ASSESSMENT OF ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF *Ipomoea mauritiana* (Palmuthukku) IN RATS

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DECLARATION

I hereby declare that the thesis entitled **"Assessment of anti-inflammatory and analgesic properties of *Ipomoea mauritiana* (Palmuthukku) in rats**" is a bonafide record of research work done by me during the course of research and that this 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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CERTIFICATE

Certified that this thesis, entitled **"Assessment of anti-inflammatory and analgesic properties of *Ipomoea mauritiana* (Palmuthukku) in rats**" is a record of research work done independently by **Dr. Priya. A.R,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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Introduction

1. INTRODUCTION

Inflammation is defined as the reaction of the vascularized living tissue to local injury. Acute inflammation is of relatively short duration, lasting for a few minutes, several hours, or a few days; and its main characteristics are the exudation of fluid and plasma proteins and the emigration of leukocytes, mainly neutrophils. 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. In such situations anti-inflammatory drugs will help to regulate it.

A variety of drugs have been used to minimize the discomfort arising due to the inflammatory process. In the past, chiefly the natural and synthetic steroids were employed in the anti-inflammatory therapy. Because of the occurrence of undesirable side effects with steroids, like adrenal insufficiency, fluid and electrolyte abnormalities, attention was directed towards the currently available non-steroidal anti-inflammatory drugs (NSAID) and concluding that most of their actions could be explained by their ability to inhibit cyclo-oxygenase, the enzyme in the pathway of prostaglandin synthesis. The simultaneous inhibition of COX-1 results in unwanted side effects like gastric ulcers, while COX-2 selective inhibition specifically alleviate pain and inflammation.

Oxygen free radicals and non radical reactive oxygen intermediates released by neutrophils and other phagocytes have been increasingly implicated in inflammation. Although free radicals perform some useful functions, they are toxic when generated in excess. The most important characteristic of toxic free radicals either *in vivo* or *in vitro* is peroxidation of lipids resulting in tissue damage and death of affected cells. The harmful effect of reactive oxygen species is neutralized by a broad class of protective agents termed as antioxidants which prevent oxidative damage by reacting with free radicals before any other molecules can become a target. The non-enzymatic antioxidants (vitamin E, C and reduced glutathione) and antioxidant enzymes (SOD, CAT, GSHPx) play an important role in the protection of cells.

Another avenue for the use of NSAIDs is the alleviation of pain. Pain can be elicited by noxious stimulus in normal persons and it is also the outstanding symptom of many diseases. Pain can be considered in two categories like nociceptive and neuropathic pain. Nociceptive pain is defined as the pain in response to an obvious stimulus, while neuropathic is defined as pain in the absence of such stimulus.

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

There has been a tremendous boom in the field of herbal medicine development in recent times. Many plants have been assessed for their action in various systems of the body. Over 80,000 species of plants are in use throughout the world. India being a treasure trove of medicinal plant, contribute much of these. Many regions in Kerala, especially the western ghats, still remain to be explored for unidentified medicinal plants.

Varieties of plants have been used for the purpose of anti-inflammatory and analgesic activity in herbal medicine. *Acacia farnesiana, Adhatoda vasica, Azardirachta indica, Cassia fistula, Curcuma amada, Emblica officinalis, Ficus racemosa, Michelia champaca, Ixora brachiata, Ocimum basilicum, Piper longum, Pongamia pinnata, Ricinus communis, Sida cordifolia, Vitex negundo etc.* are very few among the common Indian medicinal plants with analgesic and anti-inflammatory properties.

The genus **Ipomoea** is the largest in the family Convolvulaceae, with over 500 species. Most of these are called **"morning glories"**. *Ipomoea mauritiana* is one of the plants under the family Convolvulaceae that has been used for its medicinal properties since ancient times. It is found in eastern parts of India including West Bengal and Assam, and the west coast from Konkan to Kerala and also in Mauritius, growing in forests. It prefers peat with quite some water and not that much sun. The

tuberous roots will grow to ten centimetres in diameter, the vines to four meters in length. The flowers vary from pink to red. The plant can be reproduced both by seeds and cuttings, both of which form a caudex.

The bitter, tuberous roots of *Ipomoea digitata* are very much used in native medicine in India, being regarded as tonic, alterative, aphrodisiac, demulcent and purgative (Kirtikar and Basu, 1935). As a galactagogue, the powdered root-stock is given with wine. Nadkarni (1976) says that the powdered root is given for the treatement of spleen and liver enlargement as a cholagogue. The powdered sun-dried root, boiled in sugar and butter, has the effect of moderating menstrual discharge. A confection made of the root and equal parts of wheat flour and barley with milk, ghee, sugar and honey is in general use as a restorative to emaciated and debilitated children. So the root of the plant is used for the treatment of conditions associated with various health abnormalities. A compound decoction called Vidarigandhadigana Quath, consisting of *Ipomoea digitata, Desmodium gangeticum, Tribulus terrestris, Asparagus racemosus, Hemidesmus indicus, Boerhavia diffusa* and *Solanum indicum* is given in one to two ounce dose twice daily in fevers, cough and bronchitis and is found very beneficial.

In addition other species of the family convolvulacea such as *Ipomoea pes-caprae* have also been reported to have anti-inflammatory and anti haemolytic properties (Pongprayoon *et al*., 1992). Souza *et al*. (2000) proved the antinociceptive properties of methanolic extract of *Ipomoea pes-caprae*. Other member of family convolvulacea, *Ipomoea imperati* is also found to have anti-inflammatory and antispasmodic activities (Paula *et al*., 2003). *Ipomoea cairica* ethanolic extract induced dose-dependent reduction of response in inflammatory phase in the formalin test in mice and it also possess antinociceptive effect (Ferreira *et al*., 2006). Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas*) constituents was proved by Huang et al. (2004).

The present study was undertaken to evaluate scientifically the anti-inflammatory and analgesic property of the root extract of *Ipomoea mauritiana.*

Review of Literature

2. REVIEW OF LITERATURE

Ipomoea mauritiana is a common medicinal plant seen in India and distributed in various parts of Kerala, also known as *Ipomoea digitata* as well as *Ipomoea paniculata*. The plant is commonly known as Vidari-kanda (Bilai-kand Palmudukan kizhangu, Bhumikusmanda and Balaikand). The tuberous roots of the plant is commonly used for the treatment of debility, decreased milk production and for enlarged liver and spleen. It increased body weight, moderates menstrual discharge and improves digestion in human beings.

According to Kirtikar and Basu (1935) the bitter, tuberous roots of *Ipomoea mauritiana* are very much used in native medicine of India, since it is being regarded as tonic, aphrodisiac, diuretic and demulcent. As a galactagogue, the powdered root-stock is given with wine. In yunani practices it can be used as anthelmintic, expectorant as well as stomachic. *Ipomoea mauritiana* is also useful in leprosy, burning sensation, vomiting, blood diseases, gonorrhoea and in various inflammations.

Nadkarni (1976) said that the powdered root of *Ipomoea digitata* could be given for spleen and liver enlargement as a cholagogue in man. The powdered sun-dried root, boiled with sugar and butter, had the effect of moderating menstrual discharge. He also indicated that a confection made of the powdered root, equal parts of the wheat flour and barley, milk, ghee, sugar, and honey was used as a restorative for emaciated and debilitated children.

The extracts of tubers of *Ipomoea maurutiana* could be used as an immunity promoter in man (Rao, 2000).

Narayana *et al.* (2003) found that the roots of *Ipomoea mauritiana* possess sweet, cooling, aphrodisiac and could be used in agalactia, colic and emaciation in children.

The methanolic extract of *Ipomoea digitata* exhibited direct scavenging of nitric oxide radicals to some extent among the 17 commonly used Indian medicinal plants (Jagetia and Balgia, 2004).

The starchy tuber of *Ipomoea mauritiana* was effective in promoting spermatogenesis and worked faster when taken as a milk decoction. It was fairly tridoshic. It was lighter for kapha types than shatavari and bala. To treat enlarged prostate it could be combined with kapikacchu or saw palmetto. It was sweet and cooling, and it promoted ojas, muscle tone and coordination. Vidari was useful for sexual debility associated with nervous tension and adrenal stress. It was a good alternative if shatavari is either too cooling or heavy, or when ashwagandha may be too warming. It falls right between the two and was of great value as both a vata and pitta pratyanika herb. A typical dose ranges from 2 to 6 grams, 2 to 3 times daily (Vishnudass, 2007).

2.1. PHARMACOLOGICAL PROPERTIES OF OTHER PLANTS OF THE GENUS IPOMOEA

Souza *et al.* (2000) found that both methanolic extract and two fractions (ethyl acetate and aqueous) of *Ipomoea pes-caprae* exhibited considerable antinociceptive activity against two classical models of pain in mice. Methanolic extract presented a calculated effective dose value of 33.8 mg/kg, intraperitoneally against writhing test. Also inhibited both phases of pain (neurogenic and inflammatory) of the formalin test with effective dose of 37.7 and 12.5 mg/kg, intraperitonealy for the first and second phase respectively. These findings supported the popular use of *I. pes-caprae* to treat dolorous processes.

Methylene chloride and methanol extracts of 20 Indonesian plants with ethnomedical uses were assessed for *in vitro* antibacterial and antifungal properties by Goun *et al.* (2003) by disk diffusion method. According to them among the extracts of the six medicinal plants, Ipomoea species, demonstrated high activity in the bioassay system.

Hueza *et al.* (2003) evaluated the immunomodulatory effect of *Ipomoea carnea* on peritoneal cells of rats using aqueous fraction (AF) diluted in drinking water. Peritoneal macrophages were collected and subjected to the spreading, phagocytosis, and hydrogen peroxide release tests. The results suggested that low dosages of *Ipomoea carnea* induced enhanced phagocytosis activity and hydrogen peroxide production by macrophages.

Malalavidhane *et al.* (2003) investigated the oral hypoglycemic activity of *Ipomoea aquatica* in streptozotocin induced diabetic Wistar rats, and Type II diabetic patients. The shredded leaves of *Ipomoea aquatica* was fed at the rate of 3.4 g/kg for one week. The results revealed that consumption of the shredded, fresh, edible portion of *I. aquatica* for one week effectively reduced the fasting blood sugar level of streptozotocin-induced diabetic rats. And when subjected to a glucose challenge, the Type II diabetic subjects showed a significant reduction in the serum glucose concentration two hours after the glucose load.

In a study the green leaves of *Ipomoea asarifolia* were dosed to ten goats. Nine goats ingested 5 to 37 g/kg body weight daily had clinical signs such as generalized muscle tremors and incordination of movements in 4 to 38 days. One goat ingested 2.5 g/kg body weight daily during 125 days and two control goats had no clinical signs. Clinical signs were characteristic of tremorgenic syndrome. Thus Medeiros *et al.* (2003) concluded that *I.asarifolia* caused a tremorgenic syndrome due to an unknown tremorgenic phytotoxins.

Paula *et al*. (2003) investigated the anti-inflammatory activity of methanol-water extract of the *Ipomoea imperati* leaves, in experimental models of acute and sub chronic inflammation. Topical application of extract inhibited mouse ear edema, induced by croton oil and the methanolic as well as aqueous extract of *I.imperati* administered by oral route also inhibited the formation of cotton pellet induced granulomas. It also inhibited in a dose dependent manner the muscle contractions of guinea pig ileum induced by acetyl choline and histamine.

