooxygenase (Insel, 1996). Diclofenac has been found to be effective both as anti-inflammatory and analgesic agent (Todd and Sorkin, 1988).

Non-selective inhibition of both isoforms of COX by majority of the NSAIDs result in side effects like gastropathy or impairment of renal functions. Powerful immuno suppressants used to mitigate destruction of allogenic tissue graft often permits the establishment of tumors in formerly resistant host. More than 2000 times potent corticosteroids are not routinely used in clinics because of the gravity of their adverse reactions. Analysis of the cost factor and the feasibility for effectively suppressing inflammation by gold preparations and immuno- suppressive drugs entail the therapy to patient.

This directs the search of anti-inflammatory agents to the naturally occuring inflammatory regulators, which unravel the complexities of the marvelous homeostatic mechanism by adverse effects.

Therefore new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant based drugs used in the traditional medicine, have been paid great attention because they are cheap and have little side effects. According to WHO, still about 80 per cent of the world population rely mainly on plant based drugs.

From antiquity to modern times, man has been obsessed with a quest for rejuvenation and an innate desire to extend his life span. Ayurveda is an original holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation developed and perfected in India over 5000 years ago. Ayurveda (Ayur-life; Veda-knowledge) is the knowledge of healthy living and is not merely confined to the treatment of illness.

Modulation of inflammation either immune mediated or non-immunological by using various agents in order to alleviate the disease has been of interest over many years and concept of Rasayan in Ayurveda. Some Indian herbs have been shown to extend anti-inflammatory effect by modulating inflammatory mediators (Singh and Atal, 1986) and some are found to have analgesic effect or both.

Species of the genus *Tinospora* (Menispermaceae) are among the more widely employed medicinal plants throughout large part of Asia and Africa. The main species of *Tinospora* widely used in the Indian subcontinent was *T. cordifolia.* It is called guduci or Amrita in Sanskrit *T. cordifolia* is a glaborous climbing succulent herb. In the present day Ayurvedic medicine *T. cordifolia* heads the list of valuable bitter tonics and a starch of extract from the tuberous roots is often given to convalescents as it is said to be light and easily digested. It

is widely used in Ayurveda as vitaliser, anti-diabetic, hepatoprotective, antipyretic, antistress, anti-inflammatory and immunomodulatory agent (Nadkarni, 1976).

Vitex negundo Linn. (Verbenaceae) is a well known medicinal plant found in many parts of India. It is called Nirgundi in Sanskrit. The leaves of the plant are used as an indigenous drug in different diseases like rheumatic diseases, headache, catarrhal fever, cervical spondilitis and inflammatory disorders (Nadkarni, 1976).

None of the natural products may actually be a sufficient inflammalytic agent in the genuine pathophysiological context of inflammation but some of them may be likely candidates for playing the role of endogenous regulators of the acute inflammatory response.

So an attempt has been made to study the anti-inflammatory and anti-nociceptive effect of the two herbal agents *Tinospora cordifolia* and *Vitex negundo* and also the probable synergistic effects of their combinations.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PLANTS HAVING ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory agents from natural sources are said to be lacking the main side effects of the existing allopathic agents. Many studies on different plant extracts having anti-inflammatory effects were made.

Pendse *et al.* (1977) reported a significant anti-inflammatory and immunosuppressive effect of *Tinospora cordifolia* (Neem Giloe). Water extract of Neem Giloe (60 mg/100g) administered orally and intra peritoneally had shown significant reduction in carrageenin induced inflammation and inhibition of granular tissue response in granuloma pouch technique. A dose of 10 mg/100 g/day water extract had shown a significant reduction in antibody formation by Typhoid "H" antigen stimulus. This had a comparable effect with dexamethasone (0.1 mg/100g/ day).

Comparative study made by Gulati and Pandey (1982) revealed that aqueous extract of *T. cordifolia* stem possessed significant anti-inflammatory activity in male albino rats.

Anti-allergic properties of *T. cordifolia* were evaluated on histamine induced bronchospasm in guinea pigs, capillary permeability in mice and mast cell distruption in rats by Nayampalli *et al.* (1986). The aqueous extract had shown a decreased bronchospasm induced by five per cent histamine aerosol, decreased capillary permeability and reduced number of distrupted mast cells in guinea pigs, mice and rats respectively.

The ethanolic extract of the leaf of *Vitex leucoxylon* showed significant inhibition of carrageenin paw oedema and granulation tissue formation in rats (Makwana *et al.*, 1994).

Sandhika was an ayurvedic drug used in the treatment of rheumatoid arthritis. It consisted of water extract of plants namely *Commiphora mukul, Boswellia serrata, Strychnos nuxvomica,* and *Semecarpus anacardium.* Chaurasia *et al.* (1995) found a significant anti-inflammatory activity against carrageenin induced paw oedema and cotton pellet granuloma accompanied by no adverse effects.

Mengi and Deshpande (1995) evaluated the roots and leaves of *Butea frondosa* for ocular anti-inflammatory activity in rabbits. The results showed that the gel formation of *B. frondosa* leaves, prepared using a commercially available, pluronic F-127, reduced the intra-ocular pressure, decreased leucocytosis and miosis and was comparable to flubiproten gel.

The triglyceride fraction of oil of *Ocimum sanctum* (Tulsi) offered higher protection against carrageenin induced paw oedema in rats and acetic acid induced writhing in mice, as compared to the fixed oil (Singh *et al.*, 1996b).

Singh *et al.* (1996a) found that the Petroleum Ether Extract (PEE) and chloroform extract of the seeds of *Pongamia pinnata* showed potent acute anti-inflammatory effect. Whereas the aqueous suspension showed pro-inflammatory effects.

Singh and Pandey (1996) had shown the maximum anti-inflammatory effect in the bradykinin induced oedema model with the direct ethanol extract. Possible mechanism of action could be inhibition of prostaglandin synthesis and decreased capillary permeability. PEE and Acetone Extract (AE) inhibited histamine and 5-hydroxy tryptamine induced inflammation probably by their lipophilic constituents, preventing the early stages of inflammation. However, the fractions were not effective against Freund's adjuvant arthritic model indicating that the plant may not be effective in rheumatoid arthritis.

Ismail *et al.* (1997) reported that *Gmelina asiatica* root powder was effective in reducing carrageenin induced rat paw oedema model of acute

inflammation. In chronic inflammation against cotton pellet granuloma, it not only reduced the weight of the granuloma, but also the lipid peroxide and gamma-glutamyl transpeptidase (GGT) in the granuloma exudates. It also normalized serum albumin and serum acid and alkaline phosphatase levels. In liver there was a reduction of lipid peroxide, but no change in the GGT level.

Karunakar *et al.* (1997) reported that Jigrine, a polyherbal formulation possess anti-inflammatory activity by its anti-oxidant and membrane stabilizing effect against carrageenin induced acute inflammation but not against cotton pellet granuloma (subacute inflammation).

The methanolic extract of the aerial part of *Sida rhombifolia* (Atibala) showed significant oedema suppression activity in the carrageenin induced paw oedema model in rats. Probable mechanism of action may be due to its inhibitory effects on release of mediators of inflammation such as histamine, 5-hydroxytryptamine and bradykinin (Rao and Mishra, 1997).

Fixed oil of *O. sanctum* and linolenic acid were found to possess significant anti-inflammatory activity against PGE_2, leukotriene and arachidonic acid induced paw oedema. Singh and Majumdar (1997) showed that the anti-inflammatory activity of linolenic acid present in the fixed oil of *O sanctum* was probably due to blockade of both the cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism.

The water soluble parts of alcoholic extract of *Azadirachta indica* exerted significant anti-inflammatory activity in the cotton pellet granuloma assay in rats. Chattopadhyay (1998) observed reduced levels of various biochemical parameters like DNA, RNA, lipid peroxide, acid phosphatase and alkaline phosphatase in cotton pellet exudate, suggesting the mechanism of anti-inflammatory effect.

The dried stem of *T. cordifolia* produced significant anti-inflammatory effect in both acute and sub-acute models of inflammation. Jana *et al.* (1999)

found *T. cordifolia* was more effective than acetylsalicylic acid in acute inflammation. It is inferior to phenylbutazone in sub-acute inflammation.

Gaidhani *et al.* (2001) demonstrated that the alcoholic extract of *V. negundo* had significant anti-inflammatory effect in acute inflammation and the antagonist of the inflammatory mediators like promethazine, cimetidine, cyproheptadine and paracetamol also potentiated its effect.

Ethanolic extract of *V. negundo* exhibited significant anti-inflammatory effect in sub-acute inflammation. Promethazine and cyproheptadine given along with *V. negundo* significantly potentiated its anti-inflammatory activity. (Gaidhani *et al.*, 2002).

2.2 PLANTS HAVING ANTI-NOCICEPTIVE ACTIVITY

Makwana *et al.* (1994) reported that the ethanol extract and cold aqueous infusion of *Vitex leucoxylon* suppressed acetic acid writhing.

Azadirachta indica showed analgesic properties in mice. Khanna *et al.* (1995) showed that the pre-treatment with the opioid antagonist, naloxone and central noradrenaline depletor, N-2-Chloroethyl-N ethyl-2-bromobenzylamine attenuated the analgesia. The serotonin synthesis inhibitor, parachlorophenylalanine methyl ester hydrochloride also potentiated the analgesic effect. The author suggested both the central and peripheral mechanisms and complex neural pathways were involved in this effect.

Mitra *et al.* (1996) demonstrated the anti-nociceptive activity of *Panax ginseng* and its potentiating effect of both pentazocine and aspirin.

Singh *et al.* (1996a) studied the Petroleum Ether Extract (PEE) and direct Ethanolic Extract (EE) of the seeds of *Pongamia pinnata* and found that it had significant analgesic activity at doses higher than 100 mg/kg.

Krishnaveni *et al.* (1997) found that 4', 5, 6-Trihydroxy-3'7-Dimethoxy flavone from *Vicoa indica* had a potent analgesic effect against physical, chemical and thermal noxious stimuli. It was also found that flavones of *V. indica* had a peripheral analgesic action by eliciting the effect through its action on opioid receptors.

Gossypin, a bioflavonoid from the yellow petals of *Hibiscus vitifolius* (Bhasadwaji), has been shown to have anti-nociceptive activity similar to morphine and involving multineurotransmitter systems mainly the cholinergic and GABA neurotransmitter pathways. Gossypin pretreatment significantly decreased the development of acute tolerance to morphine induced anti-nociception (Ramaswamy and Viswanathan, 1997).

The Petroleum Ether Extract (PEE), Benzene Extract (BE) and Ethanol Extract (EE) of the roots of *P. pinnata* showed significant analgesic effect in the tail flick test (Singh *et al.*, 1997).

Effraim *et al.* (1998) showed that the aqueous extract of *Ziziphus spina* Christi leaves (250-1000 mg/kg) possessed anti-nociceptive effect in a dose-dependant fashion. At a dose of 250 mg/kg the extract produced comparable effect to that of 10mg/kg of pethidine hydrochloride and it was cent percent at a dose of 1000 mg/kg of extract in suppressing the number of wriths induced by acetic acid.

Different extracts of *Abies pindrow* Royle leaf (PEE, BE, AE and EE) 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 inhibitory effect on Platelet Activating Factor (PAF) and prostaglandins.

Ethanolic extract of *Martynia diandra* produced a dose dependant and significant inhibition of acetic acid induced abdominal constrictions.

Chatpalliwar *et al.* (2003) also examined its effect in formalin induced pain and found to have dose dependent anti-nociception in neurogenic pain.

Dharmasiri *et al.* (2003) suggested that the analgesic activity might be mediated via PG synthesis inhibition, antihistamine, membrane stabilizing and anti-oxidant activities. The water extract of mature fresh leaves (MFL) of *V. negundo* 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 of MFL.

2.3 OTHER PHARMACOLOGICAL PROPERTIES OF PLANTS UNDER STUDY

2.3.1 *Tinospora cordifolia*

The Indian medicinal plant *Tinospora cordifolia* (Chittamruthu) is one of the most widely used plants in various traditional medicinal systems, including Ayurvedic medicine for the treatment of jaundice, rheumatism, urinary and skin diseases, diabetes and anemia and for its antiallergic and anti-inflammatory properties.

Rege *et al.* (1989) showed the modulation of immunosuppression in obstructive jaundice by *T. cordifolia*. Pretreatment of cholestatic rats with *T. cordifolia* afforded protection against *Escherichia coli* infection. The plant not only restored the immunocompetence, but also augmented the killing activity of polymorphs.

Peer *et al.* (1990) studied the efficacy of Liv-52 and *T. cordifolia* in experimental carbon tetrachloride induced hepatotoxicity in goats and found that Liv-52 treated animals showed better regeneration of hepatic cells as compared to *T. cordifolia* treated animals.

The protective effect of *T. cordifolia* by reduction in mortality of mice caused by *E.coli* induced peritonitis from 100 per cent in control to 17.8 per cent

was assessed by Thatte *et al.* (1992). The improved bacterial clearance as well as the phagocytic capacity of neutrophils was attributed to its protective effect.

Thatte *et al.* (1994) suggested the activation of macrophage for significant increase in number of colony forming units of the granulocyte- macrophage (CFU-GM) 255 ± 49.32 vs 38.51 ± 9.98 in serum. The increase in CFU-GF in serum is brought by the treatment of *T. cordifolia* at the rate of 100 mg/kg for 10 days.

The anti-oxidant activity of *T. cordifolia* was demonstrated by Mathew and Kuttan (1996). The methanolic extract of *T. cordifolia* was found to inhibit lipid peroxide formation and 50 mg of the extract concentration is required for 100 per cent inhibition of super oxide production and 65 per cent inhibition of hydroxyl radical generation.

Methanolic extract of *T. cordifolia* significantly increased the total leukocyte count and enhanced the humoral immune response by increasing the circulating antibody titre and the number of plaque forming cells. It also increased the phagocytic activity of macrophages and also the bone marrow cellularity. The anti-tumour activity of *T. cordifolia* was significant alone and synergistic with cyclophosphamide, but *T. cordifolia* inhibited the immunosuppressive activity of the cyclophosphamide (Mathew and Kuttan, 1997).

Dhuley (1997) studied the effect of some Indian herbs on macrophage functions in Ochratoxin A treated mice. Treatment with *Asparagus racemosus*, *T. cordifolia*, *Withania Somnifera* and *Picrorrhiza kurrora* significantly inhibited Ochratoxin A induced suppression of chemotactic activity and production of IL-1 and TNF-α by macrophages. It was also found that *W. somnifera* increased macrophage chemotaxis and *A. racemosus* induced excess production of TNF-α .

Kapil and Sharma (1997) reported the immunopotentiating compounds from *T. cordifolia*. Syringin (TC-4) and Cordiol (TC-7) inhibited the *in vitro*

immuno haemolysis by inhibition of the C_3-convertase of the classical complement pathway. There was significant increase in IgG antibodies in serum macrophage activation by Cordioside (TC-2), Cordiofolioside A (TC-5) and Cordiol (TC-7).

Anti-stress activity of suspensions from different extracts of *T. cordifolia* stem was investigated by Patil *et al.* (1997). Aqueous, alcoholic, acetone and petroleum ether extracts showed a significant increase in mice swimming time in swimming endurance test and body weight gain. The petroleum ether extract on the other hand showed a comparably better protective effect against cyclophosphomide induced immunosuppression.

The effect of *T. cordifolia* extract in inhibiting pulmonary metastasis induced by B16F-10 melanoma cells was found to be 65 per cent inhibition of lung nodule formation when the extract was administered prior to B167-10 melanoma cell insulation and it was 55.2 per cent inhibition when administered simultaneously with B16F-10 melanoma cell inoculation (Mathew and Kuttan, 1998).

Jana *et al.* (1999) found the anti-inflammatory effect of *Zingiber officinale, V. negundo* and *T. cordifolia* in both acute and sub acute models of inflammation. In acute inflammation the effect of *T. cordifolia* was more than that of acetylsalicylic acid.

Aqueous *T. cordifolia* Root Extract (ATCRE) decreased the level of plasma thiobarbituric acid reactive substances, ceruloplasmin and d-tocopherol in alloxan diabetic rats. It increased the level of glutathione and Vitamin C in alloxan diabetes. TCRE showed the highest effect at a dose of 5.0 g/kg and was more effective than glibenclamide (Prince and Menon, 1999).

