ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTS OF *Withania somnifera* AND *Catharanthus roseus* IN RATS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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DECLARATION

I hereby declare that the thesis entitled "**Anti-inflammatory and antinociceptive effects of *Withania somnifera* and *Catharanthus roseus* 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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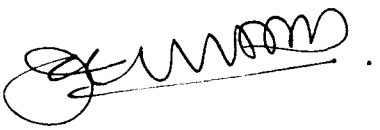
Certified that the thesis, entitled "**Anti-inflammatory and antinociceptive effects of *Withania somnifera* and *Catharanthus roseus* in rats**" is a record of research work done independently by **Shri. Arivuchelvan, A.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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ARIVUCHELVAN, A.

Dedicated to

my Sister

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Introduction

INTRODUCTION

Inflammation is the response of the living tissue to an injury, and it is fundamental to the survival of the organism. Without inflammation there could be neither protection against noxious external stimuli nor repair of damaged tissues. The stimulus for inflammation can be either immunological or non-immunological in origin, since the inflammation involves a series of defensive and reparative changes. The very purpose of inflammation is to deliver the phagocytic cells to the site of insult which destroy and remove the irritant.

The causes of inflammation can be either due to agents like physical, chemical, biological or immune reactions associated with antigen-antibody interactions which occur under various circumstances (Sastry, 1983). Inflammation is associated with cardinal signs like redness, heat, pain, swelling and disturbance or loss of functions. When a tissue is injured the body adopts this mechanism to contain the inflammation and repair the damaged tissues. A number of chemical mediators of inflammation such as histamine, kinins, prostaglandins, globulin permeability factor (GPF), complements and lysosomal enzymes are released and they are responsible for increasing the vascular permeability and also elicit certain changes in the inflammatory process. The earlier phase of carrageenin inflammation is maintained by

histamine and kinin system and sustained inflammation is maintained by prostaglandin (Dirosa *et al.*, 1971).

On the whole, inflammation is a beneficial process; however, it has been realized that inflammation, like other vital processes, may at times wander away from its beneficial path and become considerably more harmful to the organism than the noxious stimulus which initiated the reaction. Diseases which are thought to be of immunological origin such as rheumatoid arthritis, glomerulonephritis and lupus erythematosus are associated with inflammatory reactions which provide no obvious benefit, but rather inflict harm upon the host. In the process of inflammation the lysosomes present in the cells are damaged, resulting in the release of hydrolytic enzymes which bring about extensive damage to the surrounding tissues (Higgs *et al.*, 1975).

Therefore it became important to find ways for arresting such harmful inflammatory processes. A variety of drugs have been used to minimise the discomfort arising due to the inflammatory process. In the past, chiefly the natural and synthetic steroids were employed in the anti-inflammatory therapy. Because of the occurrence of undesirable side effects with steroid therapy sush as immunosuppression, adrenal insufficiency, fluid and electrolyte abnormalities etc., attention was directed towards the development of

non-steroidal anti-inflammatory agents. Most of the currently available non-steroidal anti-inflammatory drugs (NSAIDs) inhibit both cyclooxygenase 1 (Cox 1: constitutive) and cyclooxygenase 2 (Cox 2: induced in settings of inflammation) activities, and thereby synthesis of prostaglandins and thromboxane. The inhibition of Cox 2 is thought to mediate atleast in part, the antipyretic, analgesic and anti-inflammatory action of NSAIDs, but the simultaneous inhibition of Cox-1 results in unwanted side effects, particularly those leading to gastric ulcers. NSAIDs include aspirin, which irreversibly acetylates cyclooxygenase, and several other classes of organic acids viz., propionic acid derivatives, acetic acid derivatives, phenylacetic acid derivatives (diclofenac) and enolic acid derivatives, all of which compete with arachidonic acid at the active site of cyclooxygenase (Insel, 1996).

Another avenue for the use of NSAIDs is the alleviation of pain. Pain is a subjective affair though it may be accompanied by measurable physiological responses such as reflex withdrawal movements, changes in vasomotor tone, blood pressure, heart rate, breathing and sweating. Pain is an unpleasant sensory experience distinct from other sensory modalities and it can be elicited by noxious stimulation in normal persons and it is also the outstanding symptom of many

diseases. Pain depends on activation of discrete set of receptors and neuronal pathways. In animals pain has been defined as an aversive sensory and emotional experience that elicits protective motor actions, results in learned avoidance and modifies species specific traits of behaviour including social behaviour (Kitchell, 1987).

However, pain has its limitation as a protective warning system. For example, some types of acute pain after injury such as post-operative pain, serve no useful purpose and chronic pain such as that due to untreatable malignant diseases or persistent inflammation as in rheumatoid arthritis is of no value to the suffering patients. NSAIDs are useful in mild to moderate pain. The effect appears to involve peripheral and central mechanisms. Pain receptors get sensitised because of the presence of autacoid, prostaglandin, and these receptors are more prone to mechanical and other stimulation. NSAIDs arrest the sensitisation of nociceptors to such stimulation by preventing the production of arachidonic acid cascade products.

Diclofenac sodium has been found to be effective both as an anti-inflammatory and analgesic agent. But the number of drawbacks of long term use of NSAIDs such as gastrointestinal ulceration, disturbances in platlet function, changes in renal function etc. has led to the search for better alternatives

especially herbal drugs. Global estimate indicates that 80 per cent of the population cannot afford the products of the western pharmaceuticals. They have to rely upon the use of traditional medicines which are mainly derived from plant material. Thus, it is obvious that the use of herbal drugs remains a good alternative.

Varieties of plants have been used for the purpose of anti-in flammatory and anti-nociceptive activity. The medicinal properties of *Withania somnifera* (Ashwagandha) have been revealed since ancient times. The aqueous and alcoholic extracts of its root have been reported to contain many properties such as anti-arthritic, adaptogenic, immunomodulating, anti-tumour, radiosensitizing effects (Kuttan, 1996). The plant extracts are also a major ingredient of various Ayurvedic preparations. *Catharanthus roseus* and its alkaloids have reported to possess anti-neoplastic activity (Maheswari *et al.*, 1991). So, the present study has been undertaken with the view to investigate into the anti-inflammatory and antinociceptive effects of *W. somnifera* and *C. roseus*.

Review of Literature

REVIEW OF LITERATURE

2.1. Plants having anti-inflammatory activity

Many of the medicinal plants have been reported to possess anti-inflammatory activity in different types of inflammatory reactions. Much investigations have been made on such plants in India. But the reports appear to be inconclusive and contradictory as well.

Gujral *et al.* (1961) found glycyrrhizin, the active principle, obtained from *Glycyrrhiza glabra*, to be an effective anti-inflammatory agent. At a dose of 20 mg/100 g body weight orally, the drug was slightly less effective than hydrocortisone (0.5 g/100 g) in adrenalectomised rats, but in normal rats the dose was equal in potency. When glycyrrhizin was given along with hydrocortisone the effect was reported to be pronounced.

Chaturvedi and Singh (1965) found that *Dalbergia lanceolaria*, *Sida humilis*, *Paederia foetida* and *Vitex megunda* possess significant anti-inflammatory activity. Their action was comparable to those of cortisone and phenylbutazone.

Prasad *et al.* (1966) screened *Plu cheae lanceolata*, *Allium sativum*, *Alangium lamarckii* and Batchintamani (an ayurvedic preparation containing gold and pearl) for their

anti-inflammatory activity using granuloma pouch and formalin induced arthritis in rats. *P. lanceolata* was significantly active in both the tests. The effect was quantitatively similar to betamethasone. Concentrated fresh extracts of *Allium sativum* appeared to have a slight effect on arthritis but none in the granuloma pouch test. *A. lamarckii* significantly increased the inflammatory process for the first five days and thereafter reduced the foot volume upto 11 days. Batchintamani had no anti-inflammatory effect at all.

Arora *et al.* (1971) isolated a steroid from the plant *Commiphora mukul* which showed significant anti-inflammatory activity on rat paw oedema produced by carrageenin. The activity was dose dependent and more potent than the resin fraction of the same plant.

The anti-inflammatory activity of the oil obtained from *Cyperus scariosus* plant was highly significant in the carrageenin foot oedema and cotton pellet granuloma tests (Gupta *et al.*, 1972).

Bhattacharya *et al.* (1978) isolated `Withanolide S' from the leaves of *Physalis peruviana* and found that they have a greater anti-inflammatory and anti-arthritic activity when compared to other Withanolides isolated from *Withania somnifera*.

Rao *et al.* (1983) observed the anti-inflammatory and anti-cholinergic activities of *Zizyphus oenoplia*. The alcoholic extract of the bark of the plant produced significant anti-inflammatory activity against carrageenin induced pedal oedema and croton oil induced exudation in albino rats. The LD_{50} of the extract was found to be 300 mg/kg body weight in albino mice after intraperitoneal administration.

Alcoholic extract of *Bougainvillea spectabilis* leaves showed significant anti-inflammatory effect in carrageenin induced rat paw oedema but, less than that of oxyphenbutazone. Oral administration of 0.5 g/kg and 1.0 g/kg of the extract produced 26.39 per cent and 27.13 per cent inhibition against carrageenin induced oedema at 4th hour. Oxyphenbutazone (0.1 g/kg) produced 32.55 per cent inhibition. In the chronic model of cotton pellet implantation, 1 g/kg of the extract provided 33.76 per cent inhibition, when compared to oxoyphenbutazone which produced 41.67 per cent inhibition (Joshi *et al.*, 1984).

Shidore *et al.* (1985) reported the anti-diarrhoeal and anti-inflammatory activities of nutmug (*Myristica fragrans*) extract and suggested that the effect had been due to its prostaglandin synthesis inhibitory action. The petroleum ether extract showed anti-inflammatory activity with ED_{50} of

955 mg/kg against carrageenin induced rat paw oedema, while the water extract was inactive compared to the reported potency of indomethacin. Petroleum ether extract was much less potent but similar in potency to chloroquine, paracetamol and sodium salicylate.

Dhar *et al.* (1988) isolated pedilanthain, a new protease from the latex of *Pedilanthus tithymaloides* and showed the potent anti-inflammatory activity at 125 mg/kg body weight onwards after one hour of carrageenin injection. It was also evident that pedilanthain exhibits maximum activity, two hours after its oral administration.

Pandey and Das (1988) demonstrated the anti-inflammatory action of *Picrorhiza kurroa* in relation with the cell types involved in inflammation.

Total methanolic (90%) extract of *Bougainvillea glabra* leaves was fractionated by different solvents and the fractions were tested for their anti-inflammatory action in carrageenin rat paw oedema. Only petroleum ether fraction showed significant activity when given intraperitoneally at a dose of 100 mg/kg. The chromatographic studies indicated that the anti-inflammatory activity was due to the presence of steroidal component in the leaves (Giri *et al.*, 1988).

Jain *et al.* (1989) screened two new constituents from the *Limonia crenulata* plant for their anti-inflammatory activity and showed significant anti-oedema activity in carrageenin induced rat paw method.

Gomes *et al.* (1989) compared the glycosidal fraction isolated from the *Maesa chisia* with various non-steroidal anti-inflammatory drugs for their anti-inflammatory activity. *M. chisia* at a dose of 25 mg/kg orally showed 42.8 per cent inhibition in carrageenin oedema whereas ibuprofen was the most efficacious (74.1% inhibition). *M. chisia* was less active in cotton pellet granuloma where it produced only 33.55 per cent inhibition and phenylbutazone exhibited the highest activity (51.59% inhibition). In formaldehyde induced arthritis model, 53.71 per cent inhibition was produced by *M. chisia* which was comparable to indomethacin.

Viswanathan *et al.* (1990) made studies on anti-inflammatory and mast cell protective effects of *Ficus religiosa*. The aqueous extract of the bark showed significant anti-inflammatory activity in both acute and chronic models of inflammation. Degranulation of mast cell induced by various degranulators was also inhibited by the extract, and these effects may be responsible for the beneficial effect of *F. religiosa* in kumkum dermatitis and other inflammatory conditions.

Pharmacological investigations made by Bhattachary *et al.* (1990) on the root extract of *Mikania cordata* (methanolic fraction) was found to inhibit the cardinal signs of inflammation and arthritis when given orally. The extract significantly inhibited granuloma formation, leucocyte migration, peritoneal inflammation and other biochemical parameters involved in inflammatory conditions.

In the traditional system of medicine, the different parts of *Bryophyllum pinnatum* herb have been mentioned to be useful in the treatment of a variety of disease conditions. Significant anti-inflammatory action of the methanolic extract of the plant was found by Pal and Nag (1990) in different experimental models of inflammation.