Schawarz *et al.* (2003) investigated the effects of *Ipomoea carnea* aqueous fraction intake by dams during pregnancy on the physical and neuro behavioral development of rat offsprings. The effects of daily prenatal exposure to the aqueous extract (AQE) of *Ipomoea carnea* dried leaves on gestational days 5 to 21 were studied in rat pups and adult offsprings. The offsprings developmental alterations were not severe enough to produce behavioral and central monoamine level changes.

2.2. ACUTE INFLAMMATORY MODEL

2.2.1. Carrageenin induced paw oedema in rats.

In the present study on the anti-inflammatory effect of *Ipomoea mauritiana* roots, the carrageenin induced paw oedema method was used.

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

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

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

2.2.2. Croton oil induced skin inflammation in mice.

The anti-inflammatory effect of *Ipomoea mauritiana* roots was studied using the croton oil induced mice skin inflammation model.

Cochet *et al.* (1986) observed that TPA in croton oil was believed to function, at least in part, by interacting with and activating protein kinase C, an

important enzyme involved in the regulation of variety of biological processes, including cell growth and differentiation.

According to Paula *et al*. (2003) 12-O-tetradecanoyl phorbol acetate (TPA), a phorbol ester present in croton oil was shown to act as an inducer of inflammation and the mechanism of croton oil induced inflammation involved an increase in phospholipase A_2 activity which in turn lead to the release of arachidonic acid and subsequent biosynthesis of leukotrienes and prostaglandins, thus involve both cyclo-oxygenase and lipoxygenase path ways.

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

Many plant extracts have been screened for anti-inflammatory effects in acute inflammatory models especially on carrageenin induced paw oedema and croton oil induced skin inflammation.

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

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

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

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

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

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

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

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

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

The triglyceride fraction of oil from *Ocimum sanctum* (3 ml/kg, intraperitoneally) offered higher protection against carrageenin induced paw oedema in rats as compared to the fixed oil. The fixed oil of *O. sanctum* also exhibited significant anti-inflammatory activity against carrageenin, serotonin, histamine and PGE_2 induced paw oedema in rats. The *O.sanctum* fixed oil contains linoleic acid, which could contribute to dual inhibition of the arachidonic acid metabolism resulting in anti-inflammatory activity (Singh and Majumdar, 1997).

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

Oil of *Psidium guajava* leaves showed anti-inflammatory activity at the dose rate of 0.8 ml /kg body weight orally in carrageenin induced paw oedema. (Kavimani *et al.*, 1997).

Rao and Mishra (1997) reported that the methanolic extract of the aerial part of *Sida rhombifolia* (Atibala) at the dose rate of 100 mg/kg *per os* possessed significant oedema suppressant activity in the carrageenin induced paw oedema model in rats. The mechanism of action probably due to its inhibitory effects on release of mediators of inflammation such as histamine, 5- hydroxytryptamine, bradykinin etc.

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

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

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

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

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

Singh (1999) reported that the fixed oil of *Ocimum basilicum* had produced significant anti-inflammatory activity against carrageenin, histamine, serotonin, PGE_2, bradykinin and hyaluronidase induced paw oedema in rats at the dose rate of 3 mg/kg. It also inhibited the arachidonic acid and leukotriene induced oedema.

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

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

A herbal formulation named JCB containing *Alpinia galanga, Commiphora wightii, Boswellia serrata, Foenicutum vulgare, Glycyrrhiza glabra, Vitex negundo* and *Anethum graveolens* showed anti-inflammatory effect at the dose

rate of 350 mg/kg and 700 mg/kg body weight, *per os* against carrageenin induced paw oedema in rats (Venkataranganna *et al.*, 2000).

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

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

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

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

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

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

The lupeol and 19 α-H lupeol isolated from the roots of *Strobilanthus callosus* and *S. oxycephala* respectively, showed significant anti-inflammatory activity against carrageenin induced paw oedema. Both compounds in doses of 200, 400 and 800 mg/kg produced a dose dependent inhibition of paw oedema (Agarwal and Rangari, 2003).

Costa *et al*. (2003) assessed the anti-inflammatory properties of dried leaves extract from *Bouchea fluminensis* in rats by carrageenin induced paw oedema method. After the injection, the volume of the paw was determined with a plethysmometer at the1st, 2nd, 3rd and 4th hour. Oral pretreatment of animals with that crude triterpene mixture (IG) as well as the purified fraction containing ursolic, oleanolic and micromeric acids (IG-59) from *B. fluminensis* had anti-inflammatory activity. The doses ranging from 1 to 30 mg/kg significantly inhibited carrageenin induced oedema formation in all the four hours of inflammation.

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

Lakshmi *et al.* (2003) examined the antiperoxidative, anti-inflammatory, and antimutagenic activities of the ethanol extract of the mycelium of a medicinal mushroom, *Ganoderma lucidum*, found in south India. Anti-inflammatory activity was evaluated using phorbol ester-induced mouse skin inflammation. The extract showed significant inhibition of Fe^{2+}-induced peroxidation of lipid in rat liver and 37 per cent inhibition of croton oil-induced peroxidation on the mouse skin.

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

Paula *et al*. (2003) investigated the anti-inflammatory activity of methanol-water extract of the *Ipomoea imperati* leaves, in experimental models of

acute and sub chronic inflammation. Topical application of extract inhibited mouse ear oedema induced by croton oil and the methanol-water extract of *I.imperati* administered by oral route also inhibited the formation of cotton pellet induced granulomas

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

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

Ahamed *et al*. (2005) found that the oral administration of the alcoholic extracts of *Araucaria bidwillii* Hook. significantly inhibited the carrageenin (18.61, 32.12 and 45.64 per cent) and serotonin (32.81, 38.68 and 40.75 per cent) induced hind paw oedema in rats at 100, 200 and 300 mg/kg respectively.

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

The leaves of *Garcinia xanthochymus* (Guttiferae) was investigated for anti-inflammatory activity using carrageenin induced rat paw oedema method. The results demonstrated that the percentage inhibition by petroleum ether extracts and

methanolic extract was 86.4 and 80.7 per cent respectively when compared with standard drug ibuprofen (Pal *et al.,* 2005).

Rabanal *et al.* (2005) investigated the analgesic and topical anti-inflammatory activities of methanol extract and fractions of the aerial part, in blossom of *Hypericum canariense* L. and *Hypericum glandulosum* Ait. in mice. The acetic acid-induced writhing test, tail flick test and the tetradecanoylphorbol acetate (TPA)-induced ear inflammation model in mice were used to determine these effects. The results indicated the analgesic and topical anti-inflammatory activities of these plants.

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

The anti-inflammatory and antinociceptive activities of the ethanolic extract (EE) from aerial parts of *Pluchea quitoc* were evaluated in mice and rats by Barros *et al.* (2006). Oral treatment with the EE (1 to 2 g/kg, *per os*) decreased the paw oedema induced by carrageenin in rats, showed antinociceptive effects (tail-flick test and acid-induced writhing in mice) and inhibited both phases of pain (neurogenic and inflammatory) of the formalin test in rats. Topical application (EE 1.25, 2.5 and 5.0 mg) inhibited the ear oedema induced by croton oil in mice.

The root barks of *Zizyphus lotus* were extracted with water, chloroform, ethyl acetate and methanol and determined their anti-inflammatory and analgesic activities by Borgi *et al*. (2007). Aqueous extract (50, 100 and 200 mg/kg) given intraperitoneally (i.p.) showed a significant and dose-dependent anti-inflammatory activity. The intraperitoneal administration of the aqueous extract of *Z. lotus* root barks (50, 100 and 200 mg/kg) reduced significantly the paw edema induced by carrageenan by 37.81 per cent, 69.18 per cent and 72.90 per cent respectively three hours after the injection. After intraperitoneal administration of methanolic extract, significant activity was observed at the dose of 200 mg/kg, at the third hour after carrageenan injection, with 67.57 per cent reduction in paw volume.

On the contrary, only a small and not significant activity was seen at the sixth hour after the injection of ethyl acetate and chloroform extracts.

2.4 ANTI OXIDANT ENZYMES ON VARIOUS SKIN SAMPLES

The antioxidant capacity of the human epidermis is far greater than that of dermis. As the concentration of every antioxidant (referenced to skin wet weight) was higher in the epidermis than in the dermis. Among the enzymic antioxidants, the activities of superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were higher in the epidermis (Shindo *et al*., 1994).

Saleem *et al*. (2001) assessed the effect of *Cheiranthus cheiri* on 12-O-tetradecanoyl 13-phorbol acetate (TPA)-croton oil induced cutaneous oxidative stress and toxicity in 30 male mice. TPA treatment alone resulted in the depletion of cutaneous glutathione level and decreased the activities of glutathione reductase, and glutathione S-transferase by 50, 67 and 40 per cent respectively, as compared to acetone treated control animals. Pretreatment of animals with *Cheiranthus cheiri* resulted in the significant partial recovery of cutaneous glutathione levels and the activity of glutathione S-transferase and glutathione reductase ranged from 10 to 40 per cent, 8 to 30 per cent and 25 to 50 per cent, as compared with TPA treated control. Treatment with TPA alone resulted in reduced activity of cutaneous antioxidant enzymes like glutathione peroxidase and catalase to the levels 40 and 50 per cent of the acetone treated control respectively. The recovery of antioxidant enzymes ranged from 25 to 50 per cent and 12 to 38 per cent, as compared with TPA control.

Lakshmi *et al.* (2003) examined the antiperoxidative, anti-inflammatory, and antimutagenic activities of the ethanol extract of the mycelium of a medicinal mushroom, *Ganoderma lucidum*, found in south India. The extract showed significant inhibition of Fe^{2+}-induced peroxidation of lipid in rat liver and 37 per cent inhibition of croton oil-induced peroxidation on the mouse skin.

Delazar *et al*. (2003) conducted biochemical studies in mice skin tissues based on the measurement of lipid peroxidation (LPO). This study showed that Tannic Acid (TA) inhibited the carcinogenic potential of croton oil and iron dextran

significantly. TA diminished cutaneous LPO level in mice skin when compared with the untreated groups. A depletion in LPO levels in TA pretreated groups indicated that excessive generated oxidants in the mice skin tissues by croton oil were quenched by TA because of chelation of redox active iron and its faster elimination from the body.

2.5. ACUTE PAIN MODEL-TAIL FLICK METHOD:

In the present study on analgesic effect of *Ipomoea mauritiana* roots, the tail flick method was taken as a prototype of acute pain models.

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

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

2.6. PLANTS EXHIBITING ANALGESIC EFFECT IN ACUTE PAIN MODEL

The aqueous extract *Azadirachta indica* leaves was tested for analgesic potency at the dose rate of 10, 30,100 mg/kg, intraperitoneally in mice. It enhanced tail withdrawal latencies in tail flick model of nociception. Khanna *et al.* (1995) also observed that the pre-treatment with the opioid antagonist, naloxone (1mg/kg, intraperitoneally) and central nor adrenaline depleter DSP-4 (N-2-Chloroethyl-N ethyl-2-bromobenzylamine-50 mg/kg, intraperitoneally) attenuated the analgesia. The serotonin synthesis inhibitor, parachlorophenylalanine methylester hydrochloride (300 mg/kg, intraperitoneally) also potentiated the analgesic effect. These results suggested that both the central and peripheral mechanisms and complex neural pathways might be involved in this effect.

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

The petroleum ether extract, benzene extract and ethanolic extract of *Pongamia pinnata* seeds showed significant analgesic effect in the tail flick test in rats (Singh *et al.*, 1996).