Prince *et al.* (1999) demonstrated significant reduction in serum and tissue cholesterol, phospholipids and free fatty acids in alloxan diabetic rats by ATCRE.

The hypolipidaemic effect was higher at 5 g/kg body weight of root extract and it was better than glibenclamide.

The water and ethanol extracts of stems of *T. cordifolia* and *T. sinensis* inhibited immunosuppression produced by cyclophosphamide. Ethanol extract of stems of both the plants inhibited cyclophosphamide induced anemia. The water extract of *T. sinensis* was found to be more potent than the other extracts (Manjrekar *et al.*, 2000).

Evaluation of antileprosy herbal drug combination viz. *Swertia chirata, T. cordifolia* and *Achyranthes aspera* revealed that *T. cordifolia* had a wide antileprosy strain range. The inter combination of these plants and the combination with Dapsone had shown improved antileprotic activity (Asthana *et al.*, 2001).

Agarwal *et al.* (2002) reported that both the aqueous and alcoholic extract of *T. cordifolia* produced a decrease in learning scores in Hebb William maze and retention memory indicating enhancement of learning and memory. It also protected the neurodegenerative changes caused by cyclosporine in hippocampus.

Goel and Kumar (2002) investigated the role of radioprotection by *T. cordifolia* through free radical scavenging and metal chelation. Aqueous extract of *T. cordifolia* inhibited Fenton ($FeSO_4$) reaction, radiation mediated 2-deoxyribose degradation, clinically generated superoxide anion, Fe2+-bipiridyl complex formation and ferrous sulphate mediated lipid peroxidation in a dose dependant manner.

Immu-21, an ayurvedic polyherbal formulation containing extracts of *O. sanctum, W. somnifera, Emblica officinalis,* and *T. cordifolia* showed proliferative response of spleenic leukocytes to T-cell mitogens like Concavalin (Con)-A and phytohaemagglutinin. It also exhibited proliferative response to B-cell mitogen, Lipopolysaccharide (LPS) *in vitro* by (^{3}H)-thymidine uptake assay in mice. Treatment with Immu-21 (30 mg/kg i.p) once daily for 14 and 21 days

did not cause any change in body weight and spleen weight, but spleenocyte count was increased. Immu-21 (30 mg/kg i.p) for 14 days and 1 mg/kg, for 21 days significantly increased LPS induced leukocyte proliferation. Natural killer cell activity was increased in pretreatment with 10 and 30 mg/kg i.p of Immu-21 for seven days (Nemmani *et al.*, 2002).

Gupta and Sharma (2003) reported the anti-fertility effect of *T. cordifolia* stem extract in male rats. *T. cordifolia* decreased sperm motility, sperm density and interfered with spermatogenesis. The round spermatids, preleptotene, pachytene and secondary spermatocytes were decreased by 73.12, 47.60, 52.85 and 48.10 per cent respectively. The number of mature Leydig cells and the nuclear area significantly reduced with drop in serum testosterone. Biochemical parameters like protein, sialic acid, glycogen contents of testes and seminal vesicular fructose were all depleted, whereas testicular cholesterol was significantly elevated.

Anti-inflammatory activities of ethanol extracts from nine vine plants were evaluated against a panel of key enzymes like cyclooxygenase-1 (COX_1), cyclooxygenase-2 (COX_2), phospholipase A_2 (PLA_2), 5-lipoxygenase (5 LO) and 12-lipoxygenase (12 LO) by Rachel *et al.* (2003). Among the vine plants, *T. sagittata* and *T. sinensis* showed inhibitory activities only against COX_1, whereas the extract of *Tripterygium wilfordii* showed most potent inhibition against COX_1, COX_2 and 5 LO.

A polysaccharide preparation from *T. cordifolia* had been established using *Saccharomyces cerevisiae* X2180 strain as the *in vivo* test model by Subramanian *et al.* (2003). It was assessed the entire radio protective activity would be attributed to the radical scavenging capacity of the preparation as it did not enhance the expression of the protective enzymes, catalase and superoxide dismutase in the yeast cells.

2.3.2 *Vitex negundo*

Vitex negundo Linn. an aromatic shrub found in many parts of India has been used for the treatment of rheumatism and inflammatory disorders. The leaves of the plant have been claimed to be beneficial for patients of inflammatory disorders.

Makwana *et al.* (1994) compared the pharmacological activity profile of the leaves of *Vitex leucoxylon* to that of root and leaf extracts of *V. negundo*. The analgesic and anti-inflammatory activities of *V. leucoxylon* resembled that of *V. negundo*.

Anti-arthritic activity of *V. negundo* was studied by Tamhankar and Saraf (1994). The suspension of dried powdered leaves of *V. negundo* showed a dose related inhibition of primary and secondary lesions of arthritis induced by adjuvant. It also revealed inhibition of sheep red blood cell induced delayed type of hypersensitivity and the methylated Bovine Serum Albumin (BSA) induced active arthus reaction in mice.

Nair *et al.* (1994) reported the mast cell stabilizing effect of *V. negundo*. It was found that *V.negundo* had a stronger protective action against immunologically mediated granulation of mast cell than that of it induced by compound 48/80 in mice. This was found to be attributed to the selective influence of the extract on calcium fluxes across mast cell membrane.

The ethanolic extract of *V. negundo* Linn. was found to inhibit the release of histamine and products of arachidonic acid metabolism, the leukotrienes in dose dependant manner (40 mg/ml and 80 mg/ml) in the study of antigen and compound 48/80 induced contractions of guinea pig trachea by Nair and Saraf (1995).

Studies on the CNS activity of *V. negundo* Linn in mice by Gupta *et al.* (1999) revealed a depressant activity in dose dependant manner. The methanolic

extract of the leaves of *V. negundo* potentiated the sleeping time induced by pentobarbitone sodium, diazepam and chlorpromazine. It possessed analgesic properties and potentiated analgesia induced by morphine and pethidine. It also gave significant protection against strychnine and leptasole induced convulsions.

The analgesic (both central and peripheral), anti-inflammatory and the inhibitory action on oxytocin induced contractions in isolated horns of uterus by *V. negundo* leaf extract was attributed to its flavonoid contents which are known to act through inhibition of prostaglandin biosynthesis (Telang *et al.*, 1999).

Gaidhani *et al.* (2000) showed that the alcoholic extract of *V. negundo* at a dose of 1 gm/kg i.p., exhibited anti-ulcerogenic effect to an extent of 25.89 per cent of healing index on piroxicam (5 mg/kg, i.p.) induced gastric ulcers in rats.

JCB, a herbal formulation containing *Alpinia galanga, Commiphora wightii, Boswellia serrata, Foeniculum vulgare, Glycyrrhiza glabra, V. negundo* and *Anethum graveolens* was investigated for its anti-inflammatory activity by Venkataranganna *et al.* (2000). JCB dose dependently inhibited carrageenin induced paw inflammation and granuloma weight in croton oil induced granuloma pouch model in rats. A dose of 700 mg/kg body weight was found to be the optimum for anti-inflammatory activity.

Gaidhani *et al.* (2001) found that the anti-inflammatory activity of *V. negundo* on acute inflammation was due to the inhibition of the inflammatory mediators like histamine H_1, H_2, 5-hydroxytrytaine and prostaglandin. This was demonstrated by the potentiation of anti-inflammatory activity of *V. negundo* by the antagonists of these inflammatory mediators like promethazine, cimetidine, cyproheptadine and paracetamol.

Ethanolic extract of *V. negundo* (1000 mg/kg, i.p.) exhibited a significant anti-inflammatory effect on sub-acute inflammation, cotton-pellet induced granuloma in rats. Promethazine, cimetidine, cyproheptadine and paracetamol

potentiated the anti-inflammatory activity, suggesting the mechanism of action of *V. negundo* as the inhibition of inflammatory mediators (Gaidhani *et al.*, 2002).

Gupta and Tandan (2002) suggested that the analgesic activity of *V. negundo* was due to prostaglandin inhibition and reduction of oxidative stress. The non reversal of analgesia induced by *V. negundo* in naloxone treatment indicated that the central analgesic action was not mediated through opioid receptors.

Dharmasiri *et al.* (2003) investigated the anti-inflammatory and analgesic activities of mature fresh leaves of *V. negundo* at 1.25, 2.5 and 5 g/kg dose orally in rats. The leaves showed an inversely dose-dependant *in vivo* antihistamine and *in vitro* prostaglandin (PG) synthesis inhibition. It also showed a significant and directly dose dependent analgesic activity at one hour of treatment. Flowering of the *V. negundo* did not abolish the analgesic and anti-inflammatory activities of the leaves.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Eighty adult albino rats weighing 150-200 g, procured from Small Animal 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 40 animals each.

3.2 DRUGS

3.2.1 Taxonomical Identification, Collection of Plant Materials and Extraction

The plants under study namely *Tinospora cordifolia* and *Vitex negundo* Linn. growing in the premises of College of Veterinary and Animal Sciences, Mannuthy were identified and collected for the study.

3.2.1.1 Tinospora cordifolia

The vines from *Tinospora cordifolia* were collected and identified (Fig.1). It was cleaned, dried under shade and pulverized to a coarse powder. The powder was extracted with ethanol using soxhlet extraction apparatus. After extraction the extract obtained was kept open for complete evaporation of the solvent.

Hundred gram dried powder of vines of *T. cordifolia* yielded 16.18g of extract.

3.2.1.2 Vitex negundo

The leaves of *Vitex negundo* Linn. were identified (Fig.2), collected, dried under shade and pulverized to coarse powder. The powder of the leaves was

Fig. 1 *Tinospora cordifolia*

Fig. 2 *Vitex negundo*

extracted with ethanol using a soxhlet extractor. The extract was air dried to remove the traces of ethanol and kept under refrigeration.

Hundred gram of powdered dried leaves of *V. negundo* Linn. yielded 23.97 g of extract.

3.2.2 Tween-80

Three per cent of Tween-80 was used as vehicle for the experimental drugs.

3.2.3 Diclofenac Potassium

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

3.3 EXPERIMENTAL DESIGN

3.3.1 Anti-inflammatory Screening

Carrageenin induced rat paw oedema method (Winter *et al.*, 1962).

Forty rats were divided into 5 groups of eight each.

Group I	-	Vehicle alone (3 per cent Tween-80) 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	-	Ethanolic extract of *T. cordifolia* (100 mg/kg body weight) administered per os for 7 days
Group IV	-	Ethanolic extract of *V.negundo* (100 mg/kg body weight) administered per os for 7 days

Group V - Ethanolic extract of *T. cordifolia* (50 mg/kg body weight) + ethanolic extract of *V.negundo* (50 mg/kg body weight) administered per os for 7 days.

On the 7th day, 30 minutes after drug administration all the rats were given sub-planter injection of carrageenin (2 per cent w/v in sterile saline 0.05 ml).

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

The percentage inhibition of paw oedema was calculated by

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

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.

3.3.2 Anti-nociceptive Screening

Tail flick method (Dandiya and Menon, 1963).

Forty rats were divided into 5 groups of eight each. Then each group was treated as follows.

Group I - Vehicle alone (3 per cent Tween-80) 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 - Ethanolic extract of *T. cordifolia* (1000 mg/kg body weight) administered per os for 7 days

Group IV - Ethanolic extract of *V. negundo* (1000 mg/kg body weight) administered per os for 7 days

Group V - Ethanolic extract of *T. cordifolia* (500 mg/kg body weight) + ethanolic extract of *V. negundo* (500 mg/kg body weight) administered per os for 7 days.

On the 7th day after administration of drugs, reaction time for each group will be measured at 30, 60, 90 and 120 minutes using Techno analgesiometer (Davies *et al.*, 1946).

3.4 COLLECTION OF BIOLOGICAL SAMPLES

3.4.1 Blood

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.

3.4.2 Adrenal Gland

The animals were euthanized after blood collection. Adrenal glands in both sides located supra renal in position were dissected out carefully. Wet weight of the glands were taken. Left side adrenal gland was used for estimation of ascorbic acid and right side gland was used for cholesterol determination.

3.5 ESTIMATION OF VARIOUS PARAMETERS

3.5.1 Adrenal Ascorbic Acid

Adrenal ascorbic acid content was extracted by the method of Pohujani *et al.* (1969) and it was estimated by the spectrophotometric method described by Nino and Prasad (1980).

a. Reagents

1. Trichloroacetic acid – 10 g/dl solution
2. Sulphuric acid – 4.5 M solution
3. Sulphuric acid - 12 M solution
4. 2, 4-Dinitrophenyl hydrazine reagent, (2,4-D) 2 g/dl of 4.5 M sulphuric acid
5. Thiourea 5 g/dl solution
6. Copper sulfate 0.6 g/dl solution
7. Dinitrophenyl hydrazine – Thiourea – Copper Sulfate (DTC) reagent – containing 10 ml 2,4 dinitrophenyl hydrazine reagent + 5 ml thiourea solution + 5 ml Copper sulfate solution.

b. Ascorbic acid standards

1. Stock standard – 50 mg/dl
2. Intermediary standard – 5 mg/dl
3. Working standards – 0.1, 0.4, 0.8, 1.2, 2.00 and 3 mg/dl

c. Procedure

1. 1.2 ml aliquots of each working standards were taken.
2. 1.2 ml of 10 per cent trichloroacetic acid solution was taken for blank

3. 0.4 ml DTC reagent was added to each tube - mixed
4. The tubes were incubated at 37°C for 3 hours
5. The tubes were transferred to ice-water bath for 10 minutes
6. 2ml cold 12 M sulphuric acid was added slowly
7. The tubes were allowed to stand at room temperature for 20 min.
8. Spectrophotometer was at 0 absorbance at 520 nm with blank
9. The calibration graph was prepared plotting concentration against absorbance

d. Specimen processing

1. 2 ml trichloroacetic acid solution was taken in all the tubes and 0.5 ml plasma was added.
2. The content was mixed for 30 seconds and the tubes were allowed to stand for 3-4 min.
3. The contents were centrifuged for 10 min at 2000-2200 rpm
4. 1.2 ml of supernatent was pipetted out to another tube
5. It was processed in the same way as for working standards
6. Final concentration was read from the calibration graph

3.5.2 Adrenal Cholesterol

Adrenal cholesterol content was assayed by Leiberman Burchard reaction (King and Whooton, 1956).

a. Solutions

1. Redistilled glacial acetic acid
2. 10 per cent ferric chloride
3. Acetone-Alcohol mixture: Equal parts of acetone and absolute alcohol were mixed together.
4. Colour reagent – 0.5 ml of 10 per cent ferric chloride solution was added to 7.5 ml of concentrated sulphuric acid. Then the solution was mixed and diluted to 50 ml with concentrated sulphuric acid.
5. Cholesterol standard – Stock standard – 1 mg/ml (i.e., 100 mg of pure dry cholesterol in 100 ml of glacial acetic acid). Working standard - 0.2 mg/ml

b. Procedure

I. Sample

1. Adrenal gland was weighed and homogenized in acetone-alcohol solution using tissue homogeniser.
2. Homogenate in the centrifuge tube was placed in a beaker of water at 60-70°C for 10 minutes.
3. The contents were cooled and centrifuged at 2000 rpm for 3 min.
4. Two ml of the supernatent was taken in another test tube.
5. Solution was evaporated to dryness by heating the tube in a water bath at approximately 80-90°C.
6. Six ml of glacial acetic acid was added to the dry residue in the test tube.
7. The dry residue was dissolved by heating in a boiling water bath for 5 min.
8. The contents were cooled at room temperature and 4 ml of color reagent was added.
9. Read in a spectrophotometer at 570 nm

II. Blank

0.1 ml of water, 6 ml of glacial acetic acid and 4 ml of colour reagent were added in the same procedure as that of sample.

The absorbance reading in spectrophotometer is made zero by setting blank.

III. Standard

One ml of cholesterol working standard was added to 5 ml glacial acetic acid and 0.1 ml of water with 4 ml of colouring reagent were added in the same way as in the test.