Oral administration of the root extract of *Pluchea indica* had been found to possess significant anti-inflammatory activity against carrageenin induced oedema, formaldehyde induced arthritis and cotton pellet induced granuloma. Furthermore, it had been observed that the root extract also possessed significant antiulcer activity against different models of experimental gastroduodenal ulcers (Sen and Chaudhury, 1990).

Reddy and Srinivasan (1990) described the anti-inflammatory activity of nevadensin isolated from the

alcoholic extract of *Limnophila confert* (0.02% yield). The plant also possessed significant cytotoxic properties.

Subrata and Bhavsar (1990) conducted preliminary phyto-pharmacological studies on *Paederia foetida* and found that n-butanol and ethyl acetate extracts showed marked suppression of carrageenin oedema. Oral administration of methanol extract showed significant protection against carbontetra chloride induced injury to liver.

Alcoholic extract of *Iris ensata* and *Althea officinalis* prevented the development of oedema induced by carrageenin, 5-HT, Histamine, PGE_2 and Kaolin but were virtually ineffective against yeast extract induced oedema. *A. officinalis* significantly reduced the mean weight of cotton pellet granuloma whereas both were active against adjuvant poly arthritis (Sharma and Pandiya, 1990).

Barua and Wahi (1990) reported that petroleum ether and alcoholic extracts of coarse powders of the plant *Hygrophila spinosae* showed significant anti-inflammatory activity against carrageenan foot oedema in albino rats.

Sharma *et al.* (1991) made detailed studies on the anti-inflammatory activity of `TEEBURB' an indigenous veterinary product and reported that aqueous extract and alcoholic extract produced 37.55 per cent, 51.22 per cent increase in

anti-inflammatory activity respectively when compared to control. The reference drug was phenylbutazone which provides only 48.03 per cent increase in anti-inflammatory activity. In carrageenin induced paw oedema model, aqueous extract, alcoholic extract and phenylbutazone showed 26.31 per cent, 42.10 per cent and 57.89 per cent anti-inflammatory activity respectively at the end of the fourth hour.

Emodin, an anthraquinone derivative, isolated from the whole plant of *Rhamnus triquerta* at the rate of 15 mg/kg dose i.p. exhibited anti-inflammatory activity against carrageenin induced pedal inflammation in rats. In the same dosage it also showed antiulcer activity against aspirin induced ulcers in rats (Goel *et al.*, 1991).

Chawla *et al.* (1992) carried out experiments with *Vanda roxburghii* roots and reported the anti-oedema activity of the petroleum ether, chloroform and methanol extracts. The per cent anti-oedema activity was 54.3 per cent, 42.1 per cent and 21.9 per cent respectively. The reference drug used was ibuprofen which showed 66.6 per cent inhibition.

Vicolides A, B, C and D the sesquiterpene lactones isolated from *Vicoa indica* exhibited anti-inflammatory activity against cotton pellet granuloma in rats at dose level of 10 mg/kg body weight subcutaneously. They reduced the

protein content, acid and alkaline phosphatase, glutamate-pyruvate transaminase activities in liver and serum. Significant reduction in ascorbic acid content in adrenals was also observed in treated animals (Alam *et al.*, 1992).

Padmaja *et al.* (1993) reported the anti-inflammatory activity of the saponifiable fraction of the petroleum ether extract of the root of *Ixora coccinea* using carrageenan induced paw oedema in albino rats.

Tandan *et al.* (1994) did experiments with the *Ageratum conyzoides* roots. In carrageenin induced hind paw oedema in rats, the extract of the plant significantly reduced the oedema volume at 100 and 300 mg/kg oral dose. The effects shown by 300 mg/kg of alcoholic extract was comparable to 10 mg/kg of acetyl salicylic acid.

Brahmi Rasayan, an Ayurvedic preparation, was studied in rodents for its anti-inflammatory effects at p.o. doses ranging between 1 g and 10 g/kg body weight. The drug suppressed various experimentally induced inflammatory reactions and did not show any gastric irritation in anti-inflammatory doses (Jain *et al.*, 1994).

Chatterjee and Das (1994) evaluated the efficacy of `EASE', a poly herbal formulation of Indian Herbs as an anti-inflammatory agent in rats. `EASE' exhibited 47.25 per cent

protection against subacute inflammatory model which was nearly comparable to phenylbutazone.

Pharmacological studies of the lupeol isolated from the petroleum ether fraction of ethanol extract of the plant *Ixora coccinea* showed significant anti-inflammatory activity against paw oedema in albino rats (Zachariah *et al.*, 1994).

Ethanol extract and cold aqueous infusion of *Vitex leucoxylon* leaf were evaluated in a battery of tests to define the activity profile of the plant. Oral administration of ethanol extract showed significant inhibition of carrageenin paw oedema and granulation tissue formation in rats (Makwana *et al.*, 1994).

Oil of *Toddalia asiatica* leaves obtained by steam distillation was administered orally to study its effects on the exudative and proliferative phases of the inflammatory reactions, using the technique of carrageenin induced paw oedema and cotton pellet in male albino rats. In carrageenin induced paw oedema 0.8 ml/kg body weight of the volatile oil showed anti-inflammatory activity comparable to that of ketoralac. The oil was also found to be effective in cotton pellet granuloma studies (Kavimani *et al.*, 1996).

Different solvent fractionated extracts of *Pongamia pinnata* were evaluated for anti-inflammatory effect in

chemically induced paw inflammation in rats (Singh and Pandey, 1996). They found that the anti-inflammatory effects of the plant were best seen against bradykinin and PGE induced inflammation. In contrast, minimal effects were seen against histamine and 5-HT induced inflammation.

Karunakar *et al.* (1997) evaluated the anti-inflammatory activity of Jigrine (0.5 ml and 1.0 ml) a polypharmaceutical herbal formulation against acute inflammation. It showed lowering of the elevated levels of paw volume when given orally. But it could not reduce the chronic inflammation caused by cotton pellet granuloma.

Kurma *et al.* (1997) studied the effect of different parts of *Sida rhombifolia* on carrageenin induced paw oedema in rats. They found that the methanolic extract of the aerial parts showed significant oedema suppressant activity in rats.

2.2 Synthetic compounds having anti-inflammatory activity

Marked anti-inflammatory effects were displayed by Naproxen. When administered orally in rats, naproxen, indomethacin and aspirin were found to inhibit carrageenin induced paw oedema 11, 16 and 0.2 times respectively as active as phenylbutazone. Naproxen reduced granuloma formation at

levels ranging from 3.3 to 20 mg/kg/day without impairing growth in rats (Roszkowski *et al.*, 1971).

The anti-inflammatory effect of creatine and indomethacin combination as measured by the carrageenan induced paw oedema in rats had been examined. The per cent oedema inhibition caused by creatine and indomethacin was 51.3 per cent, 45.0 per cent respectively. While the combination was 63.0 per cent. The per cent oedema inhibition caused by the drug combination was more than the effect of individual compounds (Khanna and Tahashildar, 1985).

Ranitidine showed significant anti-inflammatory activity against carrageenin induced oedema, turpentine induced arthritis, formalin-induced peritonitis. Cotton wool granuloma methods. This observation supported the concept that histamine has pro-inflammatory role that is mediated via stimulation of H_2 receptors (Patwardhan *et al.*, 1986).

Synthesis and anti-inflammatory activity of Indolyl azetidinones were done by Kalsi *et al.* (1990). All the derivatives of the compound were tested for their acute anti-inflammatory activity by carrageenin paw oedema method and compared with anti-inflammatory and ulcerogenic activities of NSAIDS.

Eight substituted 2-amino-8H-indeno (1,2-d) thiazoles had been synthesised and were tested for their anti-inflammatory activity against carrageenin induced oedema in rat paw. Some of them showed moderate activity (Gupta *et al.*, 1991).

Mukhopadhyay and Lahiri (1992) evaluated the anti-inflammatory activity of newer indan-1-acid derivaties in various models of inflammation. They observed longer duration of anti-inflammatory action and lower ulcerogenic liability.

Deshpande *et al.* (1994) compared the anti-inflammatory activity of benzimidazoles by carrageenin induced rat paw oedema method with indomethacin as standard drug. They found that all the compounds showed anti-inflammatory activity to a variable degree.

Itaconic acids were synthesised by a modified stobbe condensation and were evaluated for biological activity. Some were found to possess significant anti-inflammatory and analgesic activities (Bagavant *et al.*, 1994).

Oral treatment of compound 111 A (benzylidene derivative) exhibited dose related inhibitory action in acute tests of carrageenin, histamine and dextran-induced oedema in rats. It displayed prominent anti-arthritic activity in chronic tests of adjuvant and formaldehyde-induced arthritis in rats. It

did not exhibit any analgesic, anti pyretic or ulcerogenic effect (Bani *et al.*, 1994).

Talwar *et al.* (1995) showed the anti-inflammatory activity of substituted triazoles using carrageenin-induced rat paw oedema and cotton pellet method.

Some Imidazolyl-1-(aryl/substituted aryl) esters showed pronounced anti-inflammatory activity against carrageenan induced paw oedema model. Some failed to produce inhibitory effect (Srivastava *et al.*, 1996).

2.3 Plants having antinociceptive property

Ansari *et al.* (1988) reported the antinociceptive property of N-isobutyl-4, 5-decadienamide isolated from the flowers of *Spilanthes acmella*. The test compound showed significant antinociceptive effect orally at 3 ml/kg body weight dose level in albino rats. The test adopted was tail flick response of the rats in hot wire analgesiometer.

Gomes *et al.* (1989) showed the analgesic activity of glcosidal fraction isolated from *Maesa chisia* on writhing test. They compared the results with known non-steroidal anti-inflammatory drugs and found that analgesic property of *M. chisia* was weaker than that of other NSAIDS.

Basu and Singh (1990) studied the analgesic activity and gross behavioural changes of *Argemona mexicama* seed oil. The analgesic activity was determined in mice with an analgesiometer with morphine as the standard drug. It was also confirmed that the plant seed oil did not potentiate the analgesic activity of morphine.

Abutilon hirtum is used as tonic, anthelmentic and anti-inflammatory agent. On routine phytochemical studies *A. hirtum* afforded the compound alantolactone which showed promising analgesic activity on experimental animals when given orally (Sharma and Shukla, 1990).

Sharma and Pandiya (1990) assessed the analgesic effect of alcoholic extracts *Iris ensata* and *Althea officinalis* using acetic acid induced writhing, hot plate and tail flick methods. They also showed general CNS depressant activity of the extract.

Tandan *et al.* (1994) carried out experiments with the *Ageratum conyzoides* roots. They found that the extract (30-300 mg/kg p.o) did not prolong the reaction time to thermal stimuli suggesting, lack of activity of morphine type of analgesia. However, a dose of 300 mg/kg of the extract reduced the mean acetic acid induced writhing movements significantly.

EASE, a polyherbal formulation of Indian Herbs, was evaluated for analgesic effect. EASE exhibited 39.62 per cent analgesic potency which is nearly comparable to analgin (Chatterjee and Das, 1994).

Makwana *et al.* (1994) observed suppression of acetic acid writhing following the oral administration of ethanol extract and cold aqueous infusion of *Vitex leucoxylon* leaf. LD_{50} value of ethanolic extract was more than 3000 mg/kg (ip) and cold aqueous infusion was 1050 mg/kg.

Andrographolide from *Andrographis paniculata* had been studied for its analgesic, antipyretic and antiulcerogenic activities. Andrographolide did not show any analgesic activity in hot plate test in mice, while it showed significant analgesic activity in acetic acid-induced writhing in mice and Randall Selitto's test in rats (Madav *et al.*, 1995).

An initial study of ethanolic and sequential petroleum ether, chloroform, acetone and ethanolic extracts of *Pongamia pinnata* seeds (50 mg/kg ip 30 min before) showed a tendency to increase reaction time in analgesiometer but it was significant only with petroleum ether after 45-90 min of oral administration (Singh *et al.*, 1996).

Filho *et al.* (1997) investigated the analgesic effect of the hydroalcoholic extract of the stems of *Bavhinia splendens* in chemical and thermal models of nociception in mice. They showed that the extract 3-60 mg.kg i.p. or 50-400 mg/kg orally caused dose-related, and long-lasting inhibition of acetic acid-induced abdominal constriction but failed to produce analgesic effect in the hot-plate test.