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

The hydroalcoholic extract of *Vitex negundo* leaves showed analgesic activity at the dose of 500 mg/kg and 1000 mg/kg orally against tail immersion test and acetic acid induced writhing in mice. It significantly increased the reaction time in tail immersion model and reduced the writhing movement in acetic acid induced writhing in mice (Telang *et al.*, 1999).

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

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

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

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

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

The methanolic, chloroform and petroleum ether extracts of *Cleome rutidosperma* were investigated for analgesic (narcotic, and non-narcotic) activity in tail flick method and acetic acid induced writhing in mice at a dose of 100 mg/kg administered orally. All these extracts showed significant analgesic activity compared with control and standard drug treatment with aspirin and morphine sulphate (Bose *et al*., 2004).

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

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

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

The aqueous extract of *Smilax china* at the dose rate of 1000 mg/kg, administered orally had a significant antinociceptive activity against hot plate test in

mice. Further studies were done to understand the molecular basis of mechanism of action of aqueous extract on cyclo-oxygenase -2. It revealed that the extract inhibited the prostaglandin production by suppression of cyclo-oxygenase-2 protein synthesis in whole blood assay (Shu *et al*., 2006).

2.7. DICLOFENAC:

Diclofenac has a dual mode of action via cyclo-oxygenase as well as lipoxygenase pathways. Diclofenac interacts with the arachidonic acid cascade at the level of cyclo-oxygenase and inhibits its activity. Inhibition of this key enzyme was also found in *in vitro* studies, which may consequently prevent the formation of thromboxanes, prostaglandin and prostacyclins (Menasse *et al.*, 1978).

Kyuki *et al*. (1982) determined an optimum concentration of diclofenac-sodium in cream and based on the results, they concluded that the cream preparation containing 1.0 per cent of diclofenac-sodium was more effective than others for external application. They also found that diclofenac cream produced obvious inhibition on increased vascular permeability and had a strong inhibitory effect on carrageenin-induced paw oedema and oedema in the ear of mice induced by croton oil

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

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

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

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

Diclofenac is rapidly and completely absorbed after oral administration, then a peak plasma concentration is reached within 2 to 3 hours and its plasma half-life is one to two hours. It is metabolized in the liver by cytochrome P450 isoenzyme of the CYP2C family to 4-hydroxy diclofenac and other hydroxylated forms, which undergo glucuronidation and sulfation. The metabolites are excreted in urine and bile (Roberts and Marrow 2001).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Eighty Sprague-Dawley rats of either sex weighing 150-200 gram and thirty swiss female albino mice weighing 20-25 gram, procured from Small Animals Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for the study. All the animals were maintained under identical feeding and management practices in the laboratory.

3.2 PREPARATION OF EXTRACT AND ADMINISTRATION OF DRUGS

The plant under study was *Ipomoea mauritiana.*

3.2.1 *Ipomoea mauritiana*

The root of the plant was cleaned, dried and pulverized to a coarse powder. The powder was extracted with 95 per cent ethanol using soxhlet extraction apparatus. The extract was evaporated to dryness using rotary vacuum evaporator under reduced pressure and temperature (55ºC) and was kept in an airtight container in refrigerator. The yield of the extract was 4.1 per cent on a dry matter basis.

3.2.2 Gum acacia

Five per cent of gum acacia was prepared by dissolving five gram of gum acacia powder in 100 ml of normal saline and was used as vehicle for the preparation of drugs.

A

B

Fig 1. ***Ipomoea mauritiana***
A-Whole plant,B-Root

3.2.3 Diclofenac Potassium

It was used as the reference drug (commercial tablet) at the dose rate of 3 mg/kg body weight and was administered orally, once daily in the morning on the 7th day before feeding, in five per cent gum acacia.

3.3 EXPERIMENTAL DESIGN

Eighty Sprague-Dawley rats were divided in to two groups of 40 animals each, which were again divided into ten groups of eight animals each for anti- inflammatory and analgesic studies. Thirty Swiss albino mice were divided into five groups of six animals each and was used for croton oil induced skin inflammation model of anti-inflammatory study.

3.3.1. Anti-inflammatory study

3.3.1.1. Carrageenin induced rat paw oedema method

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

Group I - 5 per cent gum acacia (vehicle) alone was administered *per os* for 7 days

Group II - Vehicle alone was administered *per os* for 6 days and diclofenac potassium @ 3 mg/kg body weight *per os* on 7th day.

Group III - Ethanolic extract of roots of *Ipomoea mauritiana* was administered @ 300 mg/kg body weight *per os* for 7 days.

Group IV - Ethanolic extract of roots of *Ipomoea mauritiana* was administered @ 600 mg/kg body weight *per os* for 7 days.

Group V - Ethanolic extract of roots of *Ipomoea mauritiana* was administered @ 1200 mg/kg body weight *per os* for 7 days.

On 7th day the paw oedema was induced after half an hour of drug administration by injecting of 0.05 ml of carrageenin (2 per cent w/v carrageenin suspension in normal saline) into plantar aponeurosis of the left hind paw of rat of all groups.

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

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

$$\text{Percentage inhibition of paw oedema} = \left(1 - \frac{V_t}{V_c}\right) \times 100$$

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.

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

3.3.1.2. *Croton oil induced skin inflammation model*

Croton oil was used topically for inducing inflammation on the skin surface. Back of each animal (female swiss albino mouse) was shaved using surgical clippers over an area with 8 mm diameter two days before the experiment. Animals with complete stoppage of hair growth were grouped into five groups of six animals each and treated as follows.

Group 1 Normal

Group 2 Croton oil (1 in 8 dilution with liquid paraffin) applied topically

Group 3 5 per cent diclofenac potassium cream applied topically 30 minutes before application of croton oil.

Group 4 Ethanolic extract 20 mg applied topically 30 minutes before application of croton oil.

Group 5 Ethanolic extract 30 mg applied topically 30 minutes before application of croton oil.

Ethanolic extract of *Ipomoea mauritiana* was applied topically to the shaved area of dorsal skin 30 minutes before application of croton oil (Lakshmi *et al*., 2003). After 24 hours, the extract and croton oil treatment was repeated on the same area. Group treated with croton oil alone was kept as inflammatory control. Diclofenac potassium (5 per cent cream) was used as the reference drug. One hour after the second treatment of croton oil, animals were sacrificed and the skin was removed. The skin punches were obtained with an 8 mm diameter cork borer. The skin punches were weighed in an analytical balance and the percentage inhibition was calculated using the formula,

$$\%\,\text{Inhibition} = \frac{[1-(\text{punch wt. of treated}-\text{punch wt. of normal})]\,x}{(\text{punch wt. of control}-\text{punch wt. of normal}}$$

After weighing, the skin punches were used for the estimation of superoxide dismutase, lipid peroxides, reduced glutathione levels and for conducting histopathological studies.

3.3.2. Analgesic activity

3.3.2.1. Tail flick method

Analgesic effect in rats was assessed by tail flick method (Dandiya and Menon, 1963) using analgesiometer. This instrument had a nichrome wire, which was heated and maintained at the required temperature by means of heat regulators. The current passing through the nichrome wire was indicated by an ammeter, which indirectly indicated the temperature of wire. A jacket surrounds the

nichrome wire through which water was circulated and that prevented the platform from getting heated up. The upper surface of the jacket served as a platform on which the tail of the rat was placed. This ensured that only the portion of the tail, which lied on the wire was heated. The ammeter was set to 4 amperes so that the heat produced in the nichrome wire was same throughout the experiment. The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting.

Forty adult rats were divided into five groups of eight each and treated as follows.

Group I - 5 per cent gum acacia (vehicle) alone was administered *per os* for 7 days.

Group II - Vehicle alone was administered *per os* for 6 days and diclofenac potassium @ 3 mg/kg body weight *per os* on 7th day.

Group III - Ethanolic extract of roots of *Ipomoea mauritiana* was administered @ 300 mg/kg body weight *per os* for 7 days.

Group IV - Ethanolic extract of roots of *Ipomoea mauritiana* was administered @ 600 mg/kg body weight *per os* for 7 days.

Group V - Ethanolic extract of roots of *Ipomoea mauritiana* was administered @ 1200 mg/kg body weight *per os* for 7 days.

On the 7th day after administration of drugs, reaction time for each group was measured at 30, 60, 90 and 120 minutes using Techno Analgesiometer. Animals with the reaction time of more than 10 seconds were discarded to avoid tissue injury. The blood was collected after 120 minutes and the biochemical parameters and hematological parameters were assessed.

3.4 SCREENING OF ETHANOLIC EXTRACTS OF *Ipomoea mauritiana* ROOTS FOR ACTIVE PRINCIPLES

The ethanolic extracts of Ipomoea roots were tested for the presence of various active principles namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure described by Harborne (1991).

3.4.1 Tests for Detection of Steroids

Salkowski test

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

Lieberman Burchardt test

Five milligram of extract was dissolved in 3 ml of chloroform in a test tube. Five drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added through the sides of the test tube. Development of a reddish ring at the junction of two layers indicates the presence of steroids.

3.4.2 Tests for Detection of Alkaloids

The extract weighing 500 mg was dissolved in 5 ml of ammonia and then extracted with equal volume of chloroform. To this, 5 ml dilute hydrochloric acid (0.2 N) was added. The acid layer obtained was used for chemical tests for the alkaloids.

Mayer's test

To 1 ml of acid extract, five drops of Mayer's reagent (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g of potassium iodide in 10 ml of water and then made the volume to 100 ml with

distilled water) was added. The development of a creamy white precipitate indicates the presence of alkaloids.

Wagner's test

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

Hager's test

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

Dragendroff's test

A few drops of Dragendroff's reagent (Stock solution (1) - 0.6 g of bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid to which 10 ml of water was added. Stock solution (2) - 6 g of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water) was mixed with 1 ml of acid extract. The development of a reddish brown precipitate indicates the presence of alkaloids.

3.4.3 Test for Detection of Phenolic compounds

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

3.4.4 Tests for Detection of Tannins

Ferric chloride test

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

Gelatin test

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

3.4.5 Tests for Detection of Flavonoids

Ferric chloride test

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

Lead acetate test

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

3.4.6 Tests for Detection of Glycosides

Sodium hydroxide test

Five milligram of extract was mixed with 1 ml water and added 6 drops of sodium hydroxide solution (10 per cent). The development of a yellow colour indicates the presence of glycosides.

Benedict's test

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

3.4.7 Test for Detection of Diterpenes

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

3.4.8 Tests for Detection of Triterpenes

Salkowski test

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

Lieberman Burchardt test

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

3.4.9 Test for the Detection of Saponins

Foam test

Five mg of the extract was shaken with 3 ml of water. The development of the foam that persists for ten minutes indicates the presence of saponins.

3.5 BIOCHEMICAL PARAMETERS

3.5.1 Estimation of serum transaminases.

3.5.1.1. Alanine Amino Transferase (ALT)

The UV-Kinetic test described by Reitman and Frankel (1957) was followed. The Kit from Merck diagnostics was used for estimation of ALT.

3.5.1.2. Aspartate Amino Transferase (AST)

The UV-Kinetic test described by Reitman and Frankel (1957) was followed. The Kit from Merck diagnostics was used for estimation of AST.