Total cholesterol content was obtained by the formula

$$\frac{\text{Reading of test}}{\text{Reading of std.}} \times \frac{0.2 \times 100}{\text{Wt. of sample}} \times \text{dilution, g/100 g}$$

3.5.3 Total Serum Cholesterol

(CHOD-PAP Method; Allain *et al.*, 1974)

(Kit from Agappe Diagnostics was used)

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

3.5.4 Thiobarbituric Acid Reactive Substances (TBARS)

The levels of lipid peroxidation in tissues/plasma were estimated by the method of Fraga *et al.* (1988).

Reagents

1. Trichloro acetic acid (TCA) - 15 percent
2. Hydrochloric acid (HCl) - 0.25 N
3. Thiobarbituric acid (TBA) - 0.38 percent in hot distilled water
4. TCA – TBA – HCl reagent – solution: 1, 2 and 3 were mixed freshly in the ratio of 1:1:1
5. Stock standard – 4.8 mM: 0.079 ml of 1, 1', 3, 3' tetra methoxy propane was diluted to 100 ml.
6. Working standard stock solution was diluted to get a concentration of 48 nM/ml.

Procedure

1. 0.5 ml of plasma was treated with 2.0 ml of TBA – TCA – HCl reagent and mixed thoroughly.

2. The mixture was kept in boiling water bath for 15 minutes.

3. After cooling, the tubes were centrifuged for 10 minutes and the supernatant was taken for measurement.

A series of standard solution in the range 2-10 n mole concentrations were treated in the similar manner. The absorbance of chromophore was read at 535 nm against the reagent blank.

Values were expressed as mM/100 g wet tissue and nM/dl plasma.

3.5.5 Estimation of Haematological Parameters

3.5.5.1 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 (Benjamin, 1985).

3.5.5.2 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 (Benjamin, 1985).

3.5.5.3 Total Erythrocyte Count

The erythrocytes were counted by standard dilution technique using Hayem's fluid diluent. Counting of erythrocytes was done under high power of the microscope in the zone for erythrocytes in the haemocytometer (Benjamin, 1985).

3.5.5.4 Haemoglobin Concentration

Haemoglobin concentration was estimated by acid haematin method (Benjamin, 1985).

3.5.6 Serum enzymes

3.5.6.1 Aspartate Amino Transferase (AST)

(UV – Kinetic test) –(Reitman & Frankel, 1957)

(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 - Dineucleotide (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.

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

3.6 STATISTICAL ANALYSIS OF DATA

Results were analyzed by using one way ANOVA test for comparison between control groups and treatment groups III, IV and V 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 deviation.

Results

4. RESULTS

4.1 CARRAGEENIN INDUCED RAT PAW OEDEMA

The results of carrageenin induced rat paw oedema which indicate the anti-inflammatory activity of alcoholic extract of *Tinospora cordifolia, Vitex negundo* and its combination viz., one hour, two hour and three hour period are presented in Tables 1, 2 and 3 respectively and Fig.3. Percentage inhibition of paw oedema, which is the measure of anti-inflammatory activity, exhibited by the treatment groups, is represented in Table 4 and Fig.4. Ethanolic extract of *Tinospora cordifolia* exhibited 32.00, 34.17 and 51.47 per cent of inhibition at first, second and third hour of carrageenin induced inflammation respectively. Anti-inflammatory effect of *Vitex negundo* was significant at first hour showing 77.33 per cent of inhibition and subsequently reduced to 59.51 per cent at second hour and 35.29 per cent at third hour. Ethanolic extract combination of *T. cordifolia* and *V. negundo* significantly inhibited the inflammation in a uniform manner at 53.33, 54.43 and 51.47 per cent in first, second and third hour of inflammation respectively.

4.2 ANTI-NOCICEPTION – REACTION TIME

The reaction time was considered as an index of nociception. The reaction times for every treatment groups at 30 minutes interval upto two hours are recorded in Table 5 and Fig.5. The increase in reaction time was gradual for all the treatment groups and it was of peak at 60 minutes except group II that reached its peak at 2 hours. The reaction time (seconds) for Group II, III, IV and V at 60 minute was 4.75 ± 0.89, 7.00 ± 1.20, 6.00 ± 1.51 and 6.88 ± 0.35 respectively. At 2 hours, the reaction time for the treatment groups II, III, IV and V were 6.00 ± 0.53, 6.25 ± 1.16, 5.25 ± 0.71 and 5.13 ± 0.35 seconds respectively.

Table 1. Effect of treatments on increase in paw volume after 1 hour of carrageenin induced paw odema in rats, ml

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.2500	0.0625	0.1563	0.0625	0.0625
2	0.2188	0.2187	0.1563	0.0625	0.2500
3	0.3750	0.1562	0.2500	0.0625	0.0937
4	0.3125	0.1562	0.2500	0.0938	0.1250
5	0.2812	0.1562	0.1563	0.0938	0.1250
6	0.3438	0.1562	0.2500	0.0625	0.0938
7	0.3438	0.0938	0.2187	0.0625	0.1563
8	0.2188	0.0938	0.1562	0.0313	0.1875
Mean ± SD	0.293± 0.06[a]	0.1367± 0.05[c]	0.1992± 0.05[b]	0.0664± 0.02[d]	0.1367± 0.06[c]

Significant at 1% level (P<0.01)

Group I : Control 3% Tween 80 per os

Group II : *Diclofenac potassium,3 mg/kg per os*

Group III : *T.cordifolia* ethanolic extract, 100 mg/kg per os

Group IV : *V.negundo,* ethanolic extract, 100 mg/kg per os

Group V : Combination of ethanolic extract of *T.cordifolia,* 50 mg/kg & *V.negundo,* 50 mg/kg per os

Table 2. Effect of treatments on increase in paw volume after 2 hours of carrageenin induced paw odema in rats, ml

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.3750	0.0937	0.2187	0.1250	0.2500
2	0.3750	0.0625	0.2500	0.1250	0.1250
3	0.2812	0.0625	0.1563	0.0937	0.2500
4	0.2812	0.1250	0.1875	0.1562	0.1562
5	0.2813	0.0625	0.2188	0.1247	0.0937
6	0.2500	0.0938	0.2188	0.1250	0.0625
7	0.3437	0.0938	0.1563	0.1250	0.0625
8	0.2812	0.1250	0.2188	0.1250	0.1250
Mean ± SD	0.3086± 0.049[a]	0.0899± 0.026[d]	0.2032± 0.033[b]	0.1250± 0.170[c]	0.1406± 0.075[c]

Significant at 1% level (P<0.01)

Table 3. Effect of treatments on increase in paw volume after 3 hours of carrageenin induced paw odema in rats, ml

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.3437	0.1875	0.1563	0.1250	0.0625
2	0.2812	0.1250	0.1563	0.1875	0.1250
3	0.1875	0.0312	0.0937	0.1875	0.0937
4	0.2813	0.1563	0.0625	0.1875	0.1875
5	0.2187	0.0937	0.2187	0.1875	0.1563
6	0.2812	0.1875	0.0937	0.1875	0.1875
7	0.2187	0.0937	0.1250	0.1250	0.0937
8	0.3125	0.0312	0.1250	0.1875	0.1250
Mean ± SD	0.2656± 0.053[a]	0.1133± 0.062[d]	0.1289± 0.049[c]	0.1719± 0.029[b]	0.1289± 0.046[c]

Significant at 1% level (P<0.01)

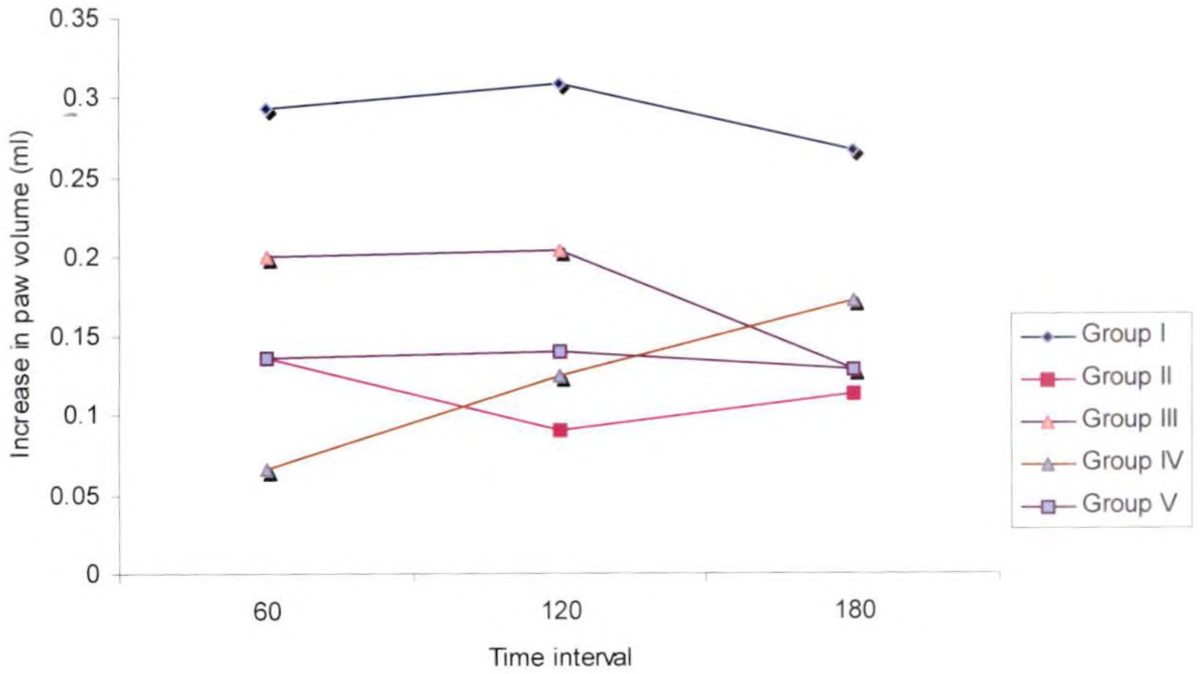

Fig. 3. Effect of treatments on mean increase of paw volume in carrageenin induced rat paw oedema

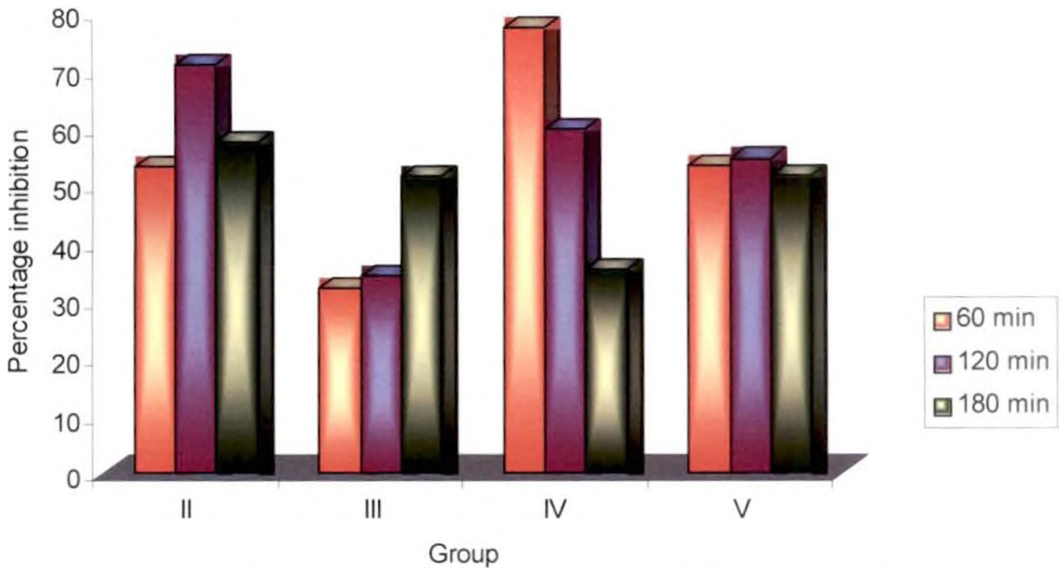

Fig.4. Effect of treatments on percentage inhibition of carrageenin induced rat paw odema

Table 4. Percentage inhibition of paw oedema by different treatments in carrageenin induced inflammation in rats, %

Time interval (min)	Group II	Group III	Group IV	Group V
60	53.34	32.00	77.33	53.33
120	70.88	34.17	59.51	54.43
180	57.36	51.47	35.29	51.47

Table 5. Anti-nociceptive effect of different treatments on reaction time by tail flick test in rats

Interval (min) / Groups	Reaction time in seconds (mean ± SD)				
	0	30	60	90	120
I	2.50 ± 0.76	3.63 ± 0.74*	3.75 ± 0.71*	3.63 ± 0.74*	3.38 ± 0.52*
II	3.25 ± 0.71	3.75 ± 0.83*	4.75 ± 0.89*	5.63 ± 0.74*	6.00± 0.53*
III	5.13 ± 1.13	6.25 ± 1.16*	7.00 ± 1.20*	6.88 ± 0.64*	6.25 ± 1.16*
IV	4.50 ± 0.76	4.88 ± 1.13*	6.00 ± 1.51*	5.75 ± 0.71*	5.25 ± 0.71*
V	4.50 ± 0.76	5.38 ± 1.19*	6.88 ± 0.35*	6.50 ± 1.20*	5.13 ± 0.35*

*Significant at 1% level (P<0.01)

Group I : Control 3% Tween 80 per os

Group II : Diclofenac potassium, 3 mg/kg per os

Group III : *T. cordifolia* ethanolic extract, 1000 mg/kg per os

Group IV : *V. negundo* ethanolic extract,1000 mg/kg per os

Group V : Combination of ethanolic extract of *T. cordifolia,* 500 mg/kg & *V. negundo,* 500 mg/kg per os

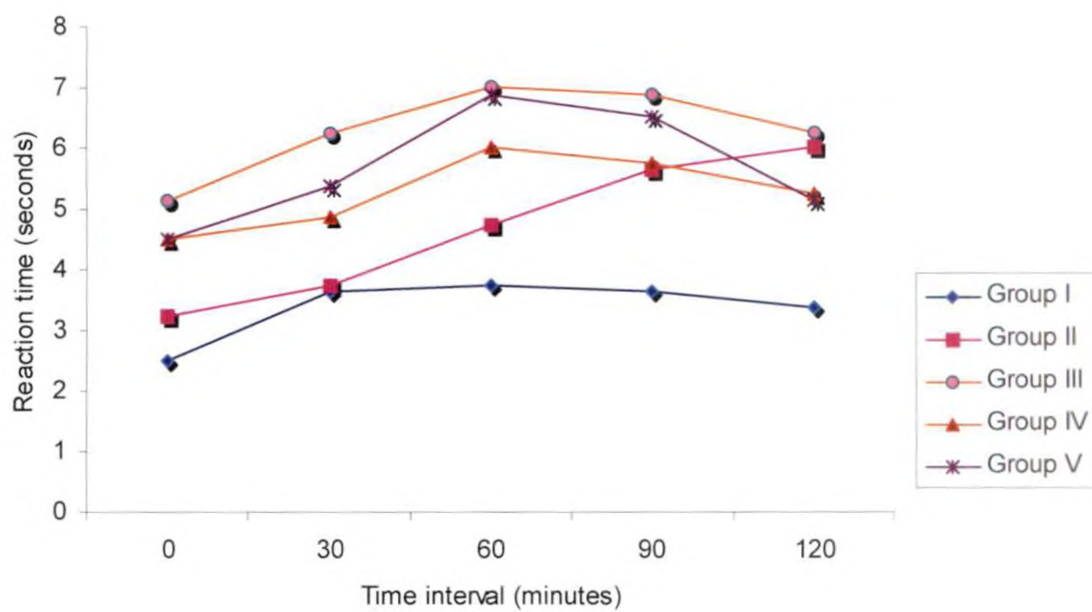

Fig. 5. Effect of treatments on reaction time by tail flick method in rats

4.3 ADRENAL GLAND WET WEIGHT

4.3.1 Anti-inflammatory Screening

The data of adrenal gland wet weight in anti-inflammatory screening are presented in Table 6. The gland weights were 0.0140, 0.0129, 0.0124, 0.0122 and 0.0130 g/100 g body weight for group I to V respectively. There was no significant difference between the groups.