Cakici *et al.* (1997) showed the antinociceptive effect of Amaryllidaceae plants in mice using P-Benzoquinone-induced abdominal constriction test. But all the plants screened were failed to show significant analgesic effect in hot-plate test.

2.4 Synthetic compounds having antinociceptive property

Roszkowski *et al.* (1977) proved the analgesic effect of naproxen by "Randall-Selitto" analgesic method in rats. A significant rise in pain threshold was obtained at 25 to 33 mg/kg p.o. dose level.

Kalsi *et al.* (1988) showed the analgesic action of newer thiobarbiturates using aconitine induced writhing method. They provided 20 to 80 per cent protection, whereas they showed substantially low ulcerogenic liabilities as compared with NSAIDS.

Cinchophen analogs as an analgesic agent was determined by thermal procedure using hot wire analgesiometer (Mishra *et al.*, 1988). Results showed compound No. CP3 to be a highly potent analgesic agent. Compound No. CP2 was also found to possess good analgesic activity when given orally.

Pharmacological studies of substituted Naphthalenes were carried out by Kaskhediker and Bagavant (1989). Analgesic activity was determined in male mice by acetic acid induced writhing model, in which oral administration of compound one to four showed significant antinociceptive activity whereas compound five to nine was devoid of analgesic activity.

Mishra *et al.* (1991) showed the analgesic effect of some derivatives of naproxen using analgesiometer. The result showed that the modification in the structure improved the analgesic effect of naproxen.

Mukhopadhyay and Lahiri (1992) tested the effect of indan-1-acids in phenylquinone analgesia in mice. Compound No. 1, 11, 111 at 200 mg/kg p.o dose level showed 81.44, 78.78 and 59.84 per cent inhibition of writhing respectively.

Substituted sydnones were tested for its analgesic activity of Satyanarayan and Rao (1995). Test method was acetic acid induced writhing and one compound C-21 was found

to have more analgesic activity than aspirin (ED_{50} 25 mg/kg p.o.).

Imidazolyl-1-ester had been synthesised and evaluated for its biological activity. The tri substituted products of this series had been found to be potent analgesics as compared to other compounds when given orally (Srivastava *et al.*, 1996).

2.5 Various effects of plants under study (*Catharanthus roseus* and *Withania somnifera*)

In unani system of practice, the tuber and root of *W. somnifera* is still used as a tonic, aphrodisiac and bronchodilator. Rajputs regarded the root to be useful in the treatment of rheumatism and dyspepsia. In Punjab, it is used for lumbar pains and is considered an aphrodisiac. The seeds are employed to coagulate milk and they are diuretic and hypnotic. In the Ayurvedic system of medicine, the leaves and roots are used in the treatment of inflammation, psoriasis, bronchitis, ulcers and insomnia. The juice of the leaves of *C. roseus* is employed in Orissa as an application to wasp stings. The macerated root is given as a tonic and stomachic. It is also used as a diabetes remedy in South Africa (Kirtikar and Basu, 1975).

Withania somnifera contains a bitter alkaloid, somniferin, having hypnotic property. Root and bitter leaves

are used as hypnotic. A decoction of the root along with milk is still recommended for curing the sterility of women. For scrofulous and other glandular swelling, fresh green root of Aswagandha is recommended. Root is used as an application in obstinate ulcers and rheumatic swelling (Nadkarni, 1976).

The powdered root of *W. somnifera* (Ashwagandha) had been investigated for its anti-inflammatory properties. In the acute-phase of inflammation, oral treatment with 1 g/kg body weight Ashwagandha caused considerable reduction in inflammation in rats. Unlike phenylbutazone, Ashwagandha influenced many modulator proteins in normal rats which suggested that several compounds present in it possibly interact with the liver protein synthetic machinery (Anbalagan and Sadique, 1981).

According to Maheswari *et al.* (1991) *C. roseus* possesses diuretic, antidysentric, antihaemorrhagic and wound healing properties. Its root, stem, and leaf contains over 60 alkaloids which are used for combating blood cancer, blood pressure and diabetes.

Water soluble fraction of *C. roseus* extracts possessed significant anti-inflammatory activity against carrageenan induced rat hind paw oedema. The anti-inflammatory activity was dose dependent. Thus 50, 100, 200 and 400 mg/kg p.o. of extract exhibited 16.66, 37.03, 46.29 and 64.81 per cent

inhibition of paw oedema respectively (Chattopadhyay *et al.*, 1992).

Devi *et al.* (1992) proved the inhibitory effect of *Withania somnifera* (Ashwagandha) on a transplantable mouse tumor, sarcoma 180. Doses of 400 mg/kg p.o. alcoholic extract of the root produced complete regression of tumour after an initial growth.

Antitumour and radiosensitizing effects of *W. somnifera* on a transplantable mouse tumor was demonstrated by Devi *et al.* (1993). The combination treatment (Gamma radiation, hyperthermia and Ashwagandha 500 mg/kg body weight p.o.) enhanced the effectiveness of therapy.

Withania somnifera administered orally at the dose of 1 g/kg produced significant anti-inflammatory activity on chronic inflammatory reaction induced by cotton pellet in rats. Cimetidine, cyprohepatadine and diclofenac significantly potentiated the anti-inflammatory activity of *W. somnifera* (Sahani and Srivastava, 1995).

The alcoholic extract of the dried roots of *Withania somnifera* as well as the active component withaferin A isolated from the extract showed significant antitumor and radiosensitizing effects in experimental tumors *in vivo* without any noticeable systemic toxicity (Devi, 1996).

Sarma *et al.* (1996) showed the anti-inflammatory and anti convulsant effect of crude extract of *Catharanthus roseus*. It exhibited 33.33, 66.67, 80.00 per cent reduction in oedema volume on carrageenin induced paw oedema at 0.5, 1, 2 g/kg doses respectively. Graded doses of the crude extracts of the plant upto 8.0 g/kg body weight failed to show anti convalescent activity by oral route of administration.

Administration of methanolic extract of the *Withania somnifera* was found to significantly increase the total WBC count in normal Balb/c mice and reduce the leucopenia induced by sub-lethal dose of gamma radiation. Major activity of *W. somnifera* seemed to be in the stimulation of stem cell proliferation (Kuttan, 1996).

Materials and Methods

MATERIALS AND METHODS

Fresh leaves of *Catharanthus roseus* were collected from the Department of Medicinal and Aromatic Plants, College of Horticulture, Kerala Agricultural University, Vellanikkara.

Roots of *Withania somnifera* were procured locally and identified by the Department of Medicinal and Aromatic Plants, Kerala Agricultural University.

Leaves with small branches of *Catharanthus roseus* were dried at room temperature and powdered using a pulverizer. The dried powder was extracted with 90 per cent methanol in a soxhlet extractor. The residue was kept at room temperature and evaporated to dryness. Yield was about 4.5 to 5 per cent w/w.

Alcoholic extract of the root of *Withania somnifera* was also prepared in the same way. The residue obtained was 4.5 to 5 per cent of the weight of dried powder.

*Diclofenac sodium was used as a reference drug for comparison. All the extracts and drug were suspended in five per cent gum acacia and administered orally.

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* Diclofenac sodium - Pure drug - Sarabhai Chemicals, Vadodara

3.1. Determination of anti-inflammatory activity of *Catharanthus roseus* by cotton pellet method (Chronic inflammatory model)

Procedure described by Meier *et al.* (1950) was adopted. Forty adult apparently healthy albino rats weighing 100-150 g were procured from the Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. They were randomly divided into five groups of eight animals each. Each group was kept in a single cage. Feed and water were provided *ad lib.*

The composition of the feed:

Bengal gram	-	30 per cent
Gingely oil cake	-	30 per cent
Wheat	-	30 per cent
Black gram bran	-	8 per cent
Salt	-	0.5 per cent
Supplevite-M*	-	1.5 per cent

All the animals were kept under observation for a period of one week before the experiment, in order to familiarise laboratory handling and management.

Cotton pellets weighing 20±1 mg made of absorbent surgical cotton were used for implantation. The pellets

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*Supplevite-M - Sarabhai Chemicals, Baroda

were made by hand, sterilized with 70 per cent alcohol and dried in hot air oven at 50°C for two hours. The weight of the pellets were found to be unchanged after the sterilization procedures.

The rats were divided into five groups of eight animals each and treated as follows:

Group No.	Treatment
I	Kept as control - 5% gum acacia administered orally
II	Alcoholic extract of *Catharanthus roseus* 200 mg/kg, administered orally
III	Alcoholic extract of *Catharanthus roseus* 400 mg/kg, administered orally
IV	Alcoholic extract of *Catharanthus roseus* 600 mg/kg, administered orally
V	Diclofenac sodium 3 mg/kg administered orally

The animals were anaesthetised by anaesthetic ether and two sterile cotton pellets were implanted subcutaneously at the two symmetrical ventrolateral sites of the abdomen by a single mid line skin incision. The incision was closed by simple interrupted suture with a cotton thread. Every day, the drugs were administered to the rats in the treatment groups and the vehicle alone to the control group in the

morning, as mentioned above. The treatment was carried out for seven consecutive days. Blood samples were collected before and after the experiment from the orbital sinus for estimating haematological parameters viz. erythrocyte count, total leucocyte count, differential count and haemoglobin concentration as described by Schalm (1975). The animals were then sacrificed using chloroform on eight day and the granuloma along with the pellets were dissected out. The pellets from each group were kept in separate petri dishes with identification marking. They were then dried at 60°C overnight. In the morning, they were weighed once and dried again at 60°C for one hour before being finally weighed. Reduction in the weight of the granuloma when compared to control was considered as a measure of anti-inflammatory activity. Percentage of anti-inflammatory activity was calculated using a formula

$$\left(1 - \frac{Wt}{Wc}\right) \times 100$$

Wt - Mean weight of the granuloma in treated group

Wc - Mean weight of the granuloma in control group

3.2. Determination of anti-inflammatory activity of *W. somnifera* by cotton pellet method (Chronic inflammatory model)

The rats were divided into five groups of eight animals each and treated as follows:

Group No.	Treatment
I	Control - 5% gum acacia administered orally
II	750 mg/kg alcoholic extract of *W. somnifera* administered orally
III	1000 mg/kg alcoholic extract of *W. somnifera* administered orally
IV	1500 mg/kg alcoholic extract of *W. somnifera* administered orally
V	Diclofenac sodium 3 mg/kg administered orally

The same procedure described in the case of *C. roseus* was followed.

3.3. Determination of anti-inflammatory activity of *C. roseus* by carrageenin induced rat paw oedema method (acute inflammatory model)

The procedure described by Winter *et al.* (1962) and Zachariah *et al.* (1994) was adopted.

Forty rats were divided into five groups of eight each. Animals were fasted for 18 hours prior to the test. The test groups were given orally 200 mg/kg, 400 mg/kg and 600 mg/kg of *C. roseus* in five per cent gum acacia solution and control was given vehicle alone. Diclofenac sodium 3 mg/kg was given as a standard drug for comparison. The paw oedema was induced half an hour after the drug administration by injecting 0.1 ml of a 1 per cent suspension of carrageenin in normal saline into the plantar aponeurosis of the left hind paw of the rat. The paw thickness was measured by using vernier calipers in three planes and the average was recorded immediately after carrageenan injection and three hours later.

Percentage of inhibition of oedema was calculated by using the formula.

$$\left(1 - \frac{Tt}{Tc}\right) \times 100$$

Tt - Mean paw thickness of treated group

Tc - Mean paw thickness of control group

3.4. Determination of anti-inflammatory activity of *W. somnifera* by carrageenin induced rat paw oedema method (acute inflammatory model)

Forty rats were divided into five groups of eight each. Group 1 remained as a control. Group II, III, IV were

administered 750 mg/kg, 1000 mg/kg, 1500 mg/kg of alcoholic extract respectively. Group V was administered with diclofenac sodium 3 mg/kg. The same procedure described in the case of *C. roseus* was followed. The percentage of inhibition of oedema was calculated as mentioned earlier. All the results obtained were analysed statistically using analysis of variance.

3.5. Investigation of antinociceptive effect of *C. roseus* and *W. somnifera*

The antinociceptive effect of *C. roseus* and *W. somnifera* was determined by thermal stimulus (Dandiya and Menon, 1963).

Antinociceptive effect in rats was assessed by tail flick method using Analgesiometer. This instrument has a nichrome wire which would be heated to the required temperature and maintained by means of heat regulators. The current passing through the nichrome wire is indicated on ammeter which indirectly gives the temperature of the wire. A jacket surrounds the nichrome wire and water is circulated through it. The upper surface of the jacket serves as a platform on which the tail of the rat can be placed. The water circulating through the jacket prevents the platform from getting heated up. This ensures that only that portion of the tail which lies just above the hot wire is heated. The ammeter was set to four Amperes so that the heat produced in

the nichrome wire was constant throughout the experiment. The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (Reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting.