3.5.2 Evaluation of Antioxidant property

3.5.2.1 Estimation of Lipid peroxides

The levels of lipid peroxides in skin tissue were estimated by the method of Fraga *et al.* (1988).

a. Reagents

1. Trichloro acetic acid (TCA)-15 per cent
2. Hydrochloric acid (HCl)-0.25 N
3. Thiobarbituricacid ((TBA) -0.38 per cent in hot distilled water
4. TCA-TBA-HCl reagent solution: 1,2 and 3 were mixed freshly in the ratio 1:1:1
5. Standard solution -4.8 Mm: 0.079 ml of 1,1,3,3 tetra methoxy propane was diluted to 100 ml.
6. Tris –HCl buffer (pH 7.5).

b. Procedure

1. 50 mg of skin tissue was homogenized with Tris –HCl buffer (pH 7.5)
2. 1.0 ml of the tissue homogenate was treated with 2.0 ml of TBA – TCA- HCl reagent and mixed thoroughly.
3. The mixture was kept in boiling water bath for 15 minutes.
4. After cooling, the tubes were centrifuged at 3200 rpm for 10 minutes and the supernatant was taken for measurement.
5. The standard solution was also treated in the similar manner.
6. The absorbance of the chromophore was read at 535 nm against the TBA-TCA- HCl reagent blank using genesis spectrophotometer.

Values were expressed as Mm /100g wet tissue.

3.5.2.2 Determination of Tissue Protein

Protein content in the tissue was determined according to the method of Lowry *et al.* (1951)

a. Reagents

1. Alkaline Copper sulphate (0.5 per cent Copper sulphate in 1 per cent Sodium potassium tartrate and 2 per cent sodium carbonate in 0.1 N sodium hydroxide in the ratio 1:50)
2. 1N Folin phenol reagent

b. Procedure

1. 0.01 ml of the homogenate was made up to 1 ml of distilled water, 5 ml of alkaline copper sulphate was added and kept for ten minutes at room temperature.

2. 0.5 ml of 1 N folin phenol reagent was added to the mixture

3. The absorbance was measured after 20 minutes at 660 nm against the reagent blank. (Distilled water treated in similar manner as that of homogenate.)

4. Protein content was calculated from the standard graph prepared using different concentrations (0.1 to 0.5 mg/ml) of bovine serum albumin (BSA)

3.5.2.3 Estimation of reduced Glutathione

Reduced glutathione was estimated by the method of Ellman (1959).

a. Reagents

1. Phosphate buffer-0.2 M, pH 8.0

2. Trichloro acetic acid: 5 per cent

3. Ellman's reagent: 19.8 mg of dinitro bis benzoic acid in 100 ml of 1 % sodium citrate solution.

4. Standard glutathione solution: 10 mg of reduced glutathione was dissolved in 100 ml of distilled water.

b. Procedure

1. 50 mg of skin tissue was homogenized with phosphate buffer.

2. From this, 0.5 ml was pipetted out and precipitated by adding 2.0 ml of 5 per cent trichloro acetic acid.

3. 1.0 ml of the supernatant was taken out after centrifugation and 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer were added to it.

4. The yellow colour developed was read at 412 nm using 'Genesys' spectrophotometer.

5. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer. The amount of glutathione was expressed as mg/100g of tissue.

3.5.2.4 Estimation of superoxide dismutase

superoxide dismutase was estimated by the method of Minami and Yoshikawa (1979).

a. Reagents

1. EDTA -0.1 M (Containing 0.0015 per cent Sodium cyanide)
2. NBT (Nitroblue tetrazolium)-1.5 mM
3. Phosphate buffer (67 mM, pH 7.8)
4. Riboflavin

b. Procedure

1. 50 mg skin tissue was homogenized with phosphate buffer (67 mM, pH 7.8)
2. 0.1 ml of homogenate was mixed with 0.2 ml of 0.1 M EDTA, 0.1 ml of 1.5 mM NBT and phosphate buffer in a total volume of 2.6 ml.
3. Added 0.05 ml of riboflavin
4. The absorbance of the solution was determined against distilled water as blank at 560 nm
5. All the tubes were uniformly illuminated for 15 minutes
6. The absorbance of the blue colour formed was measured again at 560 nm.

7. Percentage of inhibition was calculated after comparing absorbance of sample with that of control

Values were expressed in U/mg of Protein.

3.6 HAEMATOLOGICAL PARAMETERS

3.6.1 Total Leukocyte Count

The leukocytes were counted (A) by standard dilution technique using Thomas fluid diluent (1:20). Counting of leukocytes was done in the zone of leukocytes in the haemocytometer focused under low power of the microscope (Schalm, 1986).

Total WBC s= A×50

3.6.2 Total RBC count

1:200 dilution of blood with Hayem's fluid was done with RBC diluting pipette. After mixing and loading on haemocytometer, kept for 5 minutes. RBC located in four corner and one central squares were counted (A) (Schalm, 1986).

Total RBC s= A×10,000

3.6.3 Haemoglobin

Haemoglobin level was determined by the standard procedure of Ferri haeme hydrochloride method (Sasthri, 1998).

3.6.4 Volume of packed red cells

PCV was estimated by micro haematocrit method (Schalm, 1986).

3.6.5 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 through light microscope under oil immersion (Schalm, 1986).

3.7 HISTOPATHOLOGICAL EXAMINATION

Excised skin was fixed in 10 per cent formalin and then embedded in paraffin. Microtome sections were prepared from each skin and stained with hematoxylin-eosin to study the histopathological changes in skin.

3.8 STATISTICAL ANALYSIS OF DATA

The results obtained were analyzed using Analysis of Co-variance method followed by Duncan's multiple range test for comparison between groups as described by Snedecor and Cochran (1985).

Results

4. RESULTS

4.1. PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF *Ipomoea mauritiana.*

Active principles in *Ipomoea mauritiana* root extract are presented in Table 1.

4.1.1 Steroids

No red colour was obtained through the Salkowski test and no reddish ring at the junction via Lieberman Burchardt. Thus it could be concluded that the steroids were not present in ethanolic extract of roots of *Ipomoea mauritiana*.

4.1.2 Alkaloids

A creamy white precipitate was obtained in Mayer's test and a reddish brown coloured precipitate was obtained in Wagner's test. Dragendroff's test yielded a reddish brown precipitate. Hager's test produced yellow precipitate in the extract. Thus, the tests revealed the presence of detectable level of alkaloids in Ipomoea root extracts.

4.1.3 Phenolic Compounds

A dark blue colour was produced, when extract was mixed with ten per cent ferric chloride solution which indicated the presence of phenolic compounds.

4.1.4 Tannins

Intense blue colour was obtained when the extract was treated with ferric chloride and a white precipitate in gelatin test. These results indicated the presence of tannins in Ipomoea roots.

4.1.5 Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in lead acetate test indicated the presence of flavonoids in the ethanolic extract of roots of *Ipomoea mauritiana*.

4.1.6 Glycosides

In the Benedict's test, brown colour was obtained indicating the presence of glycosides. The extract gave a yellow colour when mixed with sodium hydroxide, which also indicated the presence of glycosides in ethanolic extract of Ipomoea roots.

4.1.7 Diterpenes

Diterpenes were detected in the extract of roots as indicated by the green colour, when mixed with copper acetate solution.

4.1.8 Triterpenes

Lieberman Burchardt test produced a deep red ring at the junction of two layers, which indicated the presence of triterpenes in ethanolic extract of root extract.

4.1.9 Saponins

In the foam test, the foam did not persist for 10 minutes when the root extract was shaken with water, which indicated the absence of saponins.

Table 1. Active principles of ethanolic extract of *Ipomoea mauritiana* root

Sl. no	Active Principles	Results
1	Steroids	Not Detected
2	Alkaloids	Detected
3	Tannins	Detected
4	Flavonoids	Detected
5	Glycosides	Detected
6	Phenolic compounds	Detected
7	Diterpenes	Detected
8	Triterpenes	Detected
9	Saponins	Not Detected

4.2. INVESTIGATION OF ANTI-INFLAMMATORY PROPERTY OF ETHANOLIC EXTRACT OF *Ipomoea mauritiana* (PALMUDUKKU) ROOTS

4.2.1. Carrageenin induced paw oedema

The change in foot pad volume and percentage of inhibition of paw oedema in rats administered with the ethanolic extract of roots of *Ipomoea mauritiana* after first, second and third hours of carrageenin administration are presented in Tables 2, 3 and 4 respectively and also in Fig.2. Group III fed with ethanolic extract of Ipomoea roots, at the dose rate of 300 mg/kg exhibited 42.3, 11.4 and 36.26 per cent inhibition of oedema induced by carrageenin during first, second and third hour respectively (Table 5). For Group IV (fed with 600 mg/kg extract) the percentage of inhibition of oedema was 38.46, 47.5 and 36.26 per cent (Table 5) at first, second and third hour respectively. Group V (fed with 1200 mg/kg extract) exhibited 21.30 and 34.06 percentage of inhibition of oedema (Table 5) during second and third hour respectively, where as, it did not show any reduction in paw volume during the first hour after carrageenin injection. The reference drug diclofenac (Group II) showed 57.69, 75.40 and 65.97 per cent inhibition (Table 5) during first hour, second hour and third hour respectively after carrageenin injection.

4.2.2. Croton oil induced skin inflammation

The change in mouse skin thickness induced by croton oil and the ethanolic extract of roots of *Ipomoea mauritiana* after 24 hour of croton oil application are presented in Table 6 and in Fig.3. Group 4 treated with 20 mg extract, 30 minutes before croton oil application, topically to the shaved area of dorsal skin (8 mm diameter) exhibited only 7.4 per cent inhibition of thickness induced by croton oil, where as the Group 5 (30 mg extract before croton oil application) exhibited 14.91 per cent inhibition of oedema. The treatment with reference drug diclofenac showed significant ($P<0.05$) reduction in skin thickness where the percentage of inhibition was 33.4.

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

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.0	0.5	0.0	0.0	0.3
2	0.3	0.0	0.5	0.3	0.2
3	0.4	0.3	0.5	0.2	0.3
4	0.7	0.1	0.1	0.6	0.3
5	0.1	0.0	0.2	0.3	0.3
6	0.2	0.0	0.0	0.1	0.3
7	0.5	0.0	0.0	0.0	0.7
8	0.4	0.2	0.2	0.1	0.3
Mean ± SE	0.32 ± 0.22^{a}	0.14 ± 0.09^{a}	0.19 ± 0.001^{a}	0.20 ± 0.20^{a}	0.34 ± 0.15^{a}

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

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

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	0.0	0.5	0.2	0.1	0.6
2	1.2	0.0	0.7	0.3	0.5
3	0.8	0.3	1.2	0.5	0.6
4	1.4	0.1	0.3	0.6	0.6
5	0.4	0.0	1.1	0.5	0.5
6	0.6	0.2	0.6	0.6	0.6
7	1.1	0.0	0.4	0.5	0.9
8	0.6	0.4	0.9	0.1	0.5
Mean ± SE	0.76 ± 0.46^{a}	0.19 ± 0.19^{b}	0.68 ± 0.36^{a}	0.40 ± 0.21^{b}	0.60 ± 0.13^{ab}

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

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

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	1.1	0.5	0.2	0.9	0.9
2	1.4	0.2	0.7	0.5	0.7
3	0.9	0.5	1.4	0.7	0.7
4	1.7	0.4	0.3	0.6	1.0
5	0.5	0.4	1.1	0.9	0.5
6	1.0	0.6	0.6	0.7	0.6
7	1.6	0.0	0.6	0.8	1.1
8	0.9	0.5	0.9	0.7	0.5
Mean ± SE	1.14 ± 0.40^{a}	0.39 ± 0.19^{b}	0.73 ± 0.39^{ab}	0.73 ± 0.14^{bc}	0.75 ± 0.23^{bd}

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

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

Time Interval (min.)	Group II	Group III	Group IV	Group V
60	57.69	42.30	38.46	0.00
120	75.40	11.40	47.50	21.30
180	65.97	36.26	36.26	34.06

Group I - 5 per cent gum acacia (vehicle) alone was administered *per os* for 7 days.