4.3.2 Anti-nociceptive Screening

The data of adrenal gland wet weight in anti-nociceptive screening are presented in Table 7. The mean gland weights were 0.0136, 0.0118, 0.0136, 0.0142 and 0.0130 g/100 g body weight for group I to V respectively. There was no significant difference for adrenal gland weight between the groups.

4.4 BIOCHEMICAL PARAMETERS

4.4.1 Adrenal Ascorbic Acid

4.4.1.1 Anti-inflammatory Screening

The data obtained are presented in Table 8 and Fig.6. The mean adrenal ascorbic acid values for the groups I, II, III, IV and V were 148.74 ± 17.71, 244.93 ± 26.80, 282.94 ± 10.21, 321.44 ± 20.79 and 247.43 ± 11.48 mg/100 g respectively. All the treatment groups showed significant increase in adrenal ascorbic acid compared to the control (P<0.01). Adrenal ascorbic acid level of animals given *Vitex negundo* at the rate of 100 mg/kg was 321.44 ± 20.79, which was significantly higher than all other groups. Combination treated group V had more or less similar adrenal ascorbic acid value with diclofenac treated group II.

Table 6. Effect of treatments on adrenal gland wet weight in carrageenin induced inflammation in rats, g/100 g body weight

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.0155	0.0146	0.0130	0.0129	0.0128
2	0.0186	0.0108	0.0124	0.0129	0.0130
3	0.0090	0.0134	0.0121	0.0127	0.0129
4	0.0149	0.0155	0.0124	0.0129	0.0121
5	0.0118	0.0098	0.0113	0.0111	0.0148
6	0.0141	0.0111	0.0125	0.0119	0.0112
7	0.0111	0.0128	0.0127	0.01035	0.0120
8	0.0179	0.0155	0.0129	0.0133	0.0133
Mean ± SD	0.0140	0.0129	0.0124	0.0122	0.0130

Table 7. Effect of treatments on adrenal gland wet weight in tail flick model of nociception in rats, g/100 g body weight

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.0159	0.0104	0.0148	0.0169	0.0102
2	0.0176	0.0109	0.0114	0.0146	0.0107
3	0.0126	0.0110	0.0110	0.0135	0.0145
4	0.0136	0.0119	0.0133	0.0138	0.0142
5	0.0133	0.0119	0.0164	0.0141	0.0133
6	0.0131	0.0103	0.0160	0.0125	0.0141
7	0.0119	0.0149	0.0123	0.0130	0.0127
8	0.0125	0.0128	0.0134	0.0150	0.0145
Mean ± SD	0.0136	0.0118	0.0136	0.0142	0.0130

Mean ± SD are not significant between groups

Table 8. Effect of treatments on adrenal ascorbic acid level in carrageenin induced inflammation in rats, mg/100 g of gland

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	134.0336	222.5000	292.4324	294.9383	258.5740
2	174.2647	255.5491	272.2857	341.1966	236.0869
3	153.5032	234.8718	277.1429	290.9091	236.5625
4	159.8684	199.1667	270.7317	340.0855	259.3023
5	168.0374	262.3913	286.3158	380.1493	258.0153
6	128.1768	276.0766	288.2353	322.5000	332.0670
7	130.4969	273.9234	277.0270	342.2308	244.1667
8	141.5058	234.9829	299.3506	309.4805	254.6903
Mean ± SD	148.736 ± 17.71[d]	244.933 ± 26.80[c]	282.940 ± 10.21[b]	321.436 ± 20.79[a]	247.433 ± 11.48[c]

Table 9. Effect of treatments on adrenal ascorbic acid level in tail flick method of nociception in rats, mg/100 g of gland

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	153.1460	303.5338	254.0704	323.8300	318.1439
2	147.3500	294.8193	290.6736	307.7483	362.6490
3	201.8181	305.3571	283.5165	317.8941	337.2517
4	158.6956	252.2460	288.9831	284.8402	279.0980
5	142.3809	256.1224	258.3582	304.9057	307.7330
6	161.6822	304.0541	243.8662	332.9592	319.1414
7	165.5555	299.4186	283.6364	295.6344	318.0370
8	206.9565	264.6259	302.8571	328.4351	364.0796
Mean ± SD	167.198 ± 24.18[c]	285.022 ± 23.14[b]	275.745 ± 20.85[b]	311.997 ± 16.76[a]	325.767 ± 28.35[a]

Means bearing same superscript do not differ significantly at P<0.01

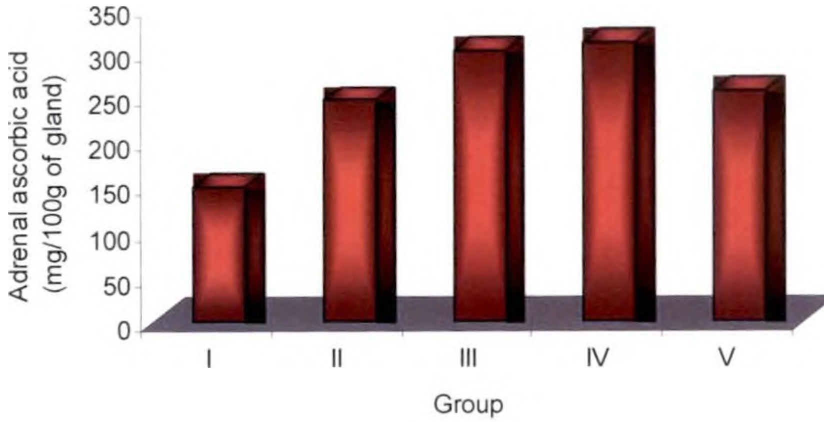

Fig. 6. Effect of treatments on adrenal ascorbic acid in carrageenin induced inflammation in rats

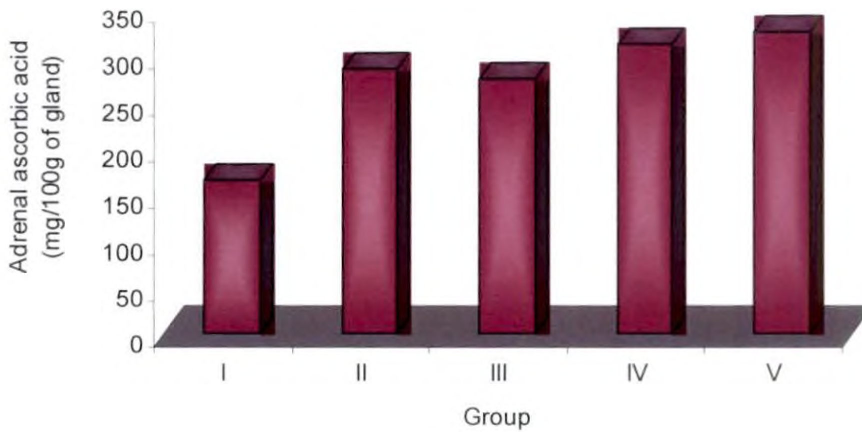

Fig. 7. Effect of treatments on adrenal ascorbic acid in tail flick method of nociception in rats

4.4.1.2 Anti-nociceptive Screening

The results are given in Table 9 and Fig.7. Mean adrenal ascorbic acid value for control group in anti-nociceptive screening was 167.198 ± 24.18 mg/100 g of gland. All treatment groups showed a significant increase in adrenal ascorbic acid value from control. Group IV and V did not differ significantly between them but showed a peak increase in adrenal ascorbic acid and they were 311.997 ± 16.76 and 325.767 ± 28.35 mg/100 g of gland respectively. *T. cordifolia* treated group III with adrenal ascorbic acid values of 275.745 ± 20.85 mg/100 g of gland was found not significantly different from diclofenac treated group (285.022 ± 23.14 mg/100 g of gland)

4.4.2 Adrenal Cholesterol

4.4.2.1 Anti-inflammatory Screening

The results are given in Table 10 and Fig.8. The mean adrenal cholesterol levels of control and diclofenac treated groups were 5.511 ± 0.46 and 3.414 ± 0.36 g/100 g of gland respectively. The adrenal cholesterol level of 5.581±0.89 g/100 g of gland in *V.negundo* (100mg/kg) treated group was on par with that of control. Group III treated with a dose of 100mg/kg *T.cordifolia* gave a significantly high value of 6.893 ± 0.50 g/100g of gland, adrenal cholesterol from the control. Whereas the combination of extract treated group showed a significant decrease in value of 2.798 ± 0.37 g/100 g of gland.

4.4.2.2 Anti-nociceptive Screening

The results are presented in Table 11 and Fig. 9. The mean adrenal cholesterol levels for control group and diclofenac treated group were 10.592 ±0.52 and 9.416 ± 0.85 g/100 g of gland respectively. All the treatment groups show a significant decrease ($P<0.01$) from control. Group III and V have the lowest adrenal cholesterol values and they did not differ significantly between

Table 10. Effect of treatments on adrenal cholesterol level in carraggenin induced inflammation in rats, g/100 g of gland

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	5.3958	3.0511	7.2373	5.6368	3.1945
2	6.0256	3.4768	7.0805	6.3154	3.4111
3	4.7504	3.866	6.2056	5.5125	2.5521
4	5.5847	3.7958	6.7336	5.0898	2.6398
5	5.9652	2.8334	6.4438	5.1905	2.6579
6	5.9819	3.2999	7.2372	5.6920	2.7656
7	5.2493	3.6364	6.5016	5.4706	2.2436
8	5.1318	3.3521	7.7013	5.7417	2.9172
Mean ± SD	5.511 ± 0.46^b	3.414 ± 0.36^c	6.893 ± 0.50^a	5.581 ± 0.38^b	2.798 ± 0.37^d

Table 11. Effect of treatments on adrenal cholesterol level in tail flick method of nociception in rats, g/100 g of gland

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	10.5695	10.3649	4.1835	5.8771	4.7308
2	10.0266	9.3829	4.5471	5.4768	4.5178
3	11.2843	9.0154	4.9149	5.1715	4.5959
4	11.0977	9.2166	4.6414	6.1679	5.0141
5	10.0436	8.3807	4.9151	6.5603	4.8959
6	11.1612	10.9803	4.7478	6.8248	4.7499
7	10.2807	9.0722	4.9815	5.8733	4.7352
8	10.2699	8.8142	4.8498	6.0192	4.9171
Mean ± SD	10.592 ± 0.52^a	9.416 ± 0.85^b	4.713 ± 0.26^d	5.996 ± 0.54^c	4.770 ± 0.17^d

Means bearing same superscript do not differ significantly at $P<0.01$

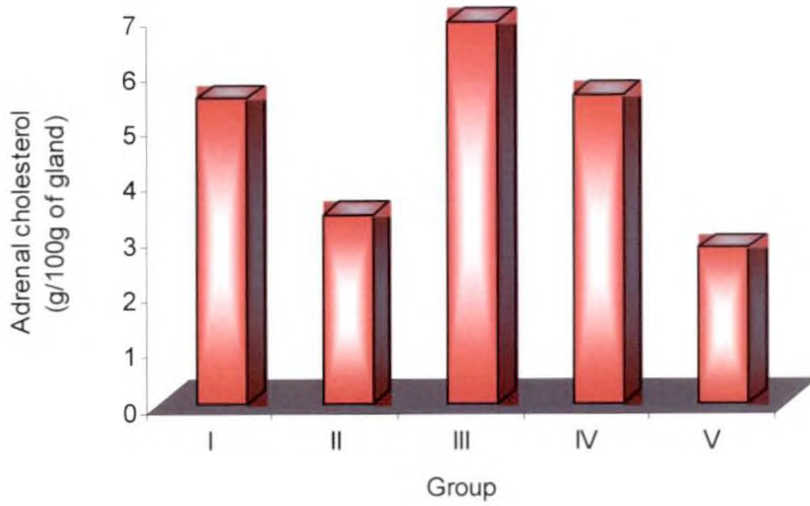

Fig. 8. Effect of treatments on adrenal cholesterol in carrageenin induced inflammation in rats

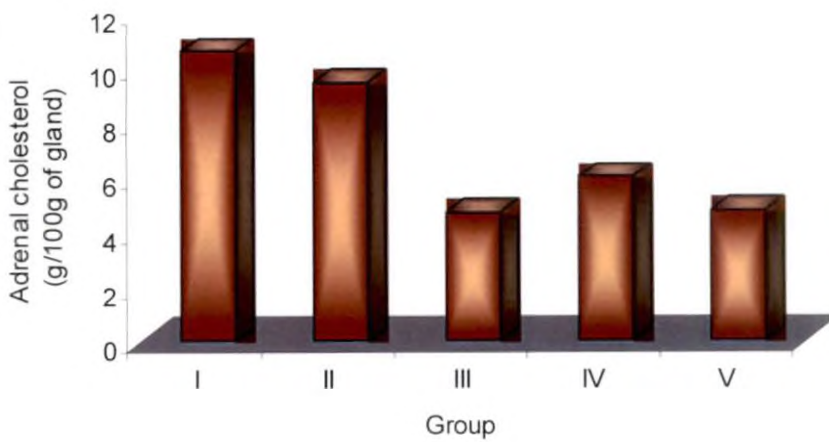

Fig. 9. Effect of treatments on adrenal cholesterol in tail flick method of nociception in rats

them. The mean adrenal cholesterol values for group III and V were 4.713 ± 0.26 and 4.770 ± 0.17 g/100 g of gland respectively.

4.4.3 Serum Cholesterol

4.4.3.1 Anti-inflammatory Screening

The values of serum cholesterol for anti-inflammatory screening are presented in Table 12 and Fig.10. Mean serum cholesterol level for group I and II was 24.88 ± 3.44 and 29.50 ± 3.44 mg/dl respectively. All the treatment groups showed a significant increase in serum cholesterol level from that of control. *T.cordifolia* (100 mg/kg) treated, group III and combination treated group V were significantly same, but both the groups reported a significant increase in serum cholesterol level from control. The values were 46.88 ± 2.30 and 46.00 ± 2.45 mg/dl respectively. However, *V.negundo* (100 mg/kg) treated group showed a highest value of 54.00 ± 3.34 mg/dl ($P<0.01$).

4.4.3.2 Anti-nociceptive Screening

The results are presented in Table 13 and Fig.11. The mean serum cholesterol level for control group and diclofenac treated group were 72.63 ± 4.44 and 97.88 ± 8.95 mg/dl. All the other treatment groups showed a significant decrease ($P<0.01$) in serum cholesterol level. Mean serum cholesterol level for group IV and V are 37.75 ± 2.25 and 42.25 ± 3.24 mg/dl respectively. They did not differ significantly between them. *T.cordifolia* treated group III had a mean serum cholesterol level of 28.38 ± 2.07 mg/dl.