Experimental design

Seven groups consisting of eight rats each were used for the study and treated as follows:

Group I - 3 mg/kg body weight of diclofenac sodium
Group II - 200 mg/kg body weight of *C. roseus*
Group III - 400 mg/kg body weight of *C. roseus*
Group IV - 600 mg/kg body weight of *C. roseus*
Group V - 750 mg/kg body weight of *W. somnifera*
Group VI - 1000 mg/kg body weight of *W. somnifera*
Group VII - 1500 mg/kg body weight of *W. somnifera*

The drug preparation used for analgesic study was similar to those used in the anti-inflammatory study. Reaction time, that is the time taken for the characteristic tail lift was measured in seconds before administration of drugs in all the rats. Animals with the reaction time of less than three

seconds and more than 10 seconds were discarded. After the administration of the drugs, reaction time for each drug was measured at 30, 60, 90 and 120 minutes. Results were analysed using student's "t" test (Snedecor and Cochran, 1967).

Results

RESULTS

In the present study, anti-inflammatory and antinociceptive activities of the two plants, *Catharanthus roseus* and *Withania somnifera* were compared with the diclofenac sodium (a standard nonsteroidal anti-inflammatory drug NSAID). The results obtained were analysed and presented in Tables 1 to 8.

4.1. Anti-inflammatory activity

4.1.1. Cotton pellet method

Alcoholic extract of both the plants *C. roseus* and *W. somnifera* were found to exhibit significant anti-inflammatory activity ($P<0.01$) when compared with the untreated controls in cotton pellet granuloma method.

The mean weight of granulation tissue in the control group was 84.39 ± 1.18 mg. Alcoholic extract of *C. roseus* (200 mg/kg) showed 67.56 ± 0.63 mg weight of granulation tissue (Fig.1). Mean difference in weight of the granulation tissue when compared to control was 16.38 mg. The per cent anti-inflammatory activity was calculated as 19.94 (Table 1c, Fig.2).

Alcoholic extract of *C. roseus* at a dose rate of 400 mg/kg body weight produced 64.73 ± 0.85 mg mean weight of granulation tissue. Difference in weight of the granulation tissue when compared to control was 19.66 mg. The per cent anti-inflammatory activity was 23.29. 400 mg/kg body weight dose did not differ significantly from 200 mg/kg dose in producing anti-inflammatory effect ($P>0.05$). Higher dose 600 mg/kg alcoholic extract of *C. roseus* produced significant anti-granuloma activity. The dose rate of 600 mg/kg produced 54.11 ± 0.63 mg weight of granulation tissue (Table 1c; Fig.1). Mean difference in the weight of granulation tissue when compared to control was 30.28 mg. It showed 35.88 per cent antigranuloma activity (Table 1c, Fig.2).

When compared with the anti-inflammatory effect of standard NSAID agent diclofenac sodium, all the above tested doses of extract were lesser in their activity in cotton pellet method. Difference in weight of the granulation tissue in case of diclofenac sodium when compared to control group was 38.32 mg. The per cent anti-inflammatory activity showed by diclofenac sodium was 45.41 (Table 1c, Fig.2).

Alcoholic extract of *W. somnifera* produced dose dependent anti-inflammatory effect in cotton pellet method. The lower dose tested (750 mg/kg) produced 73.58 ± 1.47 mg weight of

granulation tissue. Untreated control group exhibited 87.05 ± 1.44 mg, weight of granulation tissue. Difference in the weight of the granulation tissue was 13.47 mg and the dose showed only 15.47 per cent antigranuloma activity (Table 2c, Fig.4).

Alcoholic extract of *W. somnifera* 1000 mg/kg body weight produced 49.32 ± 0.75 mg weight of granulation tissue. The dose produced 37.73 mg difference in weight of the granuloma when compared to control group. The per cent anti-inflammatory activity was calculated as 43.34 (Table 2c, Fig.4).

Higher dose 1500 mg/kg produced superior anti-inflammatory activity. This group exhibited mean difference in the weight of the granuloma as 46.94 mg. The per cent anti-inflammatory activity was 53.92. Reference drug diclofenac sodium 3 mg/kg produced 44.21 ± 1.36 mg weight of granulation tissue (Fig.3). Difference in the weight of the granulation tissue when compared to control was 42.84 mg. The per cent anti-granuloma effect shown by diclofenac sodium was 49.21. Statistically 1500 mg/kg body weight extract and diclofenac sodium at the dose of 3 mg/kg body weight did not differ significantly in producing anti-granuloma effect (Table 2c, Fig.4).

Results showed that both *C. roseus* and *W. somnifera* failed to produce significant difference in erythrocyte count,

leucocyte count, haemoglobin concentration and differential count ($P>0.05$). Standard drug diclofenac sodium also failed to produce significant difference in the haematological parameters after the experiment (Table 3 and 4).

4.1.2. Carrageenin induced paw oedema

In the Carrageenin induced paw oedema method also, both the plants exhibited significant anti-inflammatory activity when compared to control group ($P<0.01$).

Control group produced 1.787 ± 0.1 mm increase in paw thickness, after carrageenin injection (Table 5). Alcoholic extract of *Catharanthus roseus* at the dose of 200 mg/kg bodyweight dose produced 1.050 ± 0.14 mm increase in paw thickness after carrageenin injection. So, reduction in paw thickness when compared to control group was 0.737 mm. The per cent inhibition of oedema was calculated as 41.24 (Table 5; Fig.5). *C. roseus* alcoholic extract at a dose rate of 400 mg/kg produced 0.787 ± 0.08 mm increase in paw thickness, and reduction in paw thickness when compared to control was 1.0 mm. The per cent inhibition of paw oedema was 55.96. *C. roseus* 600 mg/kg exhibited 0.575 ± 0.07 mg increase in paw thickness and reduction in paw thickness when compared to control was 1.212 mm. Per cent inhibition of oedema was 67.82 (Table 5).

Reference drug diclofenac sodium exhibited 65.69 per cent anti-inflammatory activity. In this experimental model, *C. roseus* produced dose dependent anti-inflammatory activity. Between 600 mg/kg extract of *C. roseus* and 3 mg/kg diclofenac sodium there was no significant difference ($P>0.05$). So the per cent anti-oedema activities were comparable (Table 5, Fig.5).

Alcoholic extract of *Withania somnifera* at a dose rate of 750 mg/kg body weight exhibited 1.40 ± 0.13 mm increase in paw thickness and the control group produced 1.737 ± 0.12 mm mean increase in paw thickness after Carrageenin injection (Table 6). Reduction in paw thickness when compared to control was 0.337 mm. Per cent anti-oedema activity was 19.40. *W. somnifera* at dose rate of 1000 mg/kg body weight and 1500 mg/kg body weight exhibited 1.125 ± 0.10 and 0.962 ± 0.11 mm increase in paw thickness respectively and the reduction in the paw thickness when compared to control was 0.612 mm and 0.775 mm. The per cent anti-oedema activity of 1000 mg/kg and 1500 mg/kg body weight extracts were 35.23 and 44.62 respectively (Fig.6). Diclofenac sodium exhibited higher anti-oedema activity. It produced 1.049 mm reduction in paw thickness when compared to control group. The per cent anti-oedema activity was 60.39 (Table 6, Fig.6).

4.2. Antinociceptive activity

Catharanthus roseus administered at the dose rates of 200 mg/kg and 400 mg/kg body weight produced no significant analgesic effect when compared to the reference drug diclofenac sodium in albino rats. *C. roseus* administered at a higher dose rate (600 mg/kg) produced mild analgesic effect but the dose was also failed to produce significant analgesic effect when compared with diclofenac sodium (Table 7). *W. somnifera* administered at different dose rates (750, 1000, 1500 mg/kg) also failed to produce statistically significant analgesic effect when compared to the reference drug diclofenac sodium (Table 8). But diclofenac sodium produced significant analgesic effect in albino rats within 30 minutes after the oral administration at a dose rate of 3 mg/kg and it persists after 120 minutes of administration (Table 7 and 8).

Table 1a. Comparative weight of the granulation tissues in *Catharanthus roseus* treated animals

Treatment	Control	*C. roseus*			Diclofenac sodium
Dose mg/kg/b.wt.	0	200	400	600	3
1	83.75	68.60	64.30	56.15	49.15
2	80.85	64.60	62.05	52.40	42.50
3	85.15	68.55	65.25	53.50	48.20
4	90.95	68.20	61.20	56.45	45.05
5	87.25	67.55	69.15	53.95	43.10
6	82.25	70.20	65.25	51.20	42.90
7	83.15	65.55	65.10	55.15	47.30
8	81.75	67.25	65.50	54.05	50.35

The figures against rows 1-8 represent the mean weight of the two granulomas from each rat in milligrams. The weight of 20 mg of cotton is included in all the readings.

Table 1b. Table for analysis of variance - *Catharanthus roseus*, cotton pellet method

Group No.	Number of animals	Sum	Average	SD	SE
1	8	675.100	84.387	3.33	1.18
2	8	540.470	67.559	1.79	0.63
3	8	517.800	64.725	2.41	0.85
4	8	432.850	54.106	1.79	0.63
5	8	368.550	46.069	3.08	1.09

CD = 3.490

	Degrees of freedom	Sum of squares	Mean square	F value	Remarks
Between	4	6770.138	1692.535	257.746 **	Highly signi-
Within	35	229.834	6.567		ficant

Table 1c. Percentage anti-inflammatory activity of *C. roseus* on cotton pellet method

Treatment	Dose mg/kg oral	Weight of granulation tissue (mg) Mean ± SE	Difference in weight of granulation tissue (mg) mean	Per cent anti-inflammatory activity
Control	-	84.39 ± 1.18	-	-
C. roseus	200	67.56 ± 0.63**	16.83	19.94
C. roseus	400	64.73 ± 0.85**	19.66	23.29
C. roseus	600	54.11 ± 0.63**	30.28	35.88
Diclofenac sodium	3	46.07 ± 1.09**	38.32	45.41

** ($P<0.01$)

Table 2a. Comparative weight of the granulation tissues in *Withania somnifera* treated rats

Treatment	Control	*W. somnifera*			Diclofenac sodium
Dose mg/kg/b.wt.	0	750	1000	1500	3
1	84.90	78.60	45.05	41.65	52.95
2	89.80	78.10	49.10	38.55	45.15
3	91.30	72.35	48.70	40.55	41.30
4	89.65	71.10	51.60	38.85	42.85
5	92.15	71.05	50.10	38.40	45.10
6	83.45	78.65	48.05	40.35	41.00
7	82.55	69.70	50.60	41.15	43.25
8	82.60	69.10	51.35	41.35	42.05

The figures against rows 1-8 represent the mean weight of the two granulomas from each rat in milligrams. The weight of 20 mg of cotton is included in all the readings.