Group II - Vehicle alone was administered *per os* for 6 days and diclofenac potassium at the rate of 3 mg/kg body weight *per os* on 7^{th} day.

Group III - Ethanolic extract of roots of *Ipomoea mauritiana* was administered at the rate of 300 mg/kg body weight *per os* for 7 days.

Group IV - Ethanolic extract of roots of *Ipomoea mauritiana* was administered at the rate of 600 mg/kg body weight *per os* for 7 days.

Group V - Ethanolic extract of roots of *Ipomoea mauritiana* was administered at the rate of 1200 mg/kg body weight *per os* for 7 days.

Table 6. Effect of treatments on skin inflammation twenty four hour after croton oil application in mice (weight in mg)

Animal No.	Group I	Group 2	Group 3	Group 4	Group 5
1	70	110	74	105	90
2	66	113	77	100	93
3	65	110	66	101	95
4	71	109	76	100	90
5	60	100	66	94	94
6	70	115	78	108	97
Mean ± SE	67 ± 1.71^{a}	109.5 ± 2.11^{b}	72.83 ± 2.22^{a}	101.33 ± 1.96^{c}	93.16 ± 1.14^{d}

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

Group 1 Normal

Group 2 Croton oil (1 in 8 dilution with liquid paraffin) applied topically

Group 3 5 per cent diclofenac potassium cream applied topically 30 minutes before application of croton oil.

Group 4 Ethanolic extract 20 mg applied topically 30 minutes before application of croton oil.

Group 5 Ethanolic extract 30 mg applied topically 30 minutes before application of croton oil.

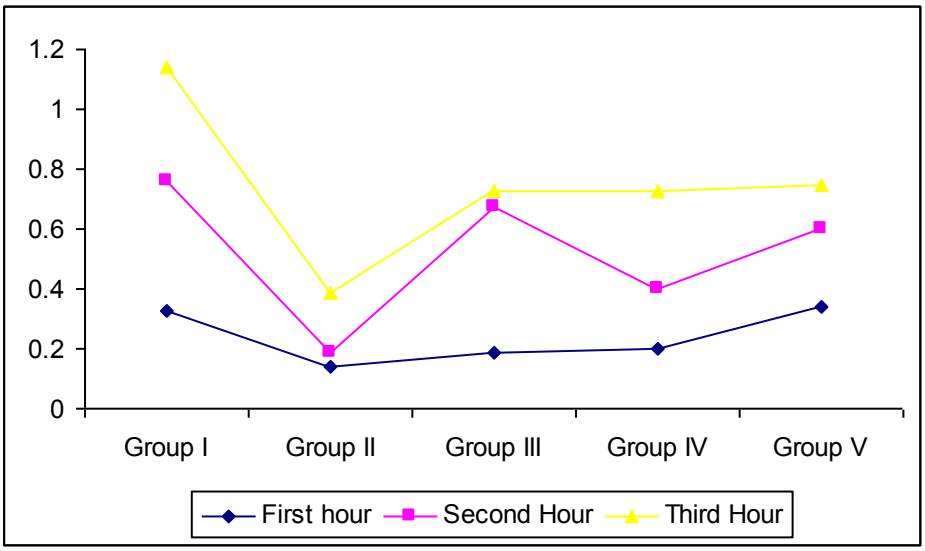

Figure 2. Effect of treatments on inhibition of carrageenin induced paw oedema in rats

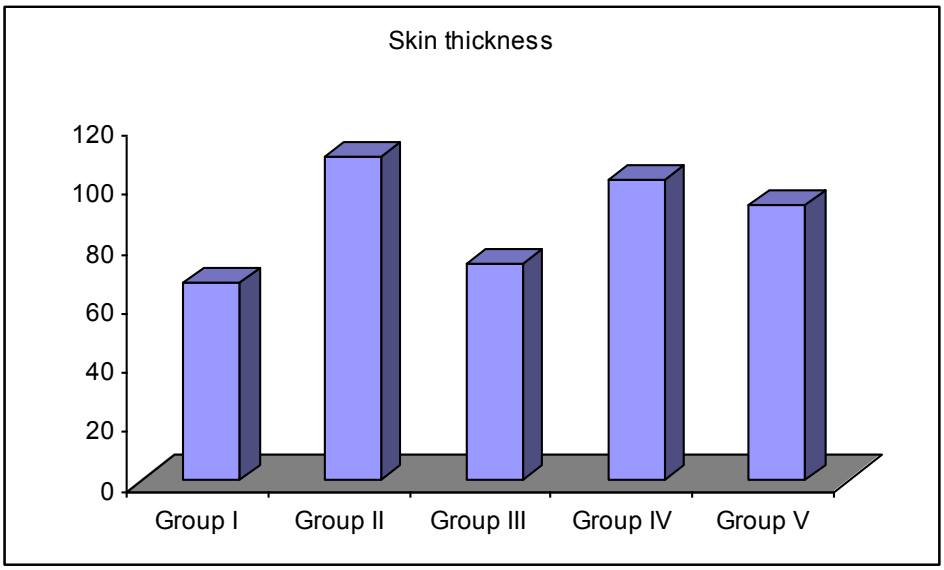

Figure 3. Effect of treatments on croton oil induced skin inflammation in mice

4.3 EFFECT ON HAEMATOLOGICAL PARAMETERS IN CARRAGEENIN INDUCED PAW OEDEMA METHOD

The results of haematological studies revealed that no significant ($P<0.05$) variation in any of the parameters like haemoglobin concentration, RBC count, total WBC count, differential leukocyte count and VPRC between groups.

4.3.1 RBC Count

The results of RBC count are presented in Table 7. For the control and diclofenac treated groups (I and II) the counts were 7.70±0.72 and 7.30±0.31 ($10^6/mm^3$) respectively. The rats administered with extract (Group III, IV and V) showed an RBC count of 7.08±0.65, 7.53±0.65 and 7.39±0.46 ($10^6/mm^3$) respectively.

4.3.2 WBC Count

WBC count is presented in Table 7. The WBC counts were 6.46±0.26, 6.29±0.38, 6.26±0.24, 6.29±0.16 and 6.27±0.26 ($10^3/mm^3$) for group I to V respectively.

4.3.3 Haemoglobin concentration

Haemoglobin concentrations are presented in Table 7. The values were 12.50±0.88, 13.43±0.49, 13.25±0.71, 12.75±1.28 and 13.00±0.92 gram percentage for group I to V respectively.

4.3.4 Volume of packed red cells

The results of VPRC are presented in Table 7. For the control and diclofenac treated groups (I and II) the values were 47.00±1.40 and 43.21±3.04 per cent, respectively. The rats administered with extract (Group III, IV and V) showed a PCV values of 43.32±4.42, 50.34±3.17 and 46.75±4.06 per cent respectively.

4.3.5 Neutrophils:

The results of neutrophil count are presented in Table 7. For the control and diclofenac treated groups (I and II) the counts were 52.00 ± 1.20, 48.25 ± 1.83 per cent respectively. The rats administered with extract, (Group III, IV and V) showed a neutrophil count of 50.63 ± 0.77, 49.75 ± 1.00 and 50.38 ± 0.73 per cent respectively.

4.3.6. Lymphocytes

The results are presented in Table 7. All the groups showed a slight decrease in lymphocyte count than the normal values. The counts were 45.50±1.18, 50.00±1.64, 46.75±0.59, 47.63±0.97 and 47.38±0.76 per cent for group I to V respectively.

4.3.7. Monocytes

Monocyte counts are presented in Table 7. The monocyte counts were 1.13±0.22, 1.00±0.32, 1.38±0.18, 1.50±0.38 and 1.38±0.18 per cent for group I to V respectively.

4.3.8. Eosinophils

The data are presented in Table 7. The eosinophil counts were 0.88±0.12, 0.50±0.20, 1.50±0.27, 1.13±0.18 and 0.88±0.22 per cent for group I to V respectively.

Table 7. Effect of treatment on haematological parameters in carrageenin induced paw oedema method

GROUPS	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (g%)	VPRC (%)	Differential leukocyte count (%)			
					Neutrophil	Lymphocytes	Monocyte	Eosinophils
Group 1	7.70±0.72^{a}	6.46±0.26^{b}	12.50±0.88^{c}	47.00±1.40^{d}	52.00± 1.20^{a}	45.50± 1.18^{b}	1.13± 0.22^{a}	0.88± 0.12^{ab}
Group 11	7.30±0.31^{a}	6.29±0.38^{b}	13.43±0.49^{c}	43.21±3.04^{d}	48.25± 1.83^{b}	50.00± 1.64^{a}	1.00± 0.32^{a}	0.50± 0.20^{ab}
Group 111	7.08±0.65^{a}	6.26±0.24^{b}	13.25±0.71^{c}	43.32±4.42^{d}	50.63± 0.77^{ab}	46.75± 0.59^{b}	1.38± 0.18^{a}	1.50± 0.27^{a}
Group 1V	7.53±0.65^{a}	6.29±0.16^{b}	12.75±1.28^{c}	50.34±3.17^{d}	49.75± 1.00^{ab}	47.63± 0.97^{ab}	1.50± 0.38^{a}	1.13± 0.18^{a}
Group V	7.39±0.46^{a}	6.27±0.26^{b}	13.00±0.92^{c}	46.75±4.06^{d}	50.38± 0.73^{ab}	47.38± 0.76^{ab}	1.38± 0.18^{a}	0.88± 0.22^{ab}

Mean±SE, n=8 Means bearing the same superscript do not differ significantly at P<0.05

4.4 BIOCHEMICAL PARAMETERS

4.4.1 Alanine Amino Transferase (ALT)

The ALT values are presented in Table 8 and in Fig 4. The mean values for the control and diclofenac treated group were 52.5±2.04 and 77.6±2.42 U/l respectively. The mean value for the groups administered with Ipomoea root extract (Group III to V) showed the values of 41.8±2.38, 37.5±2.79 and 50.6±1.72 U/l respectively.

4.4.2. Aspartate Amino Transferase (AST)

The AST values are presented in Table 9 and in Fig 4. The mean values for the control and diclofenac treated group were 83.8±2.03 and 156.0±2.39 U/l respectively. The mean value for the groups administered with Ipomoea root extract (Group III to V) showed the values of 81.0±2.25, 69.1±1.98 and 84.2±1.63 U/l respectively.

4.4.3 Effect of Ipomoea root extract on Antioxidant enzymes on mouse skin

4.4.3.1 Lipid peroxides

The results obtained are presented in Table 10 and in Fig 5. Application of croton oil produced significant ($P<0.05$) inflammation indicated by the increased values of lipid peroxides on mouse skin (7.73±0.21 mM/100 mg tissue). The normal lipid peroxide value for skin tissue was found to be 4.05±0.12 mM/100 mg tissue. The treatment with ethanolic extract of Ipomoea roots at the dose rate of 20 mg, 30 minutes before croton oil application, indicated a value of 7.50±0.15 mM/100 mg tissue which did not differ significantly ($P<0.05$) from the group treated with croton oil alone. The ethanolic extract of Ipomoea roots at the dose rate of 30 mg prior to the administration of croton oil indicated a value of 7.3 ± 0.20 mM/100 mg tissue, which also did not differ significantly ($P<0.05$) from inflammatory control group.