4.4.4 Plasma Thiobarbituric Acid Reacting Substance (TBARS)

Thiobarbituric acid reacting substance (TBARS) is a measure of lipid peroxidation. The results obtained for each group in anti-inflammatory screening were presented in Table 14 and Fig. 12. The control group and the diclofenac treated group showed a level of 7.44 ± 1.63 and 7.24 ± 0.56 nM/dl TBARS

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

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	22	34	47	53	46
2	23	29	49	60	45
3	22	31	44	53	44
4	30	26	44	58	48
5	22	25	49	54	50
6	30	28	46	52	43
7	26	34	46	52	44
8	24	29	50	50	48
Mean ± SD	24.88 ± 3.44[d]	29.50 ± 3.44[c]	46.88 ± 2.30[b]	54.00 ± 3.34[a]	46.00 ± 2.45[b]

Table 13. Effect of treatments on serum cholesterol level in tail flick method of nociception in rats, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	71	92	32	40	46
2	78	94	28	36	42
3	76	89	25	40	38
4	66	109	28	34	47
5	77	106	28	36	44
6	73	87	27	38	40
7	67	97	29	38	42
8	73	109	30	40	39
Mean ± SD	72.63 ± 4.44[b]	97.88 ± 8.95[a]	28.38 ± 2.07[d]	37.75 ± 2.25[c]	42.25 ± 3.24[c]

Means bearing same superscript do not differ significantly at $P<0.01$

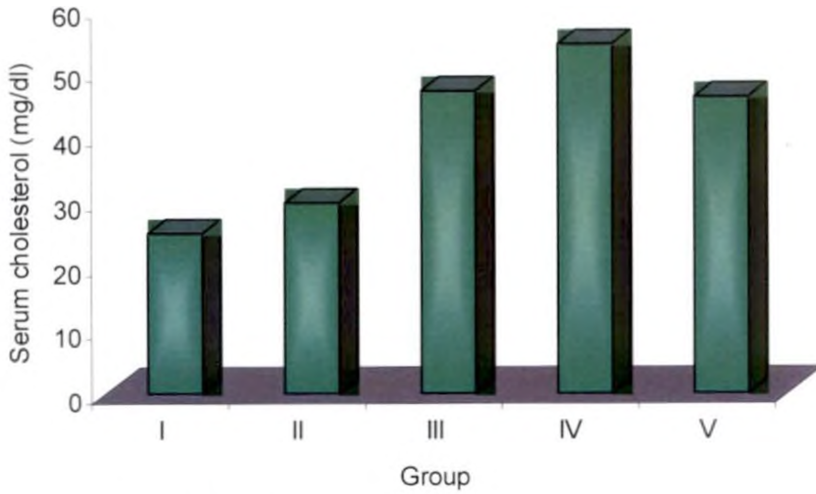

Fig. 10. Effect of treatments on serum cholesterol in carrageenin induced inflammation in rats

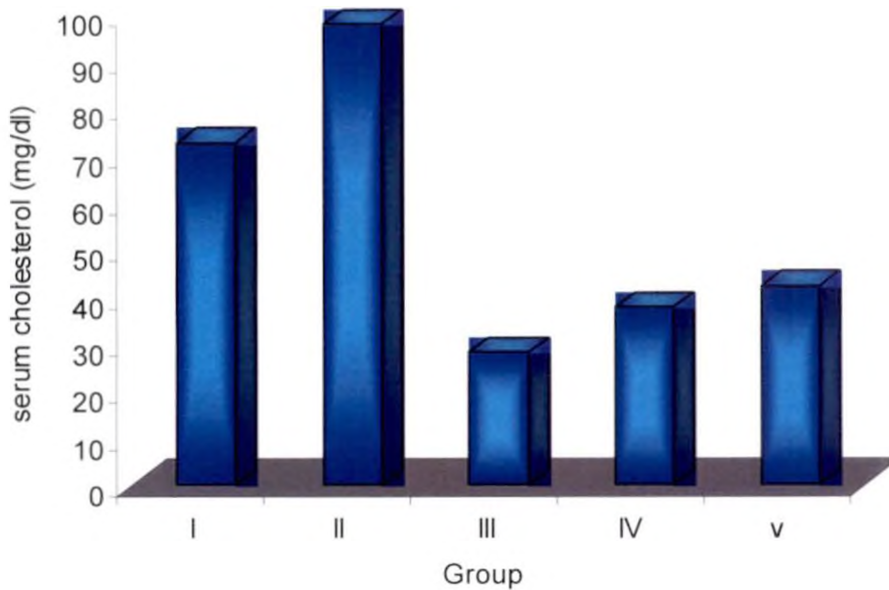

Fig. 11. Effect of treatments on serum cholesterol in tail flick method of nociception in rats

Table 14. Effect of treatments on plasma thiobarbituric acid reacting substances in carrageenin induced inflammation in rats, nM/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	7.10	7.50	19.50	14.50	22.75
2	6.00	7.75	16.25	21.50	23.25
3	6.15	7.25	17.50	22.00	21.00
4	8.00	7.00	18.00	19.50	18.25
5	6.00	7.40	11.00	18.00	20.75
6	9.00	6.25	12.50	19.50	19.25
7	6.75	8.00	15.00	20.00	22.00
8	10.75	6.75	14.50	19.75	21.25
Mean ± SD	7.44 ± 1.63[c]	7.24 ± 0.56[c]	15.53 ± 2.86[b]	19.34 ± 2.32[a]	21.06 ± 1.68[a]

Means bearing same superscript do not differ significantly at $P<0.01$

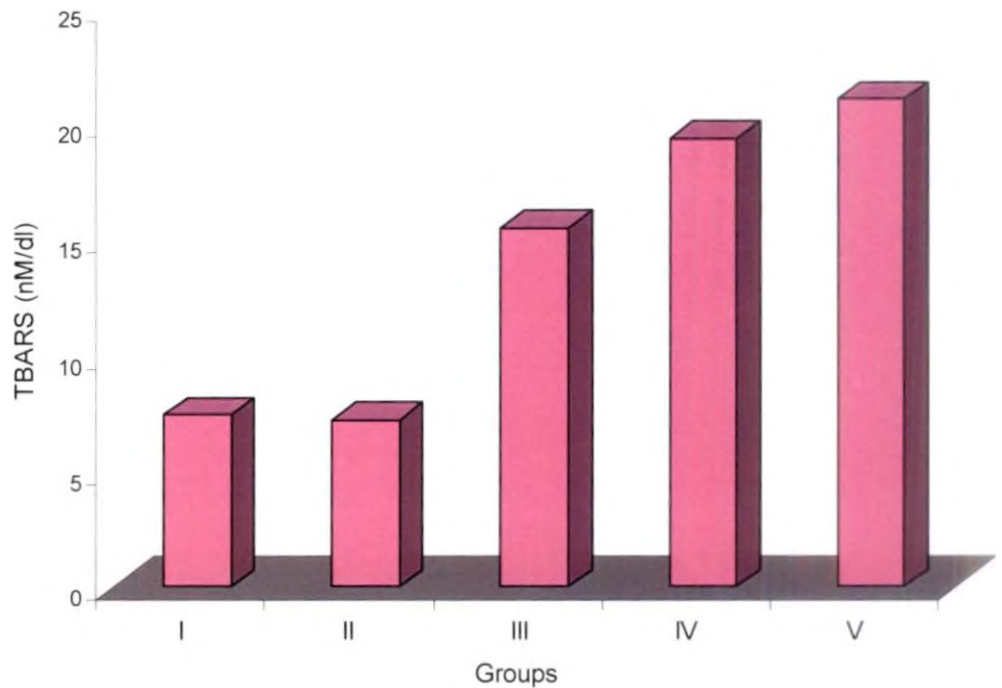

Fig. 12. Effect of treatments on plasma thiobarbituric acid reacting substance in carrageenin induced inflammation in rats

respectively. The treatment groups III, IV and V had a significant increase in TBARS level, whereas the diclofenac treated group was on par with control. TBARS level for group III and IV were 15.53 ± 2.86 and 19.34 ± 2.32 nM/dl respectively. A significant peak increase of 21.06 ± 1.68 nM/dl TBARS was observed in combination group.

4.4.5 Aspartate Amino Transferase (AST)

4.4.5.1 Anti-inflammatory Screening

The data is presented in Table 15 and Fig.13. *V. negundo* treated group IV showed a parallel AST level of 227.13 ±19.71 IU/L to that of control (230.75 ± 12.15 IU/L). There was a significant increase in the mean AST level of group III and V with values 253.13 ± 11.79 and 322.38 ± 21.82 IU/L respectively. Whereas the diclofenac potassium treated positive control group had a significant decrease in AST level and it was 178.00 ± 19.84 IU/L.

4.4.5.2 Anti-nociceptive Screening

The data of AST level for anti-nociceptive screening are presented in Table 16 and Fig.14. The mean AST level for control group was 240.38 ± 8.83 IU/L .Two parallel values of 287.88 ± 9.32 and 288.88 ± 5.84 IU/L of AST were found in *V.negundo* (1000mg/kg) treated group IV and combination (*V.negundo* 500mg/kg and *T.cordifolia* 500mg/kg) treated group V respectively. Animals treated with *T.cordifolia* (1000mg/kg) showed a similar level of AST (246.13 ± 13.44 IU/L) with that of control. A peak increase (299.75 ± 8.46 IU/L) of AST was found in diclofenac treated group ($P<0.01$)

Table 15. Effect of treatments on aspartate amino transferase level in carrageenin induced inflammation in rats, IU/L)

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	238	181	252	214	329
2	218	205	234	256	300
3	224	168	255	227	352
4	213	210	260	226	316
5	247	158	245	220	319
6	234	165	275	222	348
7	244	160	250	199	288
8	228	177	254	256	327
Mean ± SD	230.75 ± 12.15^{c}	178.00 ± 19.84^{d}	253.13 ± 11.79^{b}	227.13 ± 19.71^{c}	322.38 ± 21.82^{a}

Table 16. Effect of treatments on aspartate amino transferase level in tail flick method of nociception in rats, IU/L

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	241	309	235	293	291
2	249	300	227	281	297
3	233	296	267	286	283
4	258	314	244	303	289
5	234	303	258	286	296
6	236	292	238	280	287
7	238	294	243	286	288
8	234	290	257	288	280
Mean ± SD	240.38 ± 8.83^{c}	299.75 ± 8.46^{a}	246.13 ± 13.44^{c}	287.88 ± 7.32^{b}	288.88 ± 5.84^{b}

Means bearing same superscript do not differ significantly at $P<0.01$

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4.4.6 Alanine Amino Transferase (ALT)

4.4.6.1 Anti-inflammatory Screening

The data of Alanine amino transferase are given in Table 17 and Fig.13. A significant increase in ALT level was noticed in all the treatment groups compared to control ($P<0.01$). Mean ALT level of control was 46.88 ± 2.41 IU/L. Group IV, *V.negundo* (100mg/kg) treated group had a significant medium raise of ALT value and it was 49.75 ± 4.2 IU/L. A significant peak increase of ALT value was found in *T.cordifolia* (100mg/kg) treated group III and the value was 82.13 ± 6.63 IU/L. Combination group V showed a significant intermediate increase in ALT level and it was 52.75 ± 6.32 IU/L. However diclofenac potassium treated group II produced the ALT level of 65.75 ± 4.53 IU/L.

4.4.6.2 Anti-nociceptive Screening

The data is recorded and given in Table 18 and Fig.14. The ALT value for control group was 52.13 ± 6.13 IU/L. A significant increase in ALT value was found in all treatment groups ($P<0.01$). Group IV and V did not differ significantly between them but they had shown a peak increase in ALT values and the values were 83.63 ± 3.46 and 85.00 ± 5.53 IU/L respectively. Group III (*T.cordifolia* 1000 mg/kg) showed a medium increase of 57.5 ± 4.24 IU/L. However diclofenac treated positive control group showed a value of 67.13 ± 5.84 IU/L.

4.5 HAEMATOLOGICAL PARAMETERS

4.5.1 Total Leukocyte Count (TLC)

4.5.1.1 Anti-inflammatory Screening

The results are given in Table 19 and Fig.15. The values of total leukocyte count (TLC) of all groups were in normal range. Control and diclofenac treated group had a TLC value of 7462.5 ± 750.12 and 7250 ± 740.66/μl of blood

Table 17. Effect of treatments on alanine amino transferase level in carrageenin induced inflammation in rats, IU/L

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	48	68	90	43	60
2	45	71	92	53	57
3	43	62	77	51	50
4	49	60	75	55	45
5	50	72	83	54	61
6	45	61	76	48	55
7	46	67	78	48	46
8	49	65	86	46	48
Mean ± SD	46.88 ± 2.41[d]	65.75 ± 4.53[b]	82.13 ± 6.63[a]	49.75 ± 4.2[cd]	52.75 ± 6.32[c]

Table 18. Effect of treatments on alanine amino transferase level in tail flick method of nociception in rats, IU/L

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	55	65	56	87	82
2	52	62	54	88	84
3	59	60	63	86	83
4	57	67	55	80	80
5	56	70	54	86	98
6	48	63	55	80	84
7	40	73	58	80	86
8	50	77	55	82	83
Mean ± SD	52.13 ± 6.13[d]	67.13 ± 5.84[b]	57.50 ± 4.24[c]	83.63 ± 3.46[a]	85.00 ± 5.53[a]

Means bearing same superscript do not differ significantly at $P<0.01$

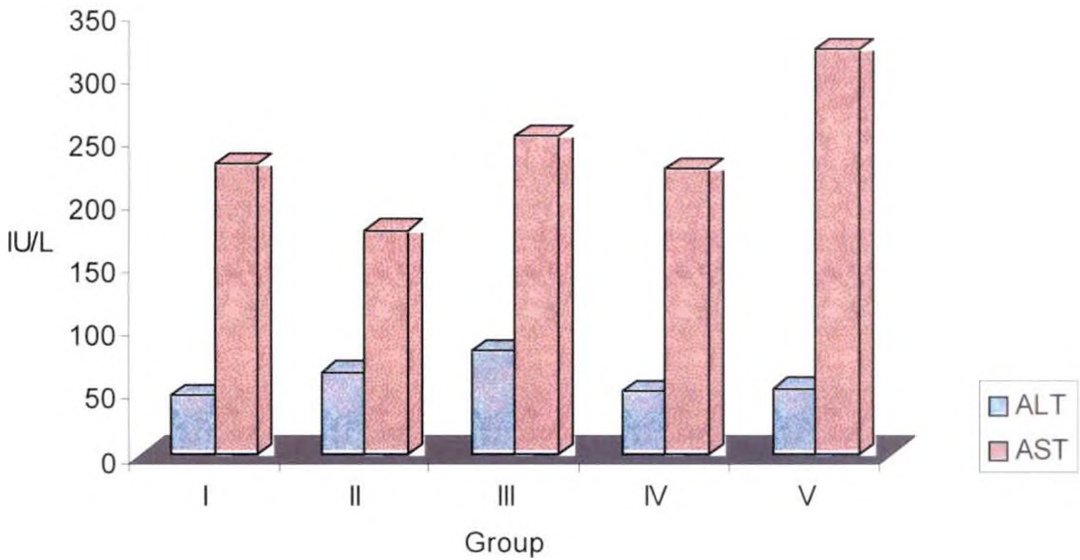

Fig. 13. Effect of treatments on alanine amino transferase & aspartate amino transferase in carrageenin induced inflammation in rats

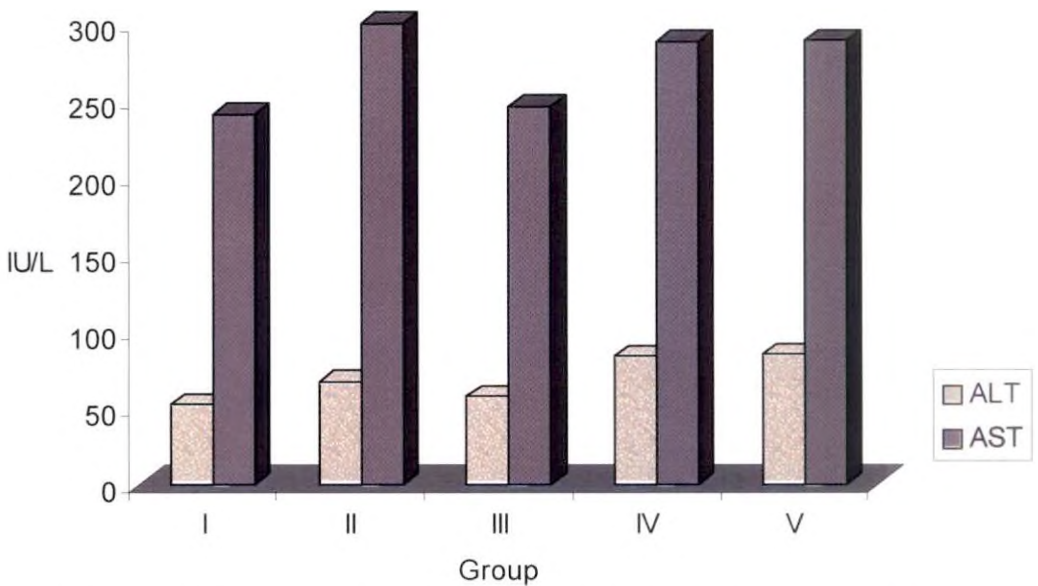

Fig. 14. Effect of treatments on alanine amino transferase & aspartate amino transferase in tail flick method of nociception in rats

respectively. They did not differ significantly between them. TLC value of Group III (6612.5 ± 253.19 µl of blood) and group V had decreased significantly (6250.0 ± 377.96/µl of blood) ($P<0.01$) from control. Whereas *V.negundo* (100mg/kg) treated group showed a significant increase in TLC, which was 8512.5 ± 322.66/µl of blood.