Table 2b. Table for analysis of variance - *W. somnifera*, cotton pellet method

Group No.	Number of animals	Sum	Average	SD	SE
1	8	696.400	87.050	4.07	1.44
2	8	588.650	73.581	4.15	1.47
3	8	394.550	49.319	2.13	0.75
4	8	320.850	40.106	1.32	0.47
5	8	353.650	44.206	3.86	1.36

CD = 4.516

	Degrees of freedom	Sum of squares	Mean square	F value	Remarks
Between	4	13350.897	3337.724	303.541**	Highly signi-
Within	35	384.859	10.996		ficant

Table 2c. Percentage anti-inflammatory activity of *W. somnifera* on cotton pellet method

Treatment	Dose mg/kg oral	Weight of granulation tissue (mg) Mean ± SE	Difference in weight of granulation tissue (mg) mean	Per cent anti-inflammatory activity
Control	-	87.05 ± 1.44	-	-
C. roseus	750	73.58 ± 1.47**	13.47	15.47
C. roseus	1000	49.32 ± 0.75**	37.73	43.34
C. roseus	1500	40.11 ± 0.47**	46.94	53.92
Diclofenac sodium	3	44.21 ± 1.36**	42.84	49.21

** ($P<0.01$)

Table 3. Haematological parameters - *Catharanthus roseus*

Treatment	Dose		Pre-administration	Post-administration
RBC (10^6/mm^3)				
Control	5% gum acacia		645.5 ± 35.58	628.5 ± 37.62
C. roseus	200 mg/kg		603.1 ± 18.51	617.5 ± 22.13
C. roseus	400 mg/kg		592.1 ± 32.64	623.0 ± 17.48
C. roseus	600 mg/kg		670.1 ± 21.00	615.1 ± 43.87
Diclofenac sodium	3 mg/kg		647.0 ± 40.58	619.1 ± 37.96
WBC (10^3/mm^3)				
Control	5% gum acacia		4456.0 ± 553.1	4539.0 ± 331.1
C. roseus	200 mg/kg		4429.0 ± 379.5	4739.0 ± 360.0
C. roseus	400 mg/kg		5113.0 ± 385.0	5300.0 ± 318.0
C. roseus	600 mg/kg			
Diclofenac sodium	3 mg/kg		4706.0 ± 275.5	4923.0 ± 218.5
Haemoglobin (g%)				
Control	5% gum acacia		9.06± 0.23	9.12± 0.43
C. roseus	200 mg/kg		9.14± 0.16	9.07± 0.18
C. roseus	400 mg/kg		9.59± 0.22	9.47± 0.16
C. roseus	600 mg/kg		8.90± 0.40	9.10± 0.31
Diclofenac sodium	3 mg/kg		8.95± 0.25	9.10± 0.30
Differential count				
Control	5% gum acacia			
		L	79.0 ± 2.90	81.1 ± 1.70
		N	20.1 ± 1.80	17.9 ± 1.40
		E	0.9 ± 0.20	1.0 ± 0.10
C. roseus	200 mg/kg			
		L	85.5 ± 1.1	82.4 ± 2.1
		N	14.1 ± 1.2	16.3 ± 2.0
		E	1.4 ± 0.3	1.3 ± 0.3
C. roseus	400 mg/kg			
		L	79.1 ± 2.3	78.9 ± 3.1
		N	18.9 ± 2.4	18.8 ± 1.63
		E	1.6 ± 0.3	1.0 ± 0.33
C. roseus	600 mg/kg			
		L	85.2 ± 2.6	82.3 ± 3.1
		N	13.2 ± 2.1	16.9 ± 2.1
		E	1.6 ± 0.4	0.8 ± 0.3
Diclofenac sodium	3 mg/kg			
		L	86.3 ± 1.4	87.1 ± 1.4
		N	13.5 ± 1.9	13.7 ± 2.0
		E	0.2 ± 0.3	0.1 ± 0.1

Table 4. Haematological parameters - *Withania somnifera*

Treatment	Dose		Pre-administration	Post-administration
RBC (10^6/mm^3)				
Control	5% gum acacia		703.1 ± 37.5	691.5 ± 31.05
W. somnifera	750 mg/kg		624.5 ± 27.3	647.7 ± 35.4
W. somnifera	1000 mg/kg		674.0 ± 40.1	699.0 ± 21.5
W. somnifera	1500 mg/kg		647.1 ± 21.5	625.0 ± 20.5
WBC (10^3/mm^3)				
Control	5% gum acacia		5224.0 ± 289.5	4974.0 ± 347.5
W. somnifera	750 mg/kg		4229.0 ± 555.4	4709.0 ± 294.5
W. somnifera	1000 mg/kg		4459.0 ± 394.0	4907.0 ± 294.0
W. somnifera	1500 mg/kg		4700.0 ± 299.0	4324.0 ± 374.5
Diclofenac sodium	3 mg/kg		4200.0 ± 402.0	4312.0 ± 294.0
Haemoglobin (g%)				
Control	5% gum acacia		8.91± 0.40	9.10± 0.50
W. somnifera	750 mg/kg		9.10± 0.50	9.30± 0.50
W. somnifera	1000 mg/kg		9.00± 0.22	9.20± 0.30
W. somnifera	1500 mg/kg		3.40± 0.30	9.20± 0.18
Diclofenac sodium	3 mg/kg		9.20± 0.15	9.00± 0.30
Differential count				
Control	5% gum acacia			
		L	80.0 ± 3.5	81.1 ± 2.1
		N	19.4 ± 2.9	18.0 ± 1.7
		E	0.6 ± 0.12	0.9 ± 0.34
W. somnifera	750 mg/kg			
		L	78.4 ± 2.7	79.5 ± 1.5
		N	20.6 ± 1.4	19.9 ± 2.1
		E	1.6 ± 0.3	0.6 ± 0.33
W. somnifera	1000 mg/kg			
		L	82.1 ± 2.7	84.1 ± 2.3
		N	17.2 ± 1.5	14.9 ± 3.7
		E	0.7 ± 0.5	1.0 ± 0.3
W. somnifera	1500 mg/kg			
		L	85.5 ± 4.3	87.0 ± 2.1
		N	13.6 ± 2.2	11.9 ± 3.9
		E	0.9 ± 0.39	1.1 ± 0.25
Diclofenac sodium	3 mg/kg			
		L	81.9 ± 1.4	83.1 ± 1.5
		N	17.1 ± 1.1	16.6 ± 2.0
		E	1.0 ± 0.25	0.9 ± 0.33

Table 5. Effect of *C. roseus* on carrageenin induced paw oedema

Group	Drug & dose	Mean increase in paw thickness ± S.D. (mm)	Reduction in paw thickness (mm)	% inhibition of odedma
I	Control Acacia 5% solution	1.787 ± 0.10	-	-
II	*C. roseus* 200 mg/kg body weight	1.050 ± 0.14*	0.737	41.24
III	*C. roseus* 400 mg/kg body weight	0.787 ± 0.08*	1.000	55.96
IV	*C. roseus* 600 mg/kg body weight	0.575 ± 0.07*	1.212	67.82
V	Diclofenac sodium 3 mg/kg body weight	0.613 ± 0.10*	1.174	65.69

* Significant at 1% level

Analysis of variance table

	Degrees of freedom	Sum of squares	Mean square	F value	Remarks
Between	4	7.932	1.983	192.137**	Highly significant
Within	35	0.361	0.010		

CD = 0.1362

Table 6. Effect of *W. somnifera* on carrageenin induced paw oedema

Group	Drug & dose	Mean increase in paw thickness ± S.D. (mm)	Reduction in paw thickness (mm)	% inhibition of odedma
I	Control Acacia 5% solution	1.737 ± 0.12	-	-
II	*W. somnifera* 750 mg/kg body weight	1.400 ± 0.13*	0.337	19.40
III	*W. somnifera* 1000 mg/kg body weight	1.125 ± 0.10*	0.612	35.23
IV	*W. somnifera* 1500 mg/kg body weight	0.962 ± 0.11*	0.775	44.62
V	Diclofenac sodium 3 mg/kg body weight	0.685 ± 0.08*	1.049	60.39

* Significant at 1% level

Analysis of variance table

	Degrees of freedom	Sum of squares	Mean square	F value	Remarks
Between	4	5.216	1.304	108.355**	Highly significant
Within	35	0.421	0.012		

CD = 0.1491

Table 7. Comparison between diclofenac sodium (DICLO) and alcoholic extract of *C. roseus* (AECR) for their antinociceptive effect

Time (min)	Mean (sec)			Mean (sec)			Mean (sec)		
	DICLO 3 mg/kg	AECR 200 mg/kg	't' value	DICLO 3 mg/kg	AECR 200 mg/kg	't' value	DICLO 3 mg/kg	AECR 200 mg/kg	't' value
30	10.25	4.38	18.963**	10.25	5.63	14.928**	10.25	6.38	12.507**
60	12.13	4.50	25.843**	12.13	5.25	20.376**	12.13	8.25	13.863**
90	13.37	4.25	37.169**	13.37	5.00	31.837**	13.37	9.62	14.491**
120	12.87	4.37	29.184**	12.87	4.38	29.184**	12.87	9.12	14.491**

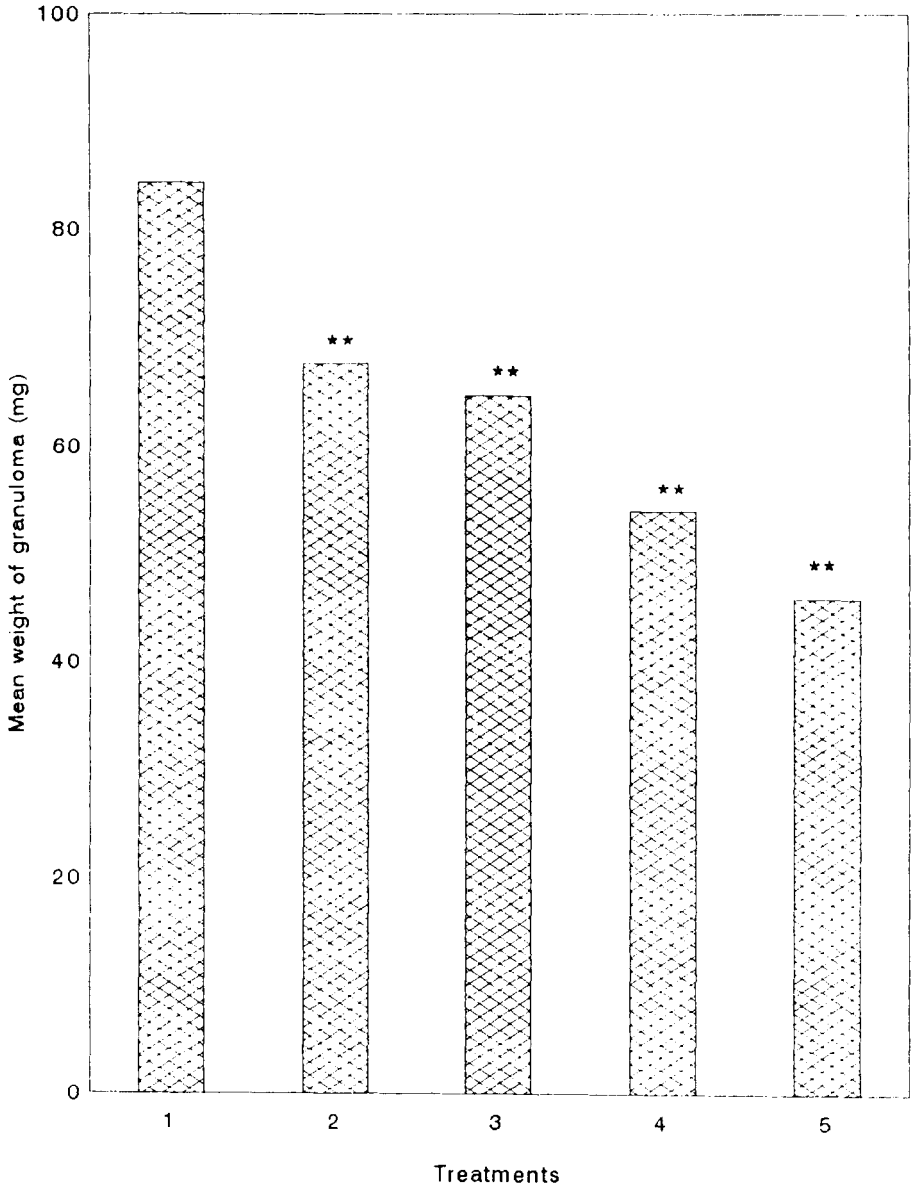
** Significant at 1 per cent level

Table 8. Comparison between diclofenac sodium (DICLO) and alcoholic extract of *W. somnifera* (AEWS) for their antinociceptive effect

Time (min)	Mean (sec)			Mean (sec)			Mean (sec)		
	DICLO 3 mg/kg	AEWS 750 mg/kg	't' value	DICLO 3 mg/kg	AEWS 1000 mg/kg	't' value	DICLO 3 mg/kg	AEWS 1500 mg/kg	't' value
30	10.25	6.38	12.507**	10.25	7.13	9.262**	10.25	8.13	6.298**
60	12.13	6.38	19.743**	12.13	7.38	16.309**	12.13	9.63	8.584**
90	13.37	7.50	22.334**	13.37	7.63	17.944**	13.37	9.13	14.592**
120	12.87	6.88	18.724**	12.87	7.00	16.764**	12.87	8.25	13.707**