Table 8. Effect of treatments on alanine amino transferase (ALT, U/l) level in carrageenin induced paw oedema in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	48	71	32	34	48
2	46	80	38	46	44
3	55	77	47	41	55
4	58	70	41	37	59
5	52	90	36	51	51
6	60	80	48	30	48
7	55	71	52	32	53
8	44	82	40	29	47
Mean ± SE	52.5 ± 2.04^{a}	77.6 ± 2.42^{b}	41.8 ± 2.38^{c}	37.5 ± 2.79^{cd}	50.6 ± 1.72^{a}

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

Table 9. Effect of treatments on aspartate amino transferase (AST, U/l) level in carrageenin induced paw oedema in rats.

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	81.4	159	81	74	82
2	82.3	164	78	78	90
3	90.1	153	83	67	76
4	85.4	161	84	71	88
5	85.6	163	76	66	83
6	91.3	158	91	61	82
7	81.06	148	70	64	84
8	73.105	146	85	72	89
Mean ± SE	83.8 ± 2.03^{a}	156.0 ± 2.39^{b}	81.0 ± 2.25^{a}	69.1 ± 1.98^{c}	84.2 ± 1.63^{a}

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

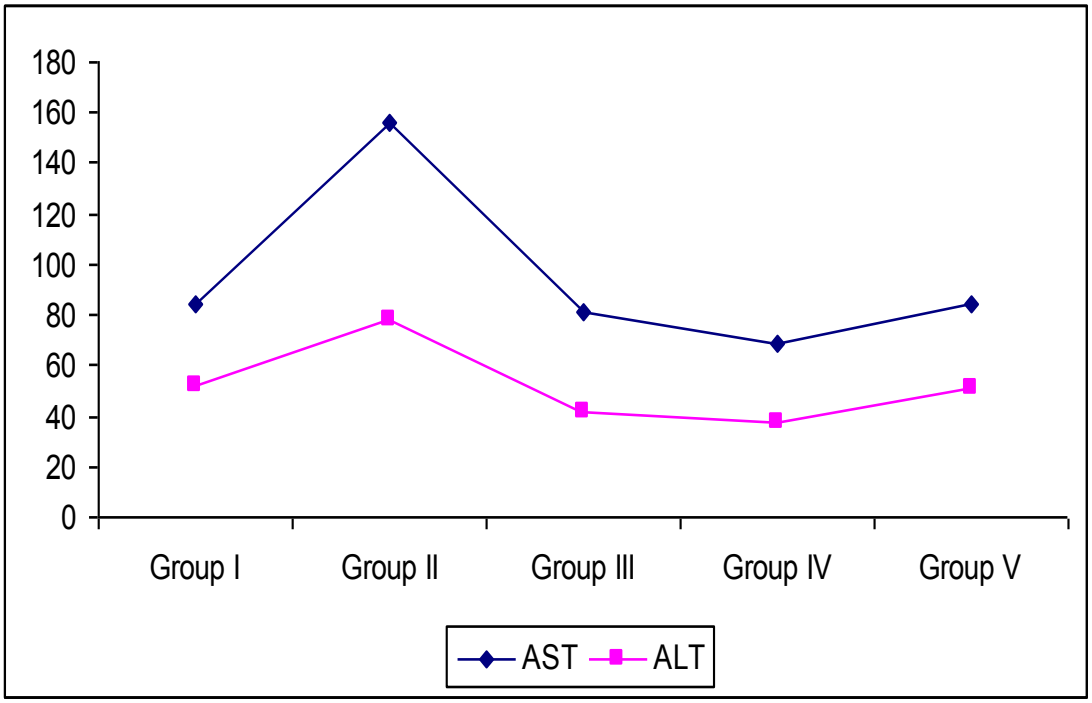

Figure 4. Effect of treatments on ALT and AST values in carrageenin induced paw oedema in rats.

The group treated with reference drug diclofenac before croton oil application produced slightly reduced value for skin lipid peroxides (5.40±0.77 mM/100 mg tissue), but it was also not significant ($P<0.05$).

4.4.3.2 Superoxide dismutase (SOD)

The results obtained are presented in Table 10 and Fig 6. Mouse skin inflammation was induced by application of croton oil and it was characterized by significantly ($P<0.05$) reduced values for skin SOD (1.60±0.31 IU/mg of protein). The normal SOD value for skin tissue was found to be 5.05±0.55 IU/mg of protein. A decrease in SOD level was noted in Group 4 (treated with 20 mg of ethanolic extract of Ipomoea roots before croton oil application) which indicated a value of 1.71±0.27 IU/mg of protein. Group 5 treated with ethanolic extract of Ipomoea roots at the dose rate of 30 mg before croton oil application gave a value of 1.80±0.31 IU/mg of protein and Group 3 treated with diclofenac cream before croton oil application produced slightly higher value for SOD (1.93±0.24 IU/mg of protein.). But all the groups (group 2, 3, 4 and 5) were significantly ($P<0.05$) different from that of normal control.

4.4.3.3 Reduced glutathione

The results obtained are presented in Table 10 and Fig 7. The normal reduced glutathione level was found to be 136.31±1.98 mg/100 g of tissue in mouse skin. Croton oil application produced significant ($P<0.05$) inflammation indicated by the decreased values of reduced glutathione (49.88±0.58 mg/100 g of tissue). All the treatment groups showed significant ($P<0.05$) reduction in reduced glutathione level when compared to group 1 (normal control). Group 4 and Group 5 indicated the values of reduced glutathione as 51.97±2.13 mg/100g of tissue and 56.08±0.90 mg/100g of tissue respectively. The group 3 (diclofenac with croton oil) produced comparatively higher value for reduced glutathione (75.49±1.27 mg/100g of tissue).

Table 10. Effect of treatments on skin superoxide dismutase, lipid peroxides and reduced glutathione after croton oil application in mice

Groups	Superoxide dismutase levels units/mg of protein (Mean±SE)	Lipid peroxide levels mM/g of tissue (Mean±SE)	Reduced glutathione levels mg/100g of tissue (Mean±SE)
Group 1	5.05±0.55[a]	4.05±0.12[a]	136.31±1.98[a]
Group 2	1.60±0.31[b]	7.73±0.21[c]	49.88±0.58[c]
Group 3	1.93±0.24[b]	5.40±0.77[b]	75.49±1.27[b]
Group 4	1.71±0.27[b]	7.50±0.15[c]	51.97±2.13[c]
Group 5	1.80±0.31[b]	7.30±0.20[c]	56.08±0.90[c]

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

Group 1 Normal

Group 2 Croton oil (1 in 8 dilution with liquid paraffin) applied topically

Group 3 5 per cent diclofenac potassium cream applied topically 30 minutes before application of croton oil.

Group 4 Ethanolic extract 20 mg applied topically 30 minutes before application of croton oil.

Group 5 Ethanolic extract 30 mg applied topically 30 minutes before application of croton oil.

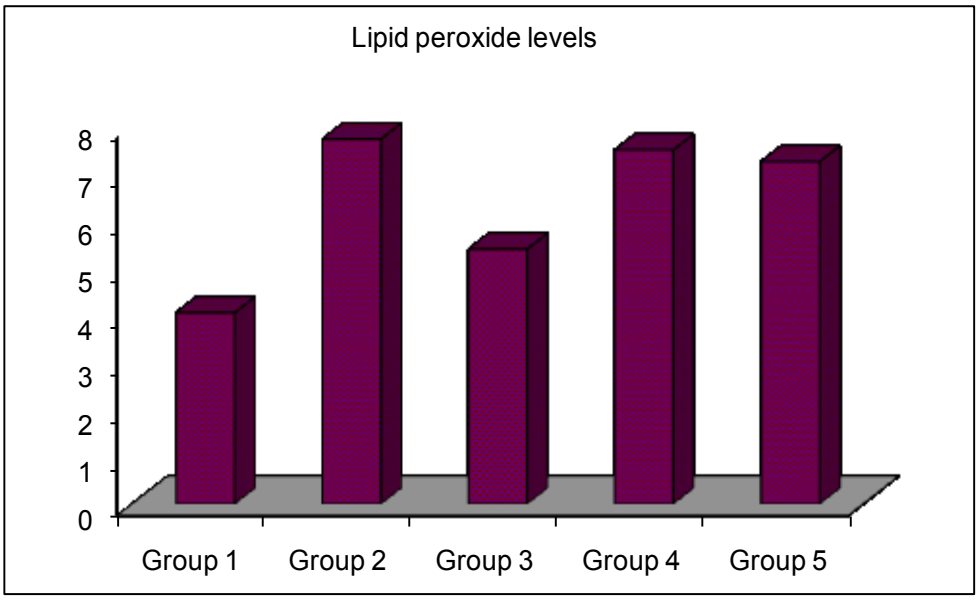

Figure 5. Effect of treatments on skin Lipid peroxide values in mice.

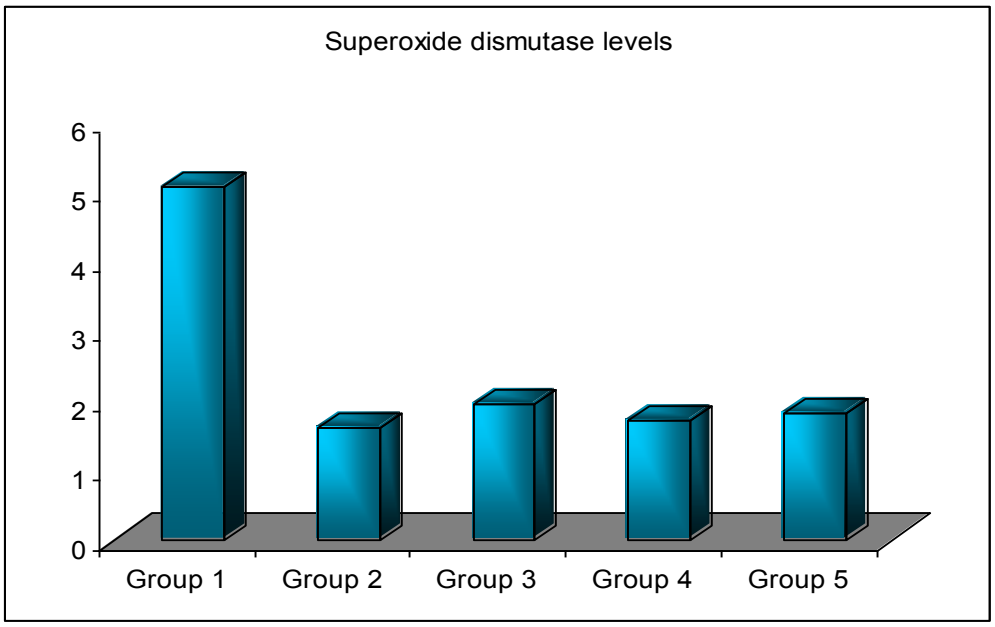

Figure 6. Effect of treatments on skin SOD values in mice.