4.5.1.2 Anti-nociceptive Screening

Total leukocyte count (TLC) was recorded after anti-nociceptive screening of each group and the data is given in Table 20 and Fig.16. Control and diclofenac treated groups showed a mean TLC of 10150 ± 1588.35 and 9250 ± 875.05/µl of blood respectively. TLC of group IV (7900 ± 440.78/µl of blood) was found to be significantly decreased from control ($P<0.01$). A mean TLC of 9912.5 ± 795.41/µl represented the *T.cordifolia* (1000mg/kg) treated group with no deviation from control. Group V had shown a significant increase in TLC and it was 11212.5 ± 2308.64/µl of blood.

4.5.2 Total Erythrocyte Count

4.5.2.1 Anti-inflammatory Screening

The results obtained are presented in Table 21 and Fig.17. Group III with a total erythrocyte count of 8.27 ± 0.66 (millions/µl of blood) show a significant increase ($P<0.01$) from the control (7.65 ± 0.34 millions/µl of blood). But the values for all the groups were in the normal range that is 7 to 10 x 10^6 cells/µl of blood.

4.5.2.2 Anti-nociceptive Screening

The data of erythrocyte count for anti-nociceptive screening are given in Table 22 and Fig.18. The diclofenac treated positive control group had an erythrocyte count of 8.31 ± 0.64 millions/µl of blood, which was significantly higher than the control (7.84 ± 0.64 millions/µl of blood). Other treatment group did not show any significant difference among them. The values for all the groups were in the normal range of 7 to 10 x 10^6 cells/µl of blood.

Table 19. Effect of treatments on total leukocyte count in carrageenin induced inflammation in rats, per microlitres of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	8300	7200	6400	8600	6000
2	6700	7500	6600	8400	6400
3	6800	7800	6900	8700	6000
4	7100	6400	6800	8500	6500
5	7700	6400	6800	8900	6000
6	8100	8000	6200	8600	5700
7	6600	6500	6400	7800	6600
8	8400	8200	6800	8600	6800
Mean ± SD	7462.5 ± 750.12[b]	7250.0 ± 740.66[b]	6612.5 ± 253.19[c]	8512.5 ± 322.66[a]	6250.0 ± 377.96[c]

Table 20. Effect of treatments on total leukocyte count in tail flick method of nociception in rats, per microlitres of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	12400	8500	10900	7800	8100
2	8400	9000	9200	7300	7500
3	10700	8600	9000	7400	10500
4	12500	10600	11000	8100	12800
5	8900	8000	9000	7800	12000
6	9400	10000	10000	8100	12800
7	8900	9500	10200	8700	12200
8	10000	9800	10000	8000	13800
Mean ± SD	10150.0 ± 1588.35[ab]	9250.0 ± 875.05[bc]	9912.5 ± 795.41[ab]	7900.0 ± 440.78[c]	11212.5 ± 2308.64[a]

Means bearing same superscript do not differ significantly at $P<0.01$

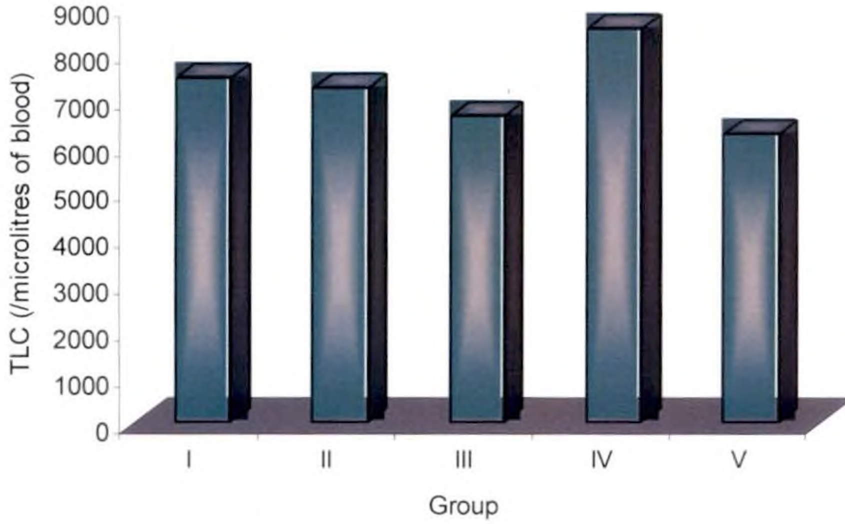

Fig. 15. Effect of treatments on total leukocyte count in carrageenin induced inflammation in rats

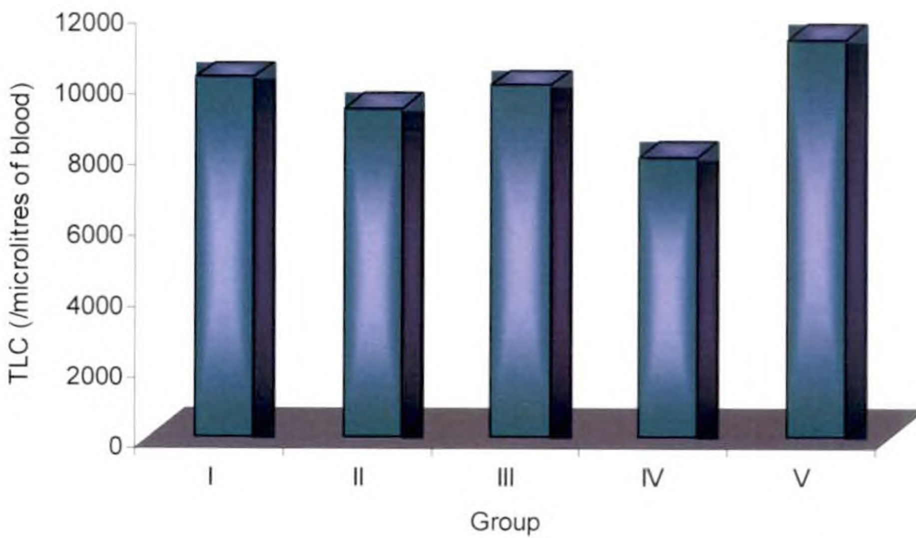

Fig. 16. Effect of treatments on total leukocyte count in tail flick method of nociception in rats

Table 21. Effect of treatments on total erythrocyte count in carrageenin induced inflammation in rats, millions per microlitre of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	7.84	7.54	8.25	8.46	7.34
2	7.38	7.60	7.75	7.80	7.10
3	7.46	7.60	7.58	7.80	7.25
4	7.35	7.82	7.92	7.66	7.20
5	8.00	8.10	9.75	7.89	7.60
6	7.90	7.25	8.30	7.42	7.72
7	7.20	7.60	8.21	8.42	7.72
8	8.10	8.00	8.42	7.24	7.80
Mean ± SD	7.65 ± 0.34^{b}	7.69 ± 0.21^{b}	8.27 ± 0.66^{a}	7.97 ± 0.51^{ab}	7.51 ± 0.35^{b}

Table 22. Effect of treatments on total erythrocyte count in tail flick method of nociception in rats, millions per microlitre of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	9.20	8.60	8.20	7.78	8.20
2	7.20	8.80	7.40	7.65	7.80
3	8.20	8.20	9.00	6.20	7.10
4	7.60	7.20	7.20	6.25	6.30
5	7.56	8.90	8.00	7.26	7.40
6	7.53	8.50	7.80	8.00	7.30
7	8.03	7.50	7.30	7.80	7.25
8	7.40	8.80	7.80	7.28	7.38
Mean ± SD	7.84 ± 0.64^{b}	8.31 ± 0.64^{a}	7.84 ± 0.59^{ab}	7.28 ± 0.70^{b}	7.30 ± 0.51^{b}

Means bearing same superscript do not differ significantly at $P<0.01$

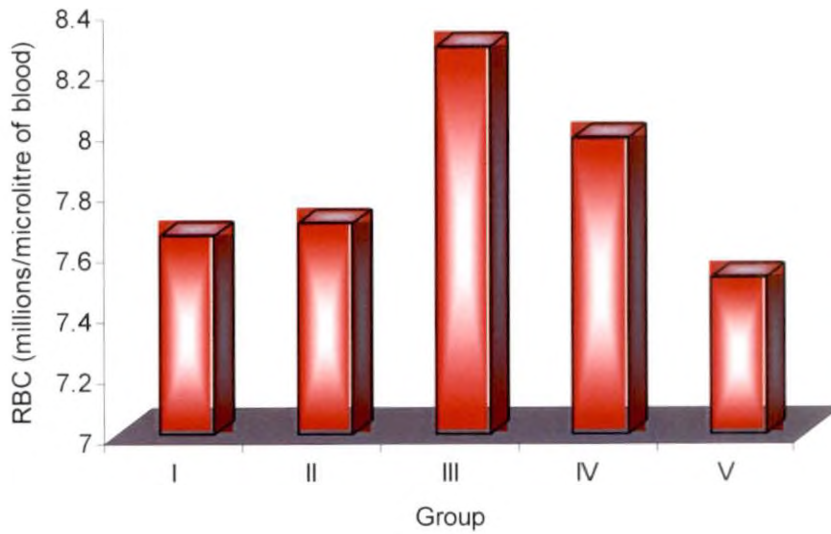

Fig. 17. Effect of treatments on total erythrocyte count in carrageenin induced inflammation in rats

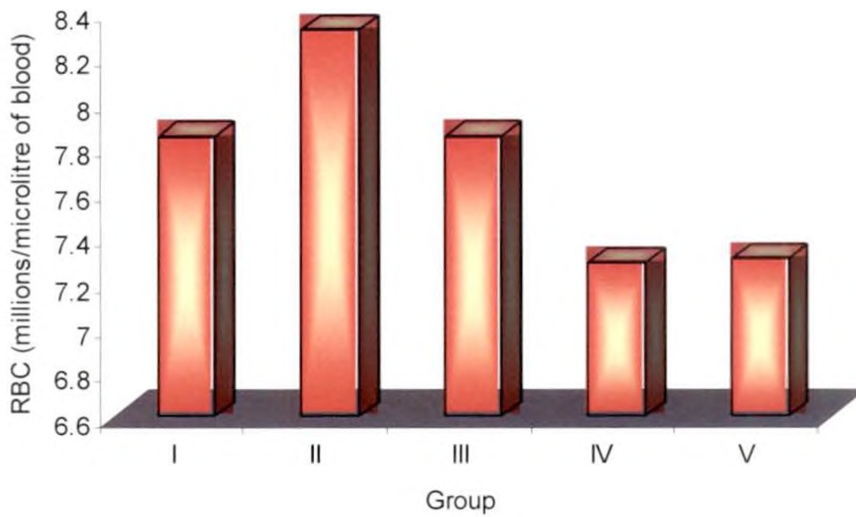

Fig. 18. Effect of treatments on total erythrocyte count in tail flick method of nociception in rats

4.5.3 Haemoglobin Concentration

4.5.3.1 Anti-inflammatory Screening

The values are presented in Table 23 and Fig.19. The mean haemoglobin concentration of group I to V were 10.88 ± 0.76, 11.03 ± 1.04, 13.63 ± 2.10, 13.80 ± 1.68 and 10.03 ± 1.02 g/dl respectively. Group V and II had no significant difference with control in haemoglobin concentration. Whereas a significant increase was found in group III and IV. But the values were within the normal range, which was 10 to18 g/dl.

4.5.3.2 Anti-nociceptive Screening

The data are given in Table 24 and Fig.20. All the treatment groups namely *T.cordifolia* (13.81 ± 1.01g/dl), *V.negundo* (10.76 ± 2.22g/dl) and combination (12.58 ± 0.78g/dl) showed a significant decrease in haemoglobin concentration compared to control (14.60 ± 1.95g/dl). Whereas the diclofenac treated group had almost similar value of 14.01 ± 1.68 g/dl.

4.5.4 Differential Leukocyte Count

4.5.4.1 Anti-inflammatory Screening

4.5.4.1.a Neutrophils

The data are presented in Table 25 and Fig.21. All the groups showed a significant increase in neutrophil than the normal count. The counts were 68.38 ± 2.20, 64.00 ± 3.89 and 62.63 ± 4.34 per cent in Group III, IV and V respectively. Group IV and V showed a significant decrease ($P<0.01$) in neutrophil count compared to control (65.00 ± 5.66).

4.5.4.1.b Lymphocytes

The results are represented in Table 25 and Fig.21. All the groups showed a significant decrease in lymphocyte count than the normal values. The counts

Table 23. Effect of treatments on haemoglobin concentration in carrageenin induced inflammation in rats, g/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	12.00	9.50	15.00	15.20	9.00
2	10.50	9.50	11.00	13.40	8.80
3	10.80	11.20	12.60	12.50	10.20
4	11.00	12.00	13.80	16.80	12.00
5	12.00	12.00	18.00	12.00	9.40
6	10.00	10.80	12.60	12.50	10.60
7	10.50	11.20	12.80	15.00	10.00
8	10.20	12.00	13.20	13.00	10.20
Mean ± SD	10.88 ± 0.76[b]	11.03 ± 1.04[b]	13.63 ± 2.10[a]	13.80 ± 1.68[a]	10.03 ± 1.02[b]

Table 24. Effect of treatments on haemoglobin concentration in tail flick method of nociception in rats, g/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	18.00	13.20	14.00	13.80	14.00
2	15.00	15.20	13.80	11.00	12.00
3	11.80	12.30	12.50	8.50	11.50
4	13.60	15.80	14.00	9.80	12.60
5	12.60	15.00	14.50	12.00	13.00
6	14.80	11.00	13.00	11.50	12.50
7	15.00	15.20	13.00	12.50	13.00
8	15.00	15.20	13.00	12.50	13.00
Mean ± SD	14.60 ± 1.95[a]	14.01 ± 1.68[ab]	13.81 ± 1.01[ab]	10.76 ± 2.22[c]	12.58 ± 0.78[b]

Means bearing same superscript do not differ significantly at $P<0.01$

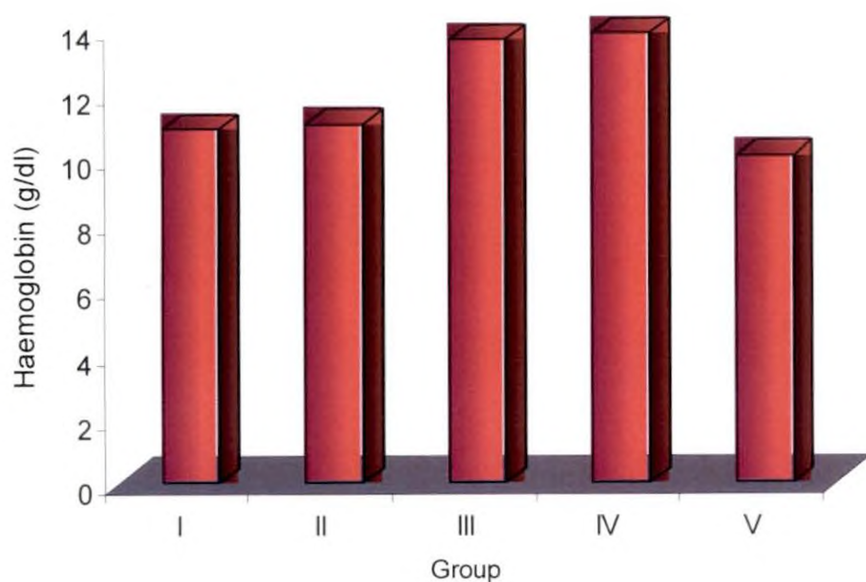

Fig. 19. Effect of treatments on haemoglobin concentration in carrageenin induced inflammation in rats