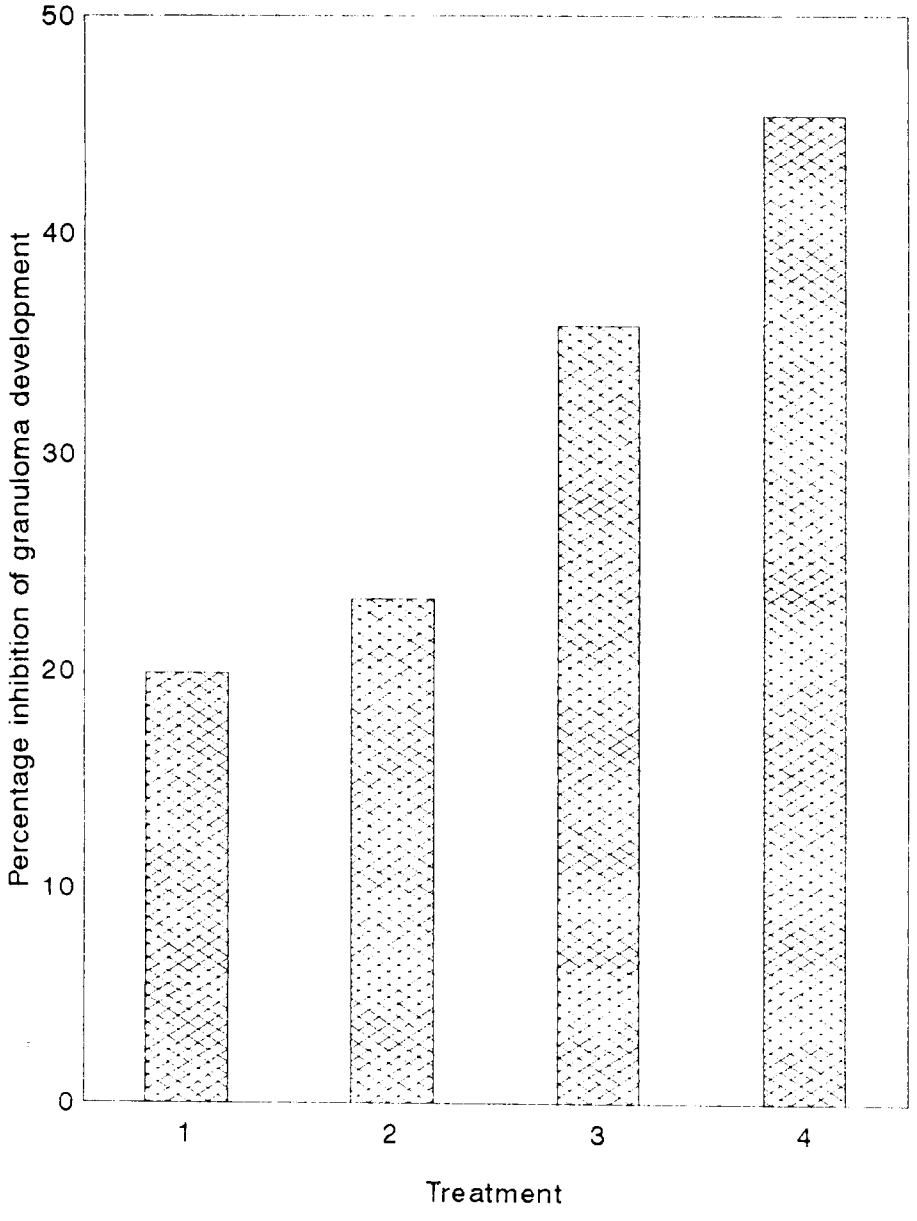
** Significant at 1 per cent level

Fig.1 Comparative anti - inflammatory effect of Catharanthus roseus - cotton pellet method



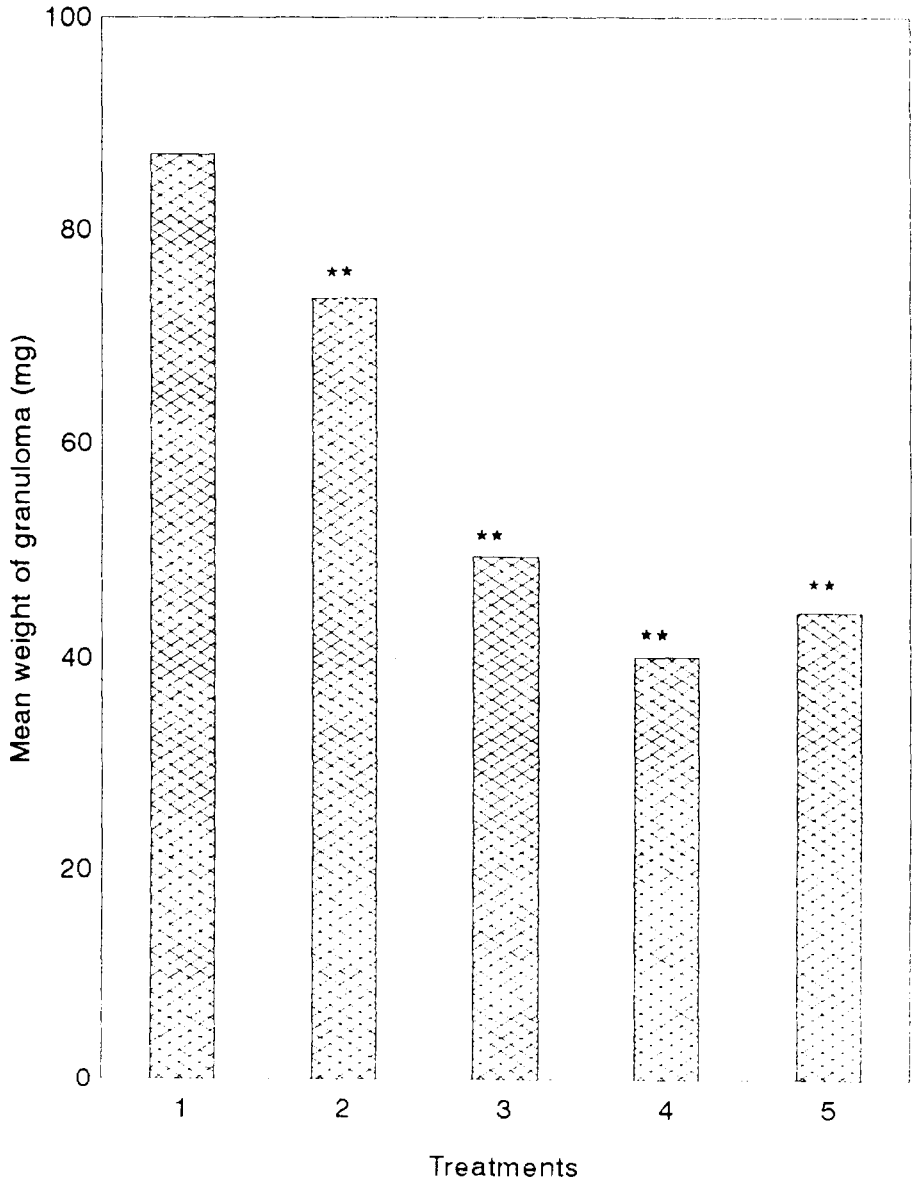
1. control 2 C. roseus 200 mg/kg 3 C. roseus 400 mg/kg
4. C. roseus 600 mg/kg 5. Diclofenac sodium 3 mg/kg
** ($p<0.01$)

Fig.2 Percentage anti - inflammatory activity of Catharanthus roseus - cotton pellet method



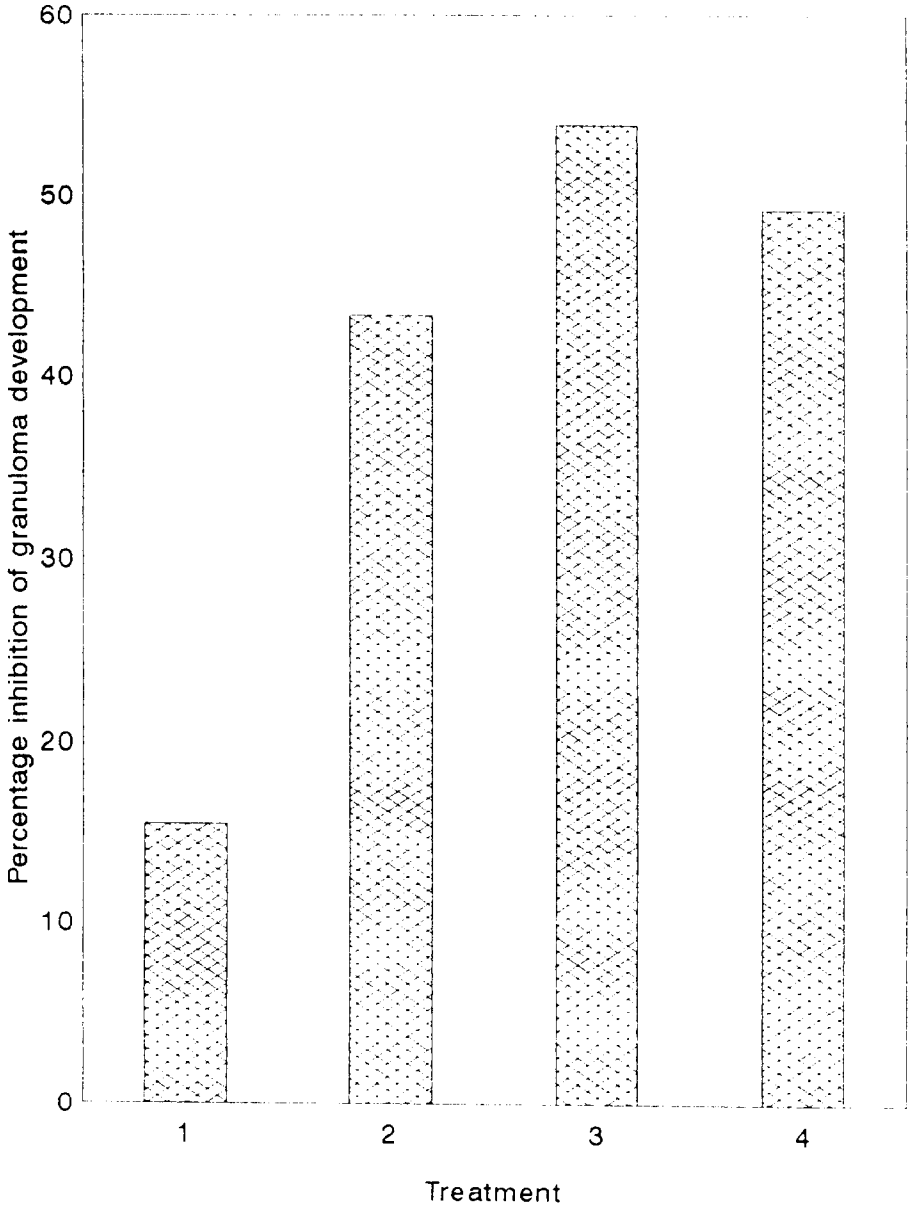
1 C. roseus 200 mg/kg 2 C. roseus 400 mg/kg
3. C. roseus 600 mg/kg 4. Diclofenac sodium 3 mg/kg

Fig.3 Comparative anti - inflammatory effect of Withania somnifera - cotton pellet method