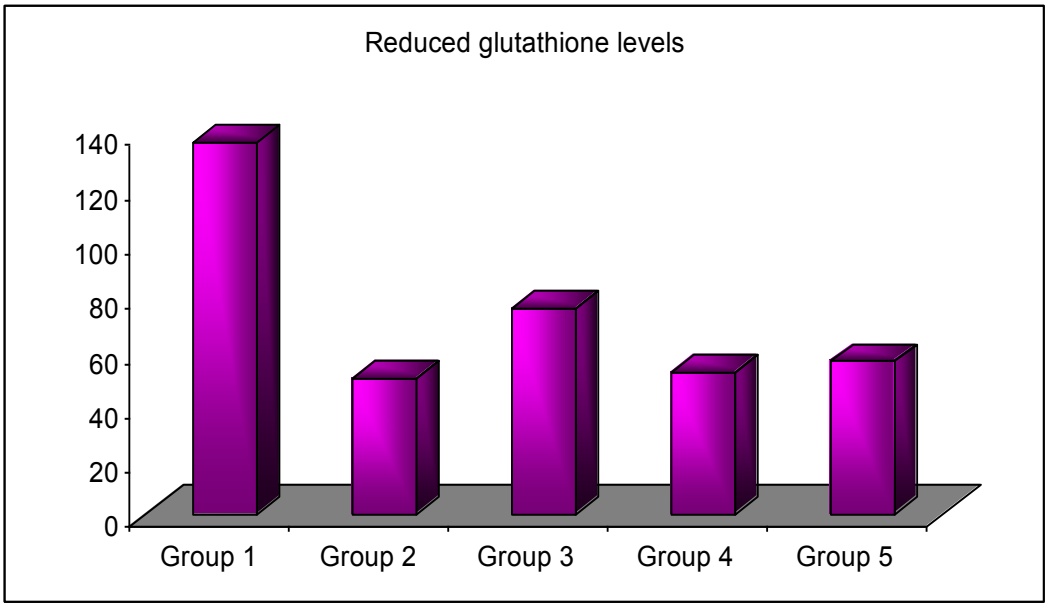

Figure 7. Effect of treatments on skin Reduced glutathione values in mice.

4.5. INVESTIGATION OF ANALGESIC PROPERTY OF ETHANOLIC EXTRACT OF IPOMOEA (*Ipomoea mauritiana*) ROOTS

4.5.1. Acute pain –Tail flick method:

The reaction time measurement (in seconds) was considered as an index of nociception. The reaction time for every treatment groups at 30 minutes interval up to two hours are recorded and presented in Table 11 and Figure 8.

The diclofenac and the ethanolic extract of Ipomoea root treated groups exhibited significant ($P<0.01$) analgesic activity compared to controls. The increase in reaction time was gradual for all the treatment groups and it was peak at 90 minutes for all groups.

The reaction time for groups I, II, III, IV and V before the experiment were 4.96±0.47, 3.75±0.36, 4.39±0.26, 4.12±0.87 and 5.00±0.73 seconds respectively. The reaction time for groups I, II, III, IV and V at 30 minutes after drug administration were 4.96±0.47, 3.75±0.36, 4.33±0.25, 4.12±0.87, 4.7±0.65 seconds respectively. The reaction time for groups I, II, III, IV and V at 60 minutes were 4.96±0.47, 5.50±0.46, 4.82±0.27, 4.46±0.94, 5.06±0.54 seconds respectively. The reaction time at 90 minutes for groups I, II, III, IV and V were 4.96±0.47, 6.00±0.56, 5.13±0.26, 4.82±0.90, 5.25+0.48 seconds respectively. At 120 minutes the reaction time for the groups I, II III, IV and V were 4.96±0.47, 4.8±0.58, 4.5±0.26, 4.12±0.87, 4.67±0.54 seconds respectively.

Table 11. Effect of treatments on reaction time in rats.

Time interval	Reaction time in seconds (mean ± SE)				
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
0 min.	4.96±0.47	3.75±0.36	4.39±0.26	4.12±0.87	5.00±0.73
30 min.	4.71±0.1 4	3.75±0.36	4.33±0.25	4.12±0.87	4.70±0.65
60 min.	4.8±0.32	5.50±0.46*	4.82±0.27	4.46±0.94	5.06±0.54
90 min.	4.99±0.28	6.00±0.56*	5.13±0.26	4.82±0.90	5.25±0.48
120 min.	4.96±0.47	4.80±0.58	4.50±0.26	4.12±0.87	4.67±0.54

* Significant at P<0.01

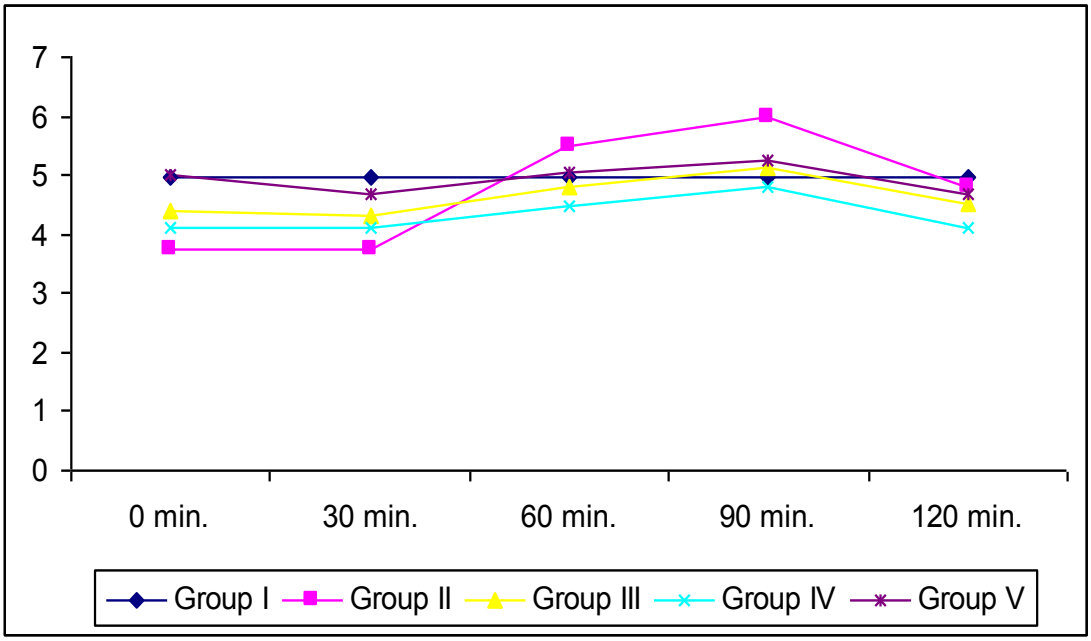

Figure 8. Effect of treatments on tail flick method of analgesic model in rats.

4.6. EFFECT ON HAEMATOLOGICAL PARAMETERS IN TAIL FLICK METHOD OF ANALGESIC MODEL IN RATS.

The result of haematological studies revealed that there was no significant ($P<0.05$) variation in any one of the parameters like haemoglobin concentration, RBC count, total WBC count, differential leukocyte count and VPRC was observed between groups.

4.6.1. RBC Count

The results of RBC count are presented in Table 12. The RBC counts were 7.40±0.11, 6.97±0.18, 7.23±0.20, 7.36±0.11 and 7.31±0.16 ($10^6/mm^3$) for group I to V respectively.

4.6.2. WBC Count

WBC count are presented in Table 12. The WBC counts were 6.43±0.08, 6.27±0.09, 6.31±0.09, 6.29±0.05 and 6.28±0.06 ($10^3/mm^3$) for group I to V respectively.

4.6.3. Haemoglobin concentration

Haemoglobin concentrations are presented in Table 12. The values were 12.5±0.42, 13.0±0.37, 13.0±0.26, 12.8±0.45 and 13.1±0.22 gram percentage for group I to V respectively.

4.6.4. Volume of packed red cells

The results of VPRC count are presented in Table 12. The PCV values were 46.75±1.32 and 45.37±1.13, 46.10±2.14, 52.01±1.35 and 50.06±0.98 per cent for group I to V respectively.

4.6.5. Neutrophils

The results of neutrophil count are presented in Table 12. The counts for group I to V were 52.75±1.60 and 50.75±2.48, 50.12±2.09, 48.88±2.33 and 50.38±1.39 per cent respectively.

4.6.6. Lymphocytes

The results are presented in Table 12. Group I to V showed the values of 45.63±1.00, 50.88±1.85, 49.13±2.11, 47.75±1.55 and 46.00±1.08 per cent respectively.

4.6.7. Monocytes

The monocyte counts were1.25±0.49, 1.13±0.23, 1.25±0.25, 1.63±0.18, 1.63±0.26 per cent for Group I to V respectively (Table 12).

4.6.8. Eosinophils

The eosinophil counts were 0.62±0.52, 0.62±0.74, 1.25±0.89, 1.12±0.64, 0.87±0.83 per cent for Group I to V respectively (Table 12).

4.7 BIOCHEMICAL PARAMETERS

4.7.2. Alanine Amino Transferase (ALT)

The ALT values are presented in Table 13 and Figure 9. The mean values for the groups (Group I to V) were 48.87±1.17, 87.25±1.61, 38.75±2.03, 37.62±1.97 and 41.25±1.26 U/l respectively

4.7.1. Aspartate Amino Transferase (AST)

The AST values are presented in Table 14 and Figure 9. The mean values for the groups (Group I to V) were 87.56±1.79, 131.37±2.75, 81.00±2.26, 83.37±2.13 and 84.20±2.89 U/l respectively.

Table 12. Effect of treatment on haematological parameters in Analgesic study

GROUPS	RBC (10^6/mm^3)	WBC (10^3 /mm^3)	Hb (g%)	VPRC (%)	Differential leukocyte count (%)			
					Neutrophil	Lymphocytes	Monocyte	Eosinophils
Group 1	7.4±0.11[a]	6.43±0.86[b]	12.5±0.42[c]	46.75±1.32[d]	52.75±1.60	45.63±1.00	1.25±0.49	0.62±0.52
Group 11	6.97±0.18[a]	6.27±0.92[b]	13.0±0.37[c]	45.37±1.13[d]	50.75±2.48	50.88±1.85	1.13±0.23	0.62±0.74
Group 111	7.23±0.20[a]	6.31±0.90[b]	13.0±0.26[c]	46.10±2.14[d]	50.12±2.09	49.13±2.11	1.25±0.25	1.25±0.89
Group 1V	7.36±0.11[a]	6.29±0.57[b]	12.8±0.45[c]	52.01±1.35[d]	48.88±2.33	47.75±1.55	1.63±0.18	1.12±0.64
Group V	7.31±0.16[a]	6.28±0.64[b]	13.1±0.22[c]	50.06±0.98[d]	50.38±1.39	46.00±1.08	1.63±0.26	0.87±0.83

Mean±SE, n=8 Means bearing the same superscript do not differ significantly at P<0.05

Table 13. Effect of treatments on alanine amino transferase (ALT, U/l) level in analgesic study.

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	45	90	30	34	34
2	44	86	48	46	44
3	50	77	45	40	40
4	54	88	37	39	45
5	51	90	37	44	43
6	47	91	40	30	40
7	50	86	39	35	44
8	50	90	34	33	40
Mean ± SE	48.87 ± 1.17^{a}	87.25 ± 1.61^{b}	38.75 ± 2.03^{a}	37.62 ± 1.97^{a}	41.25 ± 1.26^{a}

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

Table 14. Effect of treatments on aspartate amino transferase (AST, U/l) level in analgesic study.