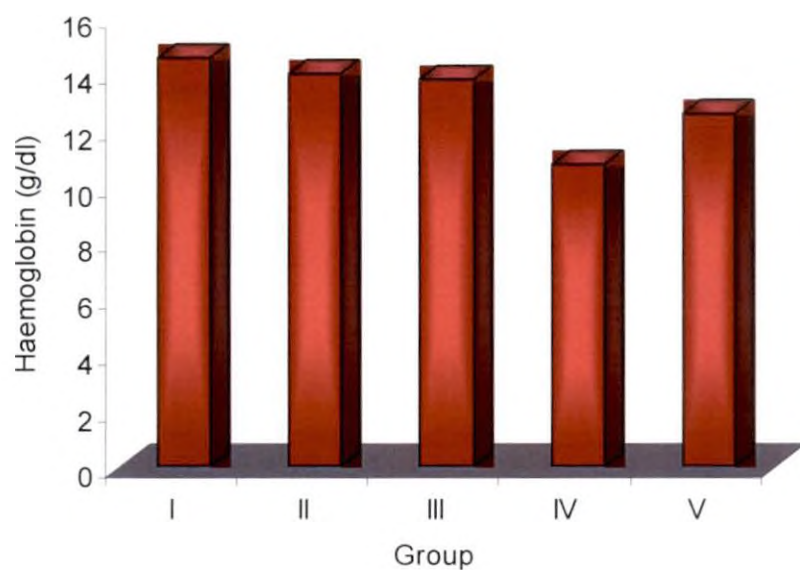

Fig. 20. Effect of treatments on haemoglobin concentration in tail flick method of nociception in rats

Table 25. Effect of treatments on differential leukocyte count in carrageenin induced inflammation in rats, %

Animal No.	Group I				Group II				Group III				Group IV				Group V			
	N	L	M	E	N	L	M	E	N	L	M	E	N	L	M	E	N	L	M	E
1	60	36	4	-	72	28	-	-	70	30	-	-	64	34	2	-	66	80	2	2
2	72	28	-	-	75	24	-	1	72	28	-	-	68	30	1	1	57	40	2	1
3	56	40	2	2	72	26	2	-	68	30	2	-	70	28	0	2	60	39	1	-
4	68	30	2	-	70	26	3	1	67	32	1	-	60	38	2	-	60	40	-	-
5	66	34	-	-	68	30	2	-	70	29	1	-	62	36	2	-	59	40	1	-
6	70	28	1	1	69	30	1	-	68	32	-	-	67	30	1	2	64	35	1	-
7	68	30	2	-	70	28	-	2	65	34	1	-	60	40	-	-	70	28	2	-
8	60	36	2	2		28	2	3	67	33	-	-	61	38	1	-	65	34	1	-
Mean ± SD	65.00 ± 5.66^{bc}	32.75 ± 4.40^{ab}	1.62 ± 1.30	0.63 ± 0.92	70.13 ± 3.00^{a}	27.50 ± 2.07^{c}	1.25 ± 1.16	0.88 ± 1.13	68.38 ± 2.20^{ab}	31.00 ± 2.07^{bc}	0.63 ± 0.74	-	64.00 ± 3.89^{c}	34.25 ± 4.46^{ab}	1.13 ± 0.83	0.63 ± 0.92	62.63 ± 4.34^{c}	35.75 ± 4.80^{a}	1.85 ± 0.71	0.38 ± 0.74

Means bearing the same superscript do not differ significantly at P<0.01

were 32.75 ± 4.40, 27.50 ± 2.07, 31.00 ± 2.07, 34.25 ± 4.46 and 35.75 ± 4.80 per cent for group I to V respectively. The counts were found increasing significantly ($P<0.01$) for group IV and V from that of control.

4.5.4.1.c Eosinophils

The data are presented in Table 25 and Fig.21. The eosinophil count of all different groups did not differ significantly. The values were within the normal range, 0 to 5 per cent.

4.5.4.1.d Monocyte

Monocyte count is presented in Table 25 and Fig.21. The monocyte count of all different groups did not differ significantly. The values were within normal range of 0 to 7 per cent.

4.5.4.2 Anti-nociceptive Screening

4.5.4.2.a Neutrophils

The data are presented in Table 26 and Fig.22. The neutrophil counts for the group I to V were 27.88 ± 8.12, 21.00 ± 6.78, 33.75 ± 3.15, 30.50 ± 6.91, 30.38 ± 5.07 per cent respectively. All the treatment groups except group II showed a significant increase from control. Whereas diclofenac treated group showed significant decrease when compared to control ($P<0.01$).

4.5.4.2.b Lymphocytes

Lymphocyte count for anti-nociceptive screening is presented in Table 26 and Fig.22. The counts for group I to V were71.5 ± 8.55, 77.88 ± 6.27, 63.75 ± 3.06, 68.38 ± 6.7 and 67.88 ± 4.09 per cent respectively. All the treatment groups except diclofenac treated group had shown a significant decrease in lymphocyte count from control ($P<0.01$).

Table 26. Effect of treatments on differential leukocyte count in tail flick method of nociception in rats, %

Animal No.	Group I				Group II				Group III				Group IV				Group V			
	N	L	M	E	N	L	M	E	N	L	M	E	N	L	M	E	N	L	M	E
1	30	69	1	-	24	76	-	-	32	65	3	-	30	68	2	-	32	66	2	-
2	28	70	1	1	26	74	-	-	40	58	2	-	36	62	2	-	20	76	3	1
3	15	84	1	-	15	84	1	-	32	65	3	-	40	60	-	-	34	64	2	-
4	36	60	3	1	28	71	1	-	35	64	1	-	18	80	2	-	32	68	-	-
5	40	60	-	-	30	69	1	-	33	66	1	-	30	69	1	-	35	65	-	-
6	24	76	-	-	18	80	2	-	30	66	2	2	28	71	1	-	30	70	-	-
7	26	74	-	-	14	84	2	-	32	66	1	1	26	74	-	-	34	64	2	-
8	20	79	1	-	13	85	2	-	36	60	2	2	36	63	1	-	26	70	4	-
Mean ± SD	27.88 ± 8.12^{b}	71.50 ± 8.55^{b}	0.88 ± 0.99	0.25 ± 0.46	21.00 ± 6.78^{c}	77.88 ± 6.27^{a}	1.13 ± 0.83	-	33.75 ± 3.15^{a}	63.75 ± 3.06^{c}	1.88 ± 0.83	0.63 ± 0.92	30.5 ± 6.91^{ab}	68.38 ± 6.70^{bc}	1.13 ± 0.83	-	30.38 ± 5.07^{ab}	67.88 ± 4.09^{bc}	1.63 ± 1.51	0.13 ± 0.35

Means bearing the same superscript do not differ significantly at $P<0.01$

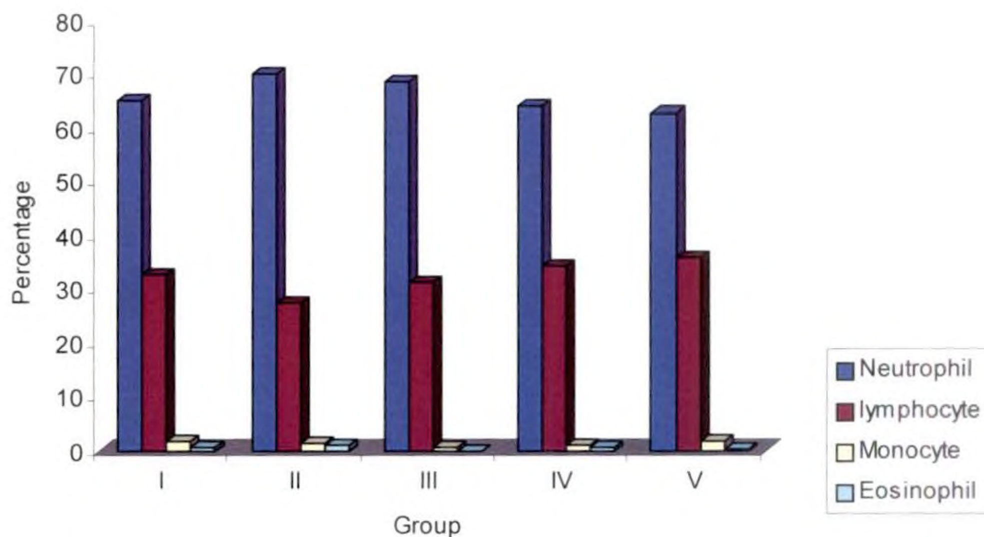

Fig. 21. Effect of treatments on differential leukocyte count in carrageenin induced inflammation in rats

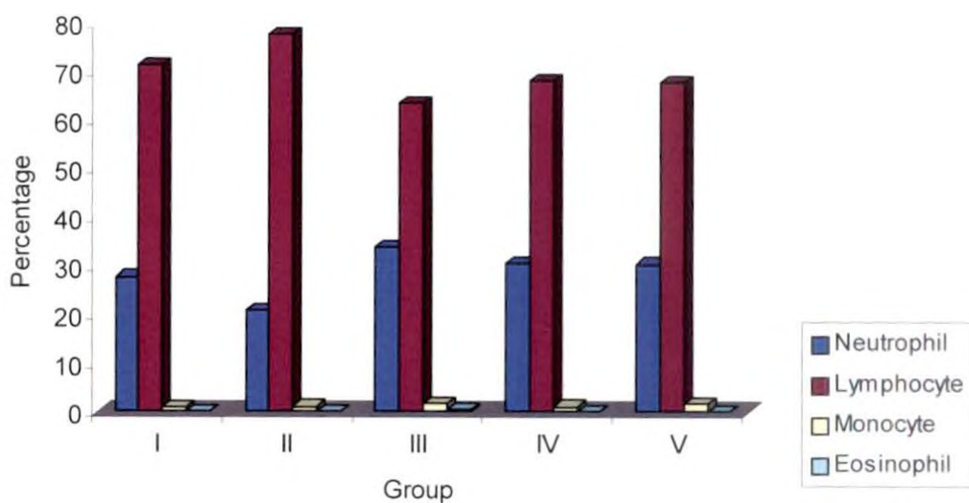

Fig. 22. Effect of treatments on differential leukocyte count in tail flick method of nociception in rats

4.5.4.2.c Eosinophils

The values are presented in Table 26 and Fig.22. The eosinophil count for both treatment and control groups were within the normal range of 0 to 5 per cent. There was no significant difference between groups.

4.5.4.2.d Monocyte

The results are given in Table 26 and Fig.22. The monocyte count for all the groups was within the normal range of 0 to 7 per cent. There was no significant difference between groups.

Discussion

5. DISCUSSION

5.1 ANTI-INFLAMMATORY STUDY

Carrageenin-induced hind paw oedema is one of the standard experimental modelsof acute inflammation. Carrageenin is the phlogistic agent of choice for testing anti-inflammatory drugs, as it is not known to be antigenic and is devoid of apparent systemic effects. Carrageenin induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 hours (Vinegar *et al.*, 1969). Complement activity is also involved throughout the carrageenin induced inflammation (Giroud and Willoughby, 1970).

The present study shows that the alcoholic extract of *Tinospora cordifolia* and *Vitex negundo* have significant anti-inflammatory effect against carrageenin induced rat paw oedema, with the former having more effect on second phase and the later having much effect on the first phase. The alcoholic extract combination of *T. cordifolia* and *V. negundo* sustained the inhibition of carrageenin induced inflammation in both the phases. The inhibition was however, less than that of the standard drug, diclofenac potassium.

Jana *et al.* (1999) have observed that *V. negundo* and *T. cordifolia* had significant anti-inflammatory effect against carrageenin induced inflammation and the effect of *T. cordifolia* was more than that of acetyl salicylic acid.

Flavonoids are known to inhibit prostaglandins which are involved in inflammation and pain perception (Rajnarayana *et al.*, 2001). Hence, the presence of flavonoids may be contributory to the anti-inflammatory activity of both *T. cordifolia* and *V. negundo. T. cordifolia* was reported with various classes of chemical constituents such as protoberberine alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids and phenols (Singh *et al.*, 2003). Presence

of various flavonoid compounds like 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone and 3,5 diethydroxy-3',4', 6,7-tetramethoxy flavonol were reported in the leaves of *V. negundo* (Telang *et al.*, 1999).

Pendse *et al.* (1981) reported that *T. cordifolia* have significant anti-inflammatory activity against carrageenin induced oedema in rats and have postulated that the anti-inflammatory activity could be due to interference with the release of histamine, 5HT and prostaglandins.

Kapil and Sharma (1997) had stated that pure compounds like syringin and cordiol isolated from *T. cordifolia* have inhibitory effect on C_3-convertase, a serine protease which indicates that inhibition of serine protease in general may be involved in anti-inflammatory activities. Peptide mediators like C_3a and C_5a derived from the complement cascade act as chemotactic mediators in leukocyte accumulation (Grant, 1973; Ward *et al.*, 1974). When C_3 convertase is inhibited, proinflammatory anaphylactic peptides are not released with the result no inflammation is observed. Thus the anti-inflammatory effect of agents that lower complement titres were found to have significant effect against carrageenin induced inflammation (DiRosa *et al.*, 1971).

The higher inhibitory effect of *T. cordifolia* on the second phase of carrageenin induced inflammation may be due to antagonistic action against prostaglandin and anti-complement activity.

Inhibition of the first phase of carrageenin induced oedema more effectively by *V. negundo* suggested an antagonistic effect against mediators like histamine and serotonin (Gaidhani *et al.*, 2001).

Dharmasiri *et al.* (2003) suggested that anti-inflammatory effect of *V. negundo* in formalin induced chronic inflammation was due to inhibition of prostaglandin synthesis. The decrease in anti-inflammatory activity of the second phase of carrageenin induced inflammation was an indication of short duration of action of *V. negundo.* This may be due to the increase of leukotrienes at the

second phase caused by the inhibition of prostaglandin synthesis, which diverts the reaction towards increase in leukotrienes synthesis (Mayes, *1996*).

Inhibition of carrageenin induced inflammation on both the phases in uniform level by alcoholic extract combination of *T. cordifolia* and *V. negundo* may be due to the added mechanism of both. They include anti-complement activity, inhibition of the synthesis, release or action of inflammatory mediators viz., histamine, serotonin and prostaglandin.

5.2 ANTI-NOCICEPTION

The results of the present study revealed the anti-nociceptive effect of both the extracts and its combination.

Prostaglandins play a significant role in different phases of inflammatory reactions. Prostaglandins elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli (Campbell, 1991). A number of flavonoids have been reported to produce analgesic activity (Hossinzadeh *et al.*, 2002) by reduced availability of prostaglandins. Hence the present anti-nociceptive activity of *T. cordifolia* and *V. negundo* may be attributed to the presence of flavonoids.

The tail flick test of nociception measures the complex response to an acute, non inflammatory, nociceptive input and can be considered a good model for studying central anti-nociceptive activity.

Significant anti-nociceptive effect in tail flick method by *T. cordifolia* and *V. negundo* exhibited the involvement of central mechanism in modulating nociception. Inhibition of cyclo-oxygenase may not be solely responsible for the central analgesic effect of anti-inflammatory drugs (Malmberg and Yaksh, 1992).

Anti-nociceptive 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).

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).

Anti-nociceptive effect of diclofenac cannot be directly explained by plasma concentration of diclofenac (Menasse *et al.*, 1978). The present study shows a gradual increase in reaction time till the end of the observation, for the diclofenac treated group. It has been suggested that the anti-nociceptive and anti-inflammatory effects of diclofenac depend on the NSAID levels at the injured site, which may not be in equilibrium with the circulation (Kyuki, 1982).

Analgesic activity of NASIDs like aspirin, diclofenac, flurbiprofen and nimesulide administered in different combination was time dependent and highest at 90 minutes post drug administration. Velankar *et al.* (1998) also found that diclofenac sodium, aspirin and their combinations exhibited rapid and pronounced analgesic activity.

Dharmasiri *et al.* (2003) reported that the mature fresh leaf extract of *V. negundo* possessed supraspinally mediated central nociception without opioid receptor mediated action. Srivastava and Sisodia (1970) reported NSAID like action for analgesic effect of *V. negundo* fruit. However, Gupta *et al.*(1999) reported a potentiating action of *V. negundo* leaf extracts on morphine and pethidine induced analgesia, besides a CNS depressant action.

Water extract of *T. cordifolia* showed a weak analgesic effect of its own and it potentiated morphine analgesia (Pendse *et al.*, 1980).

The present study on the anti-nociceptive effect of ethanolic extract combination of *T. cordifolia* and *V. negundo* reveals no synergism in action but exerts a similar range of reaction time suggesting NSAID like action and central mediated anti-nociception.