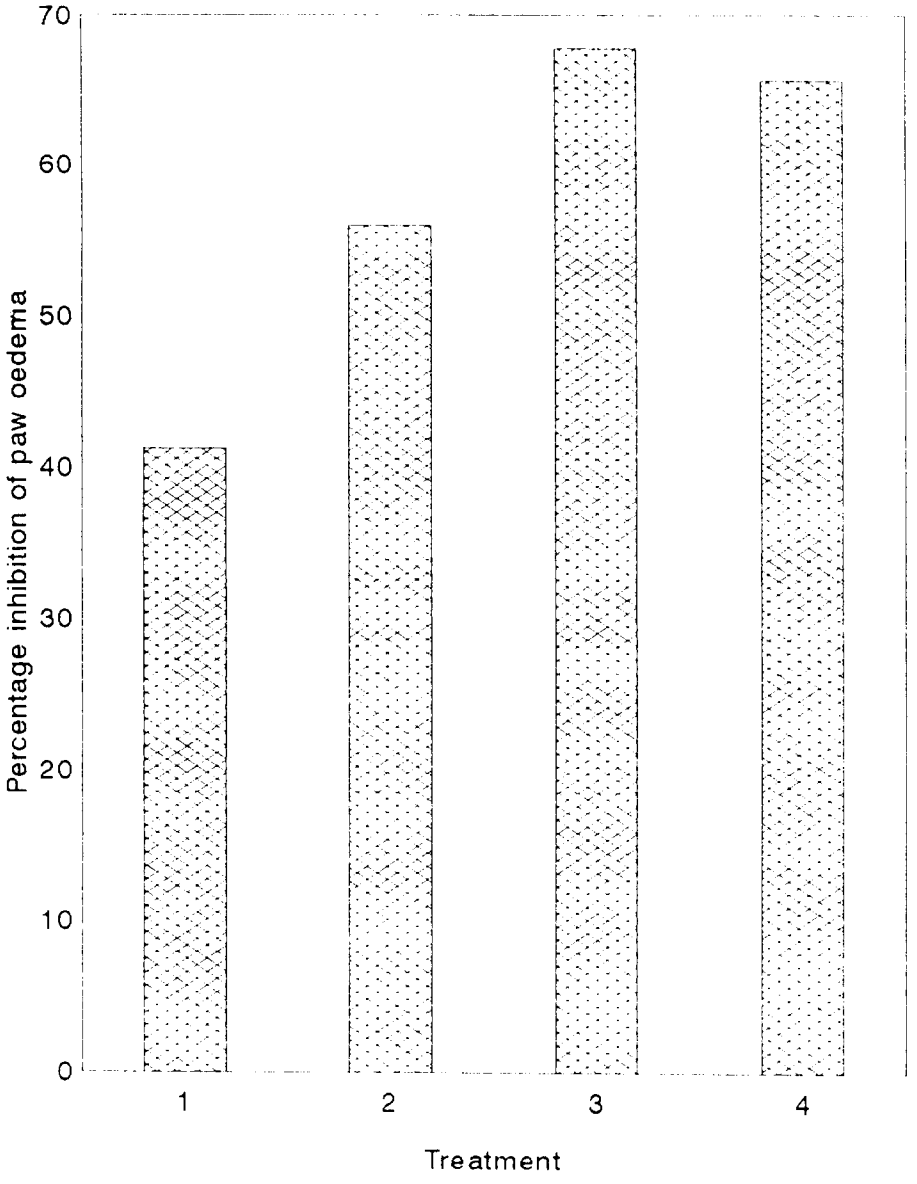
1. control 2 W. somnifera 750 mg/kg 3 W. somnifera 1000 mg/kg
4. W. somnifera 1500 mg/kg 5. Diclofenac sodium 3 mg/kg
** ($P<0.01$)

Fig.4 Percentage anti - inflammatory activity of Withania somnifera - cotton pellet method



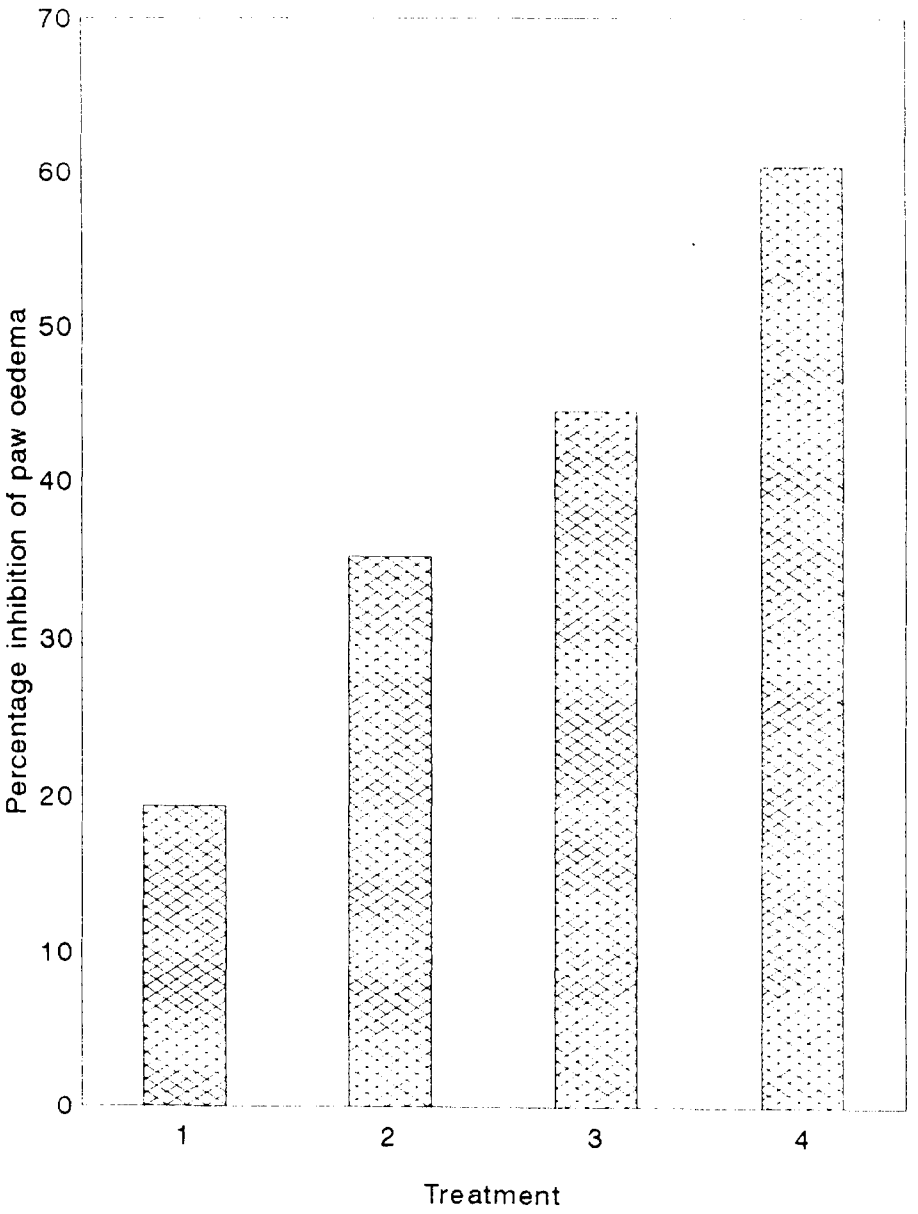
1 W. somnifera 750 mg/kg 2 W. somnifera 1000 mg/kg
3. W. somnifera 1500 mg/kg 4. Diclofenac sodium 3 mg/kg

Fig.5 Percentage anti - inflammatory activity of Catharanthus roseus - paw oedema method



1 C. roseus 200 mg/kg 2 C. roseus 400 mg/kg
3. C. roseus 600 mg/kg 4. Diclofenac sodium 3 mg/kg

Fig.6 Percentage anti - inflammatory activity of Withania somnifera - paw oedema method



1 W. somnifera 750 mg/kg 2 W. somnifera 1000 mg/kg
3. W. somnifera 1500 mg/kg 4. Diclofenac sodium 3 mg/kg

Discussion

DISCUSSION

5.1. Anti-inflammatory activity

5.1.1. Cotton pellet method

In the present study the alcoholic extract of *Catharanthus roseus* was found to produce significant anti-inflammatory effect in cotton pellet induced granuloma, a chronic inflammatory process in rats. The dose rate of 200 mg/kg and 400 mg/kg produced better anti-inflammatory activity. Between the doses of 200 mg/kg and 400 mg/kg there was no significant statistical difference. But a dose rate of 600 mg/kg extract produced superior anti-inflammatory activity. However, the percentage anti-inflammatory activity of the standard drug used diclofenac sodium was comparatively higher than all the above doses tested.

Alcoholic extract of *Withania somnifera* also produced dose dependent anti-inflammatory activity in cotton pellet method. Less anti-inflammatory activity was observed with 750 mg/kg extract whereas 1000 mg/kg and 1500 mg/kg extract exhibited better anti-inflammatory activity. Alcoholic extract of *W. somnifera* 1500 mg/kg produced equipotent anti-inflammatory effect when compared to diclofenac sodium 3 mg/kg body weight. Gomes *et al.* (1989) reported the anti-inflammatory activity of glycosidal fraction isolated from

the leaves of *Maesa chisia*. Glycosidal fraction showed 35.55 ± 2.7 per cent antigranuloma activity whereas acetyl salicylic acid showed only 20.85 ± 4.37 per cent antigranuloma activity. Naproxen also produced lesser activity when compared to glycosidal fraction. But ibuprofen and phenylbutazone produced higher anti-inflammatory activity, than glycosidal fraction (38.44 ± 4.97 and 51.59 ± 3.91 respectively). According to Sharma *et al.* (1991) anti-inflammatory activity of alcoholic extract of `Teeburb' was higher than reference drug phenylbutazone. Alcoholic extract of `Teeburb' 1000 mg/kg produced 51.22 per cent increase in anti-inflammatory activity whereas phenylbutazone 100 mg/kg exhibited 48.03 per cent anti-inflammatory activity.

Shani and Srivastava (1995) reported that *W. somnifera* at a dose rate of 500 mg/kg produced no significant anti-inflammatory activity. Dose rate of 750 mg/kg alcoholic extract showed mild activity (11.3 per cent) and 1000 mg/kg produced better anti-inflammatory activity (48.8 per cent) in cotton pellet method.

The dose dependent anti-inflammatory effect of *W. somnifera* as observed in the above findings was noticed in this study also.

There was no significant difference in RBC count, WBC count, Haemoglobin concentration and differential count

between *C. roseus* treated rats and control rats. *W. somnifera* treated rats also did not show significant difference in haematological parameters before and after treatment.

Devi (1996) observed that there was no significant difference between *W. somnifera* treated and control group in haematological parameters except slight increase in the haemoglobin level.

According to Kuttan (1996) administration of a 75 per cent methanolic extract of *Withania somnifera* significantly increased the total WBC count in mice. The extract also normalised the ratio of normochromatic erythrocytes and polychromatic erythrocytes in mice after the radiation exposure. Major activity of *W. somnifera* seemed to be in the stimulation of stem cell proliferation. She also observed there was no variation in haemoglobin level and weight gain of the animals. So further studies on haematological parameters are needed to ascertain the long term effect of these plants.

5.1.2. Carrageenin induced paw oedema method

In carrageenin induced paw oedema method *C. roseus* exhibited a dose dependent anti-inflammatory activity. Higher effect was produced by 600 mg/kg effect of *C. roseus* when compared to 200 mg/kg and 400 mg/kg of extract. In this model

anti-inflammatory effect of 600 mg/kg extract was comparable to the standard drug diclofenac sodium 3 mg/kg. Alcoholic extract of *Withania somnifera* at a dose rate of 1500 mg/kg showed higher anti-inflammatory activity when compared to other doses of extract tested. But standard drug diclofenac sodium produced maximum anti-inflammatory activity among these groups.

Sharma *et al.* (1991) showed the anti-inflammatory effect of `Teeburb' on carrageenin induced paw oedema method. In their study aqueous extract and alcoholic extract of `Teeburb' showed 28.00 and 52.00 per cent anti-inflammatory activity respectively. Reference drug phenylbutazone exhibited higher activity (64.00 per cent anti-inflammatory activity).

Chawla *et al.* (1992) demonstrated the anti-inflammatory effect of *Vanda roxburghii* roots in carrageenin induced paw oedema method. Petroleum ether extract, 500 mg/kg and chloroform extract, 500 mg/kg exhibited 54.3, 42.11 per cent inhibition of oedema respectively. But per cent inhibition of standard drug ibuprofen was higher (66.6 per cent).

Chattopadhyay *et al.* (1992) demonstrated the anti-oedema activity of *C. roseus* in acute inflammatory model. They showed the percentage inhibition of 400 mg/kg alcoholic extract was more than the standard reference drug phenylbutazone. According to Sarma *et al.* (1996) crude

extract of *C. roseus* produced dose dependent anti-inflammatory activity in acute inflammatory model. Rats that were received 0.5 g/kg, 1.0 g/kg and 2.0 g/kg crude extract produced 33.33, 66.67 and 80.00 per cent inhibition of paw oedema volume respectively. Aldeen and Nabi (1989) showed the dose dependent anti-inflammatory activity of *W. somnifera* on intact rats by measuring the suppression of carrageenan-induced paw oedema. In the present study also dose dependent anti-inflammatory effect was observed.

Anti-inflammatory activity of diclofenac sodium is well established. Unlike other NSAIDs, diclofenac has a dual mode of action i.e., it acts via cyclo-oxygenase as well as lipoxygenase pathways. Diclofenac sodium interacts with the arachidonic acid cascade at the level of cyclo-oxygenase. Inhibition of this key enzyme occurs *in vitro*. Consequently, the formation of thromboxanes, prostaglandins and prostacyclin were prevented (Menasse *et al.*, 1978).

The inhibitory effect of diclofenac sodium on leukotriene suggests a potential benefit of this agent in chronic inflammation. According to Ku and Kothari (1984) decreased arachidonic acid release and increased uptake, limit the availability of arachidonic acid flowing into the cyclo-oxygenase and lipo-oxygenase pathways.