5.3 EFFECT OF ADRENAL GLAND WEIGHT AND ASCORBIC ACID

5.3.1 Anti-inflammatory Screening

Carrageenin induced acute inflammation in the hind paw of rat led to a factor formation probably by cell distruption, protein break down or enzymatic activation. This factor is concentrated in the inflamed tissue and is slowly released into the circulation. Stimulating the hypothalamo-pituitary-adrenal axis resulting in release of corticosterone, which is responsible for anti-inflammatory action (Robinson and Robson, 1964; Leme and Schapoval, 1975).

Adrenal ascorbic acid has been implicated in the oxidative biosynthesis of the corticosteroids, the protection of adrenal enzymes active in corticosteroidogenesis from oxidative inactivation (Hechter and Pincus, 1954) and as a component part of the storage forms of corticosteroids in the adrenal gland (Lowenstein and Zwemer, 1946; Slusher and Roberts, 1957).

Pohujani *et al.* (1969) observed a constant ratio of adrenal ascorbic acid and adrenal cholesterol under varying conditions suggested a possibility of a cholesterol ascorbic acid linkage in the adrenal.

Naik and Sheth (1972) found depletion of adrenal ascorbic acid and increase in blood cholesterol level immediately after centrifugal stress. There is marked depletion of adrenal ascorbic acid in inflammation. The depletion of adrenal ascorbic acid persists for three to six hours in carrageenin induced oedema.

In the present study *T. cordifolia, V. negundo* and their combination not only reduced carrageenin induced inflammation, but also prevented inflammation induced changes in adrenal weight and ascorbic acid.

5.3.2 Anti-nociceptive Screening

Certain drugs comprise possible interaction with endogenous anti-inflammatory systems as one of its mechanisms to produce anti-nociceptive effect. The endogenous anti-inflammatory system is the so called anterior pituitary and adrenal cortex axis.

Many drugs and foreign chemicals cause a non-specific stimulation of the pituitary adrenal axis and this may be dose dependant. For example salicylates in relatively large doses cause responses both direct and indirect which are typical of adreno cortical stimulation (Smith, 1966).

In the present study, the adrenal gland weight of animals treated with *T. cordifolia* and combination of *T. cordifolia* and *V. negundo* shows weight similar to that of the control. Group treated with *V. negundo* show a slight non significant increase in adrenal gland weight. This suggests a similar change in adrenal gland with that of control.

However increase in adrenal ascorbic acid content of *T. cordifolia* and *V. negundo* treated groups reveals the protective effect against stress induced changes in adrenal gland.

5.4 ADRENAL CHOLESTEROL

5.4.1 Anti-inflammatory Screening

The increased level of adrenal cholesterol and ascorbic acid with significant decrease of adrenal gland weight by betamethasone indicated the inhibition of adrenocorticotrophin release from anterior pituitary (Rathor and Goyal, 1973).

The present study shows a significant increase in adrenal cholesterol for *T. cordifolia* and *V. negundo* treated animals under carrageenin induced inflammation. This confirms the adaptogenic property of these drugs along with

increase in adrenal ascorbic acid. Whereas the combination treated rats exhibit a decrease in adrenal cholesterol with an increase in adrenal ascorbic acid.

Gupta and Sharma (2003) found a significant elevation of testicular cholesterol followed by reduction in testosterone levels in male rats when a methanolic extract of *T. cordifolia* stem *at* a dose of 100 mg/rat /day was given for 60 days.

5.4.2 Anti-nociceptive Screening

Adrenal cholesterol levels were reduced in all treatment groups compared to control. The decrease in adrenal cholesterol with non significant increase of adrenal gland weight in anti-nociceptive study suggests involvement of endogenous modulatory system for anti-nociception. Whereas, the increase in adrenal ascorbic acid by the treatment groups was in contrary. This needs a further investigation by estimation of endogenous glucocorticoid/adrenalectomy technique to probe the involvement of endogenous modulatory system in anti-nociception.

5.5 SERUM CHOLESTEROL

5.5.1 Anti-inflammatory Screening

The values of serum cholesterol for anti-inflammatory screening as presented in Table 12 and Fig.10 are found within the normal range for all treatment groups. Mean serum cholesterol level of each treatment group was raised from the depletion caused by the inflammation. However, *V. negundo* replenish the depleted serum cholesterol effectively comparing the other treatment groups.

5.5.2 Anti-nociceptive Screening

The results of serum cholesterol for treatments in anti-nociceptive study showed a lowering effect. Control group showed a normal level of serum cholesterol. The diclofenac potassium (3 mg/kg) treated positive control flared up the level of serum cholesterol. Whereas all the extract treated groups kept the level lowered. *T. cordifolia* (1000 mg/kg) was found effective in keeping the serum cholesterol lower than the other treatment groups.

Prince *et al.* (1999) reported that aqueous extract of *T. cordifolia* possessed hypolipidaemic action in alloxan diabetic rats.

5.6 PLASMA TBARS OF ANTI-INFLAMMATORY STUDY IN RATS

Mathew and Kuttan (1996) found a 30 per cent inhibition of cyclophosphamide induced TBARS in liver homogenate incubated with 5 g/ml of aqueous extract of *T. cordifolia.*

Oral administration of an aqueous *T. cordifolia root* extract (2.5 and 5.0 g/kg) for six weeks resulted in decreased level of plasma TBARS in alloxan diabetic rats (Prince and Menon, *1999*).

Goel and Kumar (2002) explained the anti-oxidant property of aqueous extract of *T. cordifolia* by inhibition of Fenton reaction mediated lipid peroxidation (TBARS) in a dose dependant manner, 50 per cent inhibition at 1300 ug/ml concentration and 70 per cent at 2000 µl/ml concentration.

The present study on the plasma TBARS by ethanolic extract of *T. cordifolia, V. negundo* and their combination showed no inhibition. The ethanolic extract combination was found to increase the TBARS level in plasma high, compared to their individual drugs.

5.7 ASPARTATE AMINO TRANSFERASE (AST) AND ALANINE AMINO TRANSFERASE (ALT)

5.7.1. Anti-inflammatory Screening

Serum AST and ALT were found to be increased in carrageenin induced inflammation. Pretreatment with ethanolic extract of *V. negundo* only sustained the level of enzymes with that of control whereas pretreatment with ethanolic extract of *T. cordifolia* in 100 mg/kg dose and the combination of extract in the same dose had no control over the inflammation induced enzyme levels to have a protective role.

5.7.2 Anti-nociceptive Screening

The rise in serum level of AST and ALT in nociception was not reduced by any of the treatments. However ethanolic extract of *T. cordifolia* at 1000 mg/kg restored the level of enzymes with that of control.

Tandon and Gupta (2004) reported that, *Vitex negundo* induced histomorphological changes in liver and heart at higher doses, and concluded that *V. negundo* can induce changes in heart at 1000 mg/kg dose rate.

5.8 HAEMATOLOGICAL PARAMETERS

5.8.1 Anti-inflammatory Screening

Mathew and Kuttan (1996) found that *T. cordifolia* improved the haemopoietic conditions such as WBC count and bone marrow cellularity in cyclophosphamide induced toxicity.

Patil *et al.* (1997) observed a best protective effect in neutrophil count by *T. cordifolia* against cyclophosphamide induced cytotoxicity damage.

An increase in total leukocyte count was found in *V. negundo* (100 mg/kg) treated group. The ethanolic extract of *T. cordifolia* (100 mg/kg) and

ethanolic extract combination (50 + 50 mg/kg) significantly reduced the total leukocyte count in rats.

T. cordifolia raised the neutrophil count significantly from that of control at a dose rate of 100 mg/kg, whereas combination group showed a concomitant decrease in neutrophil count along with total leukocyte count.

Total erythrocyte count and haemoglobin were found to be increased by *V. negundo* and *T. cordifolia* at 100 mg/kg dose rate each. Whereas it was in control level for the ethanolic extract combination treated group.

5.8.2 Anti-nociceptive Screening

Cell proliferative effect of Immu-21, a polyherbal formulation containing *T. cordifolia* was observed by Nemmani *et al.* (2002). Immu-21 did increase the cellularity of splenic leukocytes at 1, 10 and 30 mg/kg, i.p. dose rate.

Nagarkatti *et al.* (1994) suggested that modulation of kupffer cell activity was brought about by macrophage activating property of *T. cordifolia*

Ethanolic extract combination raised the total leukocyte count significantly in anti-nociceptive study, whereas *V. negundo* (1000 mg/kg) treated group showed a decrease in total leukocyte count.

T. cordifolia at 1000 mg/kg increased the neutrophil count in nociceptive treatment whereas the *V. negundo* and combination treated group in same dose were on par with control for neutrophil count.

All the treatment groups maintained the control level for total erythrocyte count. There was a significant fall in haemoglobin concentration with *V. negundo* and the combination treatment.

Summary

6. SUMMARY

The present study was undertaken to assess and compare the individual and additive anti-inflammatory and anti-nociceptive effect of *Tinospora cordifolia* and *Vitex negundo* in rats. Diclofenac potassium was used as a standard for both anti-inflammatory and anti-nociceptive screening. Forty adult Wistar rats of 150-200 g body weight were divided into five groups of eight each and maintained for anti-inflammatory screening. All the groups were fed with *ad libitum* normal feed. Three per cent tween 80, diclofenac potassium at the rate of 3 mg/kg body weight, ethanolic extract of *T. cordifolia* at the rate of 100 mg/kg, *V. negundo* at the rate of 100 mg/kg and the combination at the rate of 50 mg *T. cordifolia* and 50 mg *V. negundo*/kg body weight were fed to group I, II, III, IV, and V respectively for 7 days except group II in which the drug was administered on the 7th day.

Similarly five other groups were maintained for anti-nociceptive study. A dose of 1000 mg/kg body weight of *T. cordifolia, V. negundo* and combination of both drugs (500 mg *T. cordifolia* and 500 mg *V. negundo*), were administered in group III, IV and V respectively for anti-nociceptive study.

Anti-inflammatory effect was studied in carrageenin induced hind paw oedema model of acute inflammation. *T. cordifolia* showed a significant inhibition of carrageenin induced oedema at the second phase and *V. negundo* at the first phase. The combination of both the drugs produced a significant uniform level of inhibition in both the phases.

Tail flick method of nociception was carried out to assess the anti-nociceptive effect of the treatments. All the treatments exhibited anti-nociceptive effect mediated through central mechanism.

Different parameters of adrenal gland namely gland weight, ascorbic acid and cholesterol levels, the serum level of enzymes ALT and AST, serum

cholesterol and haematological parameters namely total and differential leukocyte count, total erythrocytes and haemoglobin concentration were investigated in both anti-inflammatory and anti-nociceptive screening groups on the 7th day of the experiment.

There was no significant difference in adrenal gland weight among groups in anti-inflammatory study. However all the treatment groups showed a non-significant decrease in gland weight compared to the control. Adrenal ascorbic acid was increased in all the treatments, with a peak increase noticed in *V. negundo* treated group. *T. cordifolia* could significantly increase the adrenal cholesterol and *V. negundo* to get the level away from control. All the above adrenal parameters suggested the effect of the test drugs in preventing the inflammation induced changes in adrenal gland. However, the combination of *T. cordifolia* and *V. negundo* was found to decrease the adrenal cholesterol significantly from control and adrenal ascorbic acid from that of individual drugs. This conferred that the combination may lower the effect of test drugs in preventing the inflammation induced changes in adrenal gland.

All the treatment groups in anti-nociceptive study showed a similar increase in weight from that of control. There was also a significant decrease in adrenal cholesterol suggesting the endogenous modulatory system as one of the mechanism involved in anti-nociception. However the increase in adrenal ascorbic acid level by *T. cordifolia* and *V. negundo* was in contrary. An assay of endogenous glucocorticoid is necessary to probe its action.

T. cordifolia was found effective among the treatments which lowered the serum cholesterol compared to the control in anti-nociceptive study.

Control group showed low serum cholesterol in carrageenin induced inflammation. However *V. negundo* topped among the treatments, which replenished the depletion showed by control, to the half way normal.

None of the treatments were found to inhibit the lipid peroxidation induced by inflammation in plasma. *V. negundo* and the combination of extracts showed a significant increase of plasma lipid peroxide level.

The rise in serum level of AST and ALT in both inflammation and nociception was not found to be inhibited by the treatments.

A rise in neutrophil count without an increase in total leukocyte count was found in *T. cordifolia* treated group, both in carrageenin induced inflammation and tail flick nociception.

V. negundo showed a increase in total leukocyte count in anti-inflammatory study and not in anti-nociceptive study. A decrease in both total leukocyte count and neutrophil count was observed in ethanolic extract combination treated group in anti-inflammatory study. However there was an increase in total leukocyte count for tail flick nociception.

Total erythrocyte count and haemoglobin concentration were increased by *T. cordifolia* in both the studies, whereas they were in control level for the ethanolic extract combination treatment. *V. negundo* showed a increase in haemoglobin concentration and total erythrocyte count in anti-inflammatory study. It showed a decrease in haemoglobin concentration and control level of total erythrocyte count in anti-nociceptive study.

In ayurveda, combinations of plant preparations are commonly used in inflammatory conditions and other ailments. The present study with combination showed a significant additive effect against acute inflammation. The anti-nociceptive and hypolipidaemic effect are found to be significant with no additive/synergestic effect in combination. The protective effect of the herbal agents need further investigation.

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COMPARATIVE STUDY OF ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE EFFECT OF *Tinospora cordifolia* (Chittamruthu) AND *Vitex negundo* Linn. (Karinochi) IN RATS

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ABSTRACT

Anti-inflammatory and anti-nociceptive effect of *T. cordifolia*, *V. negundo* and the combination of two herbal agents were assessed and compared in rats. Carrageenin induced rat paw oedema and tail flick method of nociception were adopted for anti-inflammatory and anti-nociceptive screening respectively. Diclofenac potassium at the rate of 3 mg/kg was used as a standard drug for both the studies. Anti-inflammatory effect of *T. cordifolia* at the rate of 100 mg/kg, *V. negundo* at the rate of 100 mg/kg and the combination of these agents at 50 mg/kg of each were studied in rats..

Test drugs at the rate of 1000 mg/kg for both *T. cordifolia* and *V. negundo* and 500 mg of each agent in combination were given for anti-nociceptive study in rats.

Anti-inflammatory effect of *T. cordifolia* was found effective in first phase and *V. negundo* in the second phase of carrageenin induced inflammation. The combination of the herbal agents produced a uniform significant inhibition in both the phases.

Anti-nociceptive effect was found significant for both the herbal agents and their combination, in tail flick method of nociception. This suggested a central mediated mechanism of anti-nociception by both the agents.

Adrenal parameters like gland weight, ascorbic acid and cholesterol for anti-inflammatory study suggested that *T. cordifolia* and *V. negundo* had a preventive effect on the inflammation induced changes in adrenal gland. However combination of the herbal agents was found to decrease the preventive action of the individual agents against inflammation induced changes in adrenal gland.

The involvement of endogenous modulatory system for the anti-nociceptive effect of *T. cordifolia* and *V. negundo* was in contrary because of the anonymous increase of adrenal ascorbic acid and decrease of adrenal cholesterol.

T. cordifolia was found effective in lowering the serum cholesterol whereas combination was not that much effective.

None of the treatments were found to inhibit the lipid peroxidation induced by inflammation in plasma. Combination of *T. cordifolia* and *V. negundo* showed a significant peak increase of plasma lipid peroxide level.

The rise in serum level of AST and ALT in both inflammation and nociception were not inhibited by the treatments. Haematological parameters for all groups were within the normal range. However an increase in neutrophil count than lymphocyte was noticed in carrageenin induced inflammation.

Both the studies showed an increase in neutrophil count without an increase in total leukocyte count for *T. cordifolia. V. negundo* showed an increase in total leukocyte count in anti-inflammatory study. A decrease in total leukocyte count and neutrophil count was made by the combination of *T. cordifolia* and *V. negundo* in anti-inflammatory study. However there was an increase of total leukocyte count for combination of agents in anti-nociceptive study. Total erythrocyte count and haemoglobin concentration were increased by *T. cordifolia* in both the studies, whereas they were normal for combination of treatment.