The results of our findings show that various phases of the inflammatory reactions were affected by *C. roseus* and *W. somnifera*. Carrageenin-induced paw oedema was taken as a prototype of exudative phase of inflammation. The development of oedema has been described as biphasic (Kavimani *et al.*, 1996).

The initial phase is attributable to the release of histamine, serotonin and kinin in the first hour after injection of carrageenin. A more pronounced second phase is related to the release of prostaglandins-like-substances in second to third hour. So the significant anti-inflammatory effect of both the plants may be related to its histamine, kinin and prostaglandin inhibitory activity. In the cotton pellet granuloma model, inflammation and granuloma develop in a few days. The model is the indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources for granuloma formation. Therefore decrease in granuloma weight indicates the suppression of proliferative phase by both the plants under study. According to Jain *et al.* (1994) the anti-inflammatory activity of `Brahmi rasayan' (Ayurvedic preparation) is due to the antagonistic effect of the preparation on inflammatory mediators and inhibitory effect on proliferative stages of inflammation. The anti-inflammatory effect is also attributed to its

stabilizing effect on lysosomal membranes. Cotton pellet granuloma is also a model of non-immunological type of inflammation mediated mostly by Kinins (Warren, 1972). As the plant extracts under study showed significant protective effect against this model of inflammation, it may safely be presumed that both the plants possess anti-kinin activity and are effective to control non-immunological type of inflammation. However, to ascertain the anti-kinin activity of these plants, its effect on kinin-induced inflammation and kinin biosynthesis is proposed to be studied in future. The role of histamine, 5-HT and prostaglandins in anti-inflammatory activity of *W. somnifera* was studied by Sahni and Srivastava (1995). They noticed that promethazine, H_1 receptor antagonist, failed to produce any significant increase in anti-inflammatory activity of *W. somnifera* as compared to its per se anti-inflammatory activity. Cimetidine (H_2 receptor antagonist), cyproheptadine (5-HT antagonist) significantly increased the anti-inflammatory activity of *W. somnifera* in chronic inflammatory model. The involvement of H_2 receptors in chronic inflammatory reaction has also been reported by Pandse *et al.* (1985). The report of Lal *et al.* (1985) also suggested *W. somnifera* acts by altering 5-HT concentration in central nervous system where it produced dose-related decrease in brain 5-HT level. The anti-inflammatory activity of *Azadirachata indica* (Chattopadhyay *et al.*, 1990) and `Teeburb' (Sharma *et al.*, 1991) has also

been reported to be mediated through inhibition of 5-HT on chronic inflammatory reaction. Pretreatment with diclofenac significantly potentiated the anti-inflammatory activity of *W. somnifera* (Sahni and Srivastava, 1995). The results emanating from the present study therefore, suggest the inhibition of different mediators might be responsible for the anti-inflammatory activity of *C. roseus* and *W. somnifera*.

W. somnifera root extracts contain several alkaloids, withanolides, a few flavonoids and reducing sugars. The pharmacological and therapeutic properties of the plant have been attributed to the presence of withanolides, the most important of which is Withaferin A, a steroidal lactone (Devi, 1996).

According to Singh and Pandey (1996) the anti-inflammatory activity of *Pongamia pinnata* seed extract is attributed to the presence of flavonoids and glycosides which are known to inhibit inflammatory mediators. Tandan *et al.* (1994) reported that the anti-inflammatory activity of *Ageratum cnyzoides* root extract could be due to flavonoides reported from this plant.

5.2. Antinociceptive activity

Alcoholic extract of *C. roseus* at the dose level of 200 mg/kg, 400 mg/kg and 600 mg/kg showed no significant analgesic

action in albino rats for a period of two hours after its administration, when compared with diclofenac sodium at the dose rate of 3 mg/kg. The present study indicates a lack of activity similar to morphine type of analgesics which is a centrally acting. Sarma *et al.* (1996) observed that the crude extract of *Cataranthus roseus* produced significant analgesic effect in acetic acid induced writhing test, the results suggesting peripheral analgesic activity of the extract. Similarly *W. somnifera* upto a dose rate of 1.5 gram/kg failed to produce significant analgesic effect when compared to diclofenac sodium.

In general three types of stimuli - physical, thermal and chemical, are employed in mice or rats for evaluation of analgesic property of a compound. False positive results are also sometimes obtained with the tests. Thermic stimuli is a measure of morphine type of analgesia and writhing tests (chemical stimuli) is a measure of peripheral analgesic activity. In the present study the plants have failed to produce significant analgesis activity when compared to diclofenac. It may be revealed that both the plants may lack the morphine type of analgesia. Tandan *et al.* (1994) showed that the *Ageratum cnyzoides* root extract did not prolong the reaction time to thermic stimuli suggesting lack of activity of morphine type of analgesia. However, the extract showed significant analgesic activity in acetic acid-induced writhing

movement suggesting peripheral analgesic activity of the extract. Suresh (1992) observed no significant analgesic activity of *Tinospoora cordifolia* and *Ocimum sanctum* when compared with aspirin using analgesiometer. But *O. sanctum* showed significant analgesic activity in acetic acid induced writhing test. The results of the above findings are in accordance with the present observations.

From the results of the present study, it can be inferred that alcoholic extract of *C. roseus* and *W. somnifera* are effective anti-inflammatory agents. The efficacy of these extracts (high dose level) in producing antigranuloma and antioedema was comparable to that of diclofenac sodium as shown by the statistical analysis of the results obtained. Hence, these agents prove to be of value as anti-inflammatory agents in the future clinical trails that can be carried out in animals and also in human beings.

Summary

SUMMARY

In the present study an attempt was made to assess the anti-inflammatory and antinociceptive effect of two indigenous plants namely, *Catharanthus reseus* and *Withania somnifera* in comparison with the established non steroidal anti-inflammatory drug (NSAID) diclofenac sodium, in albino rats.

To assess the anti-inflammatory activity, two methods namely, cotton pellet induced granuloma and carrageenin induced paw oedema were adopted. Granuloma was induced by implanting 20 mg of cotton subcutaneously to all the rats. First group was kept as control animals which received vehicle alone. Second group was given alcoholic extract of *C. roseus* orally at a dose rate of 200 mg/kg body weight. The third group was treated with 400 mg/kg extract and the fourth group received 600 mg/kg alcoholic extract of *C. roseus* orally. The fifth group was taken as the positive control group, which received the known NSAID, diclofenac sodium at a dose rate of 3 mg/kg body weight orally. All the agents were tried for a period of seven days. On 8th day granuloma along with pellet was dissected out, dried in hot air oven and weighed. For alcoholic extract of *W. somnifera* also same experimental design was adopted.

Second, third and fourth group received 750, 1000, and 1500 mg/kg body weight alcoholic extract respectively.

From the results of the study, *C. roseus* at the dose level of 200 mg/kg produced 67.56 ± 0.63 mg granulation tissue and per cent anti-inflammatory activity was 19.94. *C. roseus* at the dose rate of 400 mg/kg body weight produced 64.73 ± 0.85 mg granulation tissue and percentage antigranuloma activity was calculated as 23.29. Alcoholic extract of *C. roseus* 600 mg/kg body weight produced 54.11 ± 0.63 mg granulation tissue and it produced 35.88 per cent increase in anti-inflammatory activity. Reference drug diclofenac sodium produced higher antigranuloma activity (45.41 per cent). *W. somnifera* produced dose dependent anti-inflammatory activity in this model of experiment. Alcoholic extract of *W. somnifera* 750 mg/kg body weight produced 73.58 ± 1.47 mg of granulation tissue and it was only 13.47 mg lesser than the control group. The per cent anti-inflammatory activity was calculated as 15.47 only. Alcoholic extract at the dose level of 1000 mg/kg body weight produced 49.32 ± 0.75 mg granuloma and per cent antigranuloma was calculated as 43.34. Anti-inflammatory activity of *W. somnifera* (1500 mg/kg) was comparable to the reference drug diclofenac sodium and the dose produced 53.92 per cent increase in anti-inflammatory activity.

Haematological parameters taken before and after the experiment revealed no significant difference in erythrocyte count, leucocyte count, haemoglobin level and differential count.

In carrageenin induced paw oedema method also five groups of eight rats each were used per plant. *C. roseus* 200 mg/kg alcoholic extract produced 0.737 mm reduction in paw thickness when compared to control. Per cent inhibition of oedema was calculated as 41.24. *C. roseus* at the dose level of 400 mg/kg body weight produced 1.0 mm reduction in paw thickness and anti-oedema activity was 55.96 per cent when compared to control group. *C. roseus* at the rate of 600 mg/kg body weight produced 1.212 mm reduction in paw thickness and per cent inhibition of oedema was 67.82. Anti-oedema activity at 600 mg/kg dose was comparable to the reference drug diclofenac sodium 3 mg/kg.

Alcoholic extract of *Withania somnifera* also produced dose dependent activity. *W. somnifera* at the dose level of 750 mg/kg body weight produced 0.337 mm reduction in paw thickness and per cent inhibition of oedema was calculated as 19.40. Thousand mg/kg extract produced 0.612 mm reduction in paw thickness and the per cent anti-oedema activity was 35.23. Higher dose of *W. somnifera* (1500 mg/kg) produced 44.62 per cent anti-oedema activity which was less than the

anti-oedema activity of reference drug diclofenac sodium 3 mg/kg orally.

Anti-nociceptive effect of both plants was assessed by tail flick method using analgesiometer. Totally seven groups consisting of eight rats each were used for the study. After the administration of the drugs, reaction time for each drug was measured at 30, 60, 90 and 120 minutes.

Two hundred, four hundred and six hundred milligram per kilogram dose level of alcoholic extract of *C. roseus* produced no significant analgesic effect in rats. The effects were compared with diclofenac sodium (3 mg/kg) in albino rats for a period of two hours.

The dose of 750, 1000, 1500 mg/kg body weight alcoholic extract of *W. somnifera* showed no significant analgesic effect in rats. The effects were compared with diclofenac sodium 3 mg/kg in rats for a period of two hours.

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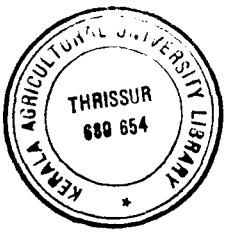

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ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTS OF *Withania somnifera* AND *Catharanthus roseus* IN RATS

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ABSTRACT OF A THESIS

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ABSTRACT

The present study was undertaken with the objective of determining the anti-inflammatory and antinociceptive effects of *Withania somnifera* and *Catharanthus roseus*. Alcoholic extract of both the plants were used for the study and the effect produced by the above plants were compared with that of the known non-steroidal anti-inflammatory drug namely, diclofenac sodium which served as the positive control drug.

To assess the anti-inflammatory effect two methods namely, cotton pellet and carrageenin induced paw oedema were adopted. In cotton pellet method five groups of eight rats each were used per plant. First group was kept as a control, which received five per cent gum acacia only. IInd, IIIrd and IVth group received 200, 400, 600 mg/kg alcoholic extract of *C. roseus* Vth group served as the positive control which received diclofenac sodium 3 mg/kg dose level. All the drugs were administered orally. *C. roseus* produced significant anti granuloma activity when compared to control group. Higher activity was produced by 600 mg/kg body weight extract (35.88 per cent anti-inflammatory activity). For *W. somnifera* also same experimental design was adopted with dose rates of 750, 1000, 1500 mg/kg body weight. *W. somnifera* produced dose dependent antigranuloma activity. Higher dose (1500 mg/kg

body weight) produced more antigranuloma activity (53.92 per cent) which was comparable to the antigranuloma activity of diclofenac sodium. Haematological parameters before and after treatment showed no significant changes for both the plants.

In carrageenin induced paw oedema method also five groups of eight rats each were used per plant. All the three doses of extract and reference drug were given thirty minutes prior to the carrageenin injection and the paw thickness was recorded three hour after injection. *C. roseus* produced significant antioedema activity in this model. Higher dose (600 mg/kg) produced equipotent effect compared to diclofenac sodium 3 mg/kg. *W. somnifera* also produced dose dependent anti oedema activity. Extract at the dose rate of 750, 1000, 1500 mg/kg produced 19.4, 35.23, 44.62 per cent antioedema activity respectively. But the reference drug diclofenac sodium produced higher antioedema activity.

For evaluating antinociceptive effect of *C. roseus* and *W. somnifera*, seven groups of eight animal each were used. All the dose rates of both the plant extracts were compared with diclofenac sodium for a period of two hours showed no significant analgesic effect.