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	81	134	81	77	90
2	88	136	78	80	95
3	90	122	83	77	83
4	80	146	84	91	84
5	96	132	76	90	76
6	90	130	91	80	91
7	88	129	70	90	70
8	87	122	85	82	85
Mean ± SE	87.56 ± 1.79^{a}	131.37 ± 2.75^{b}	81.00 ± 2.26^{a}	83.37 ± 2.13^{a}	84.20 ± 2.89^{a}

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

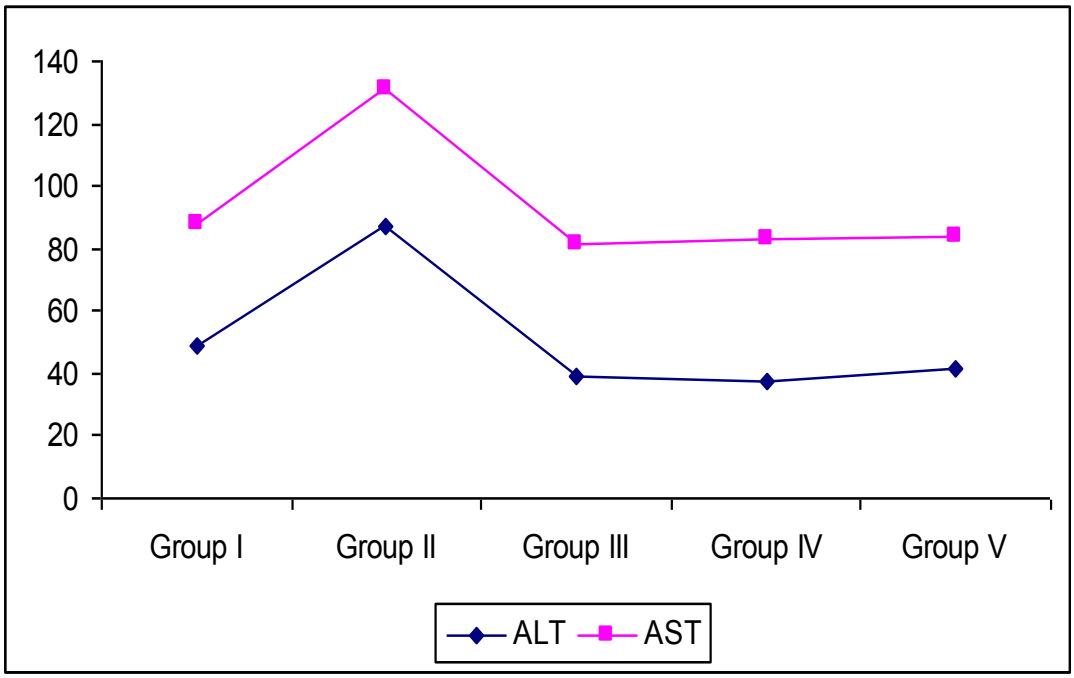

Figure 9. Effect of treatments on ALT and AST values in tail flick method of analgesic model in rats.

HISTOPATHOLOGICAL EXAMINATION OF MOUSE SKIN

Histopathological examination of skin from the mice treated with croton oil revealed the presence of numerous inflammatory cells alone with disruption of epidermis and dermal oedema (Fig.10). Skin samples from the mice treated with *Ipomoea mauritiana* root extract topically at the rate of 20 mg/kg, before croton oil application showed slight regeneration of epidermis (Fig.11). In the 30 mg/kg extract treated group, it showed marked grayish blue areas of moderately regenerating cells (Fig.12). In group 3 applied topically with 5 per cent diclofenac potassium cream 30 minutes before application of croton oil showed maximum regenerating cells, marked by grayish blue areas, and intact epidermis with out much damage (Fig.13).

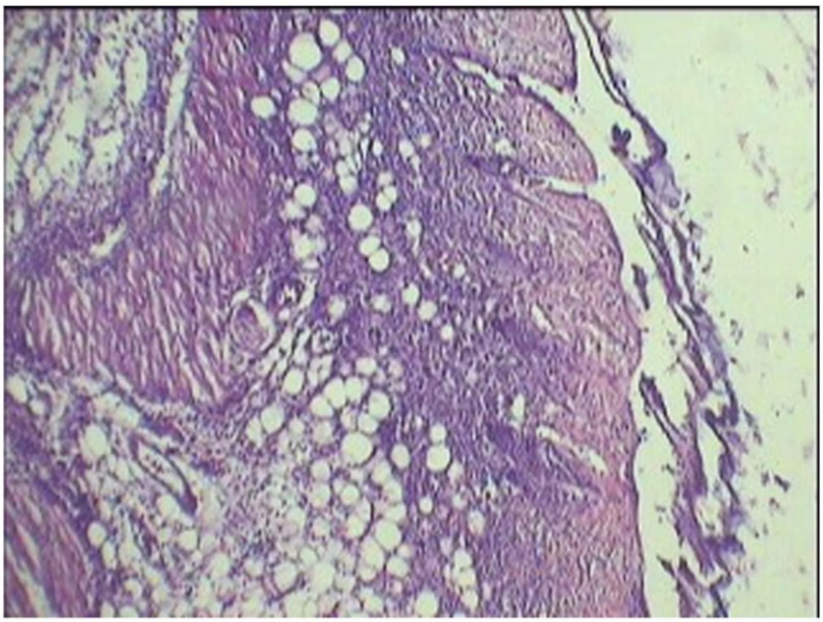

Fig.10 Skin - croton oil treated group showing dermal oedema and inflammation (H & E x 100)

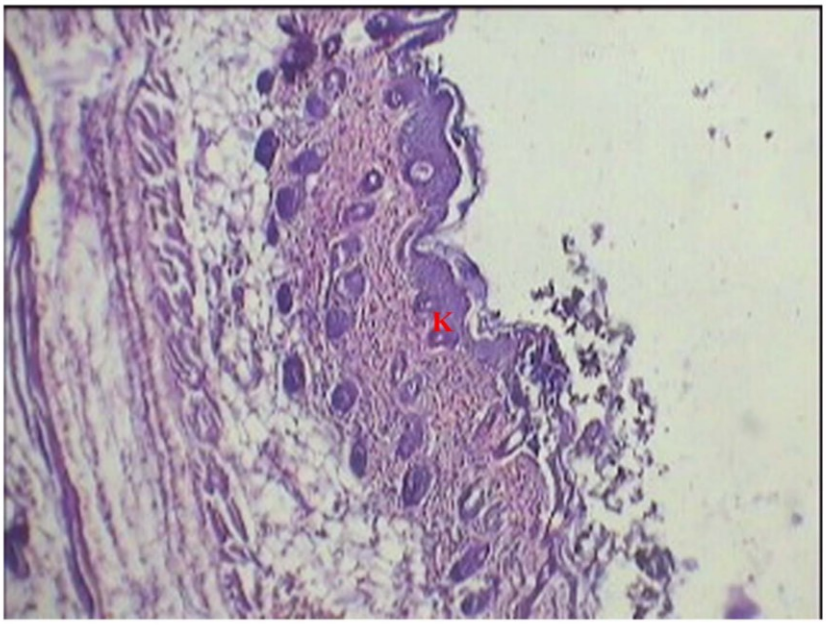

Fig. 11 Skin - *Ipomoea mauritiana* 10 mg/kg Slight regeneration (K) (H & E x 100)

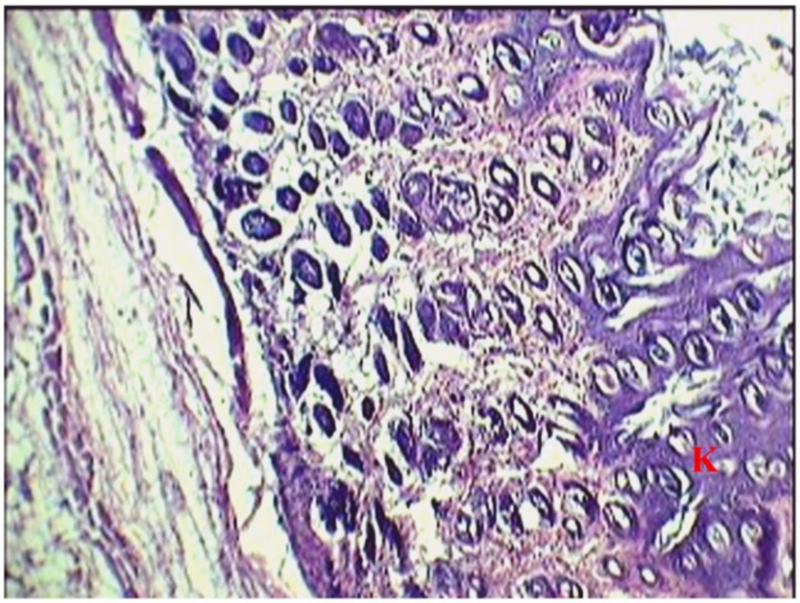

Fig.12 Skin - *Ipomoea mauritiana* 20 mg/kg Moderate regeneration (K) (H & E x 100)

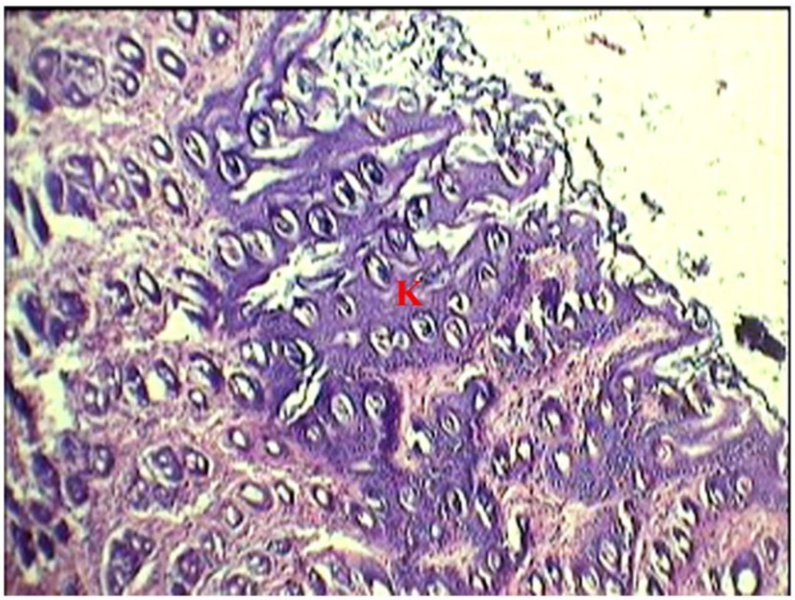

Fig.13 Skin - Diclofenac treated group showing moderate regeneration (K) and intact epidermis without much change (H & E x 100)

Discussion

5. DISCUSSION

It is well known that the traditional herbal remedies existed before the introduction of modern medicine and even now a good percentage of world population depend on herbal practices for their health care. Herbal drugs are widely used in recent years for preventive and therapeutic purposes. Ancient knowledge coupled with scientific principles has come to the forefront to provide powerful remedies to eradicate the diseases. *Ipomoea mauritiana* is one of the plants that has been used for its medicinal properties since ancient times. The bitter, tuberous roots are very much used in native medicine in India, being regarded as tonic, alterative, aphrodisiac, galactogogue, cholagogue and demulcent. Now, there is a growing interest in identifying and characterizing the natural compounds with anti-inflammatory and analgesic activity, ever since they have been suggested in modern medicine. With these objectives in mind, the present study was aimed to detect the active principles present in roots of *Ipomoea mauritiana* and their anti-inflammatory as well as analgesic properties in rats.

5.1. ANALYSIS OF IPOMOEA ROOT EXTRACT FOR ACTIVE PRINCIPLES.

The phytochemical analysis of Ipomoea roots revealed the presence of alkaloids, flavonoids, glycosides, tannins, phenolic compounds, diterpenes and triterpenes. Mishra *et al.* (1964) investigated the fixed oil from tubers of *Ipomoea digitata* Linn. It contains palmitic (8.15 per cent), oleic (60.10 per cent), linoleic (19.38 per cent), and linolenic acids (1.11 per cent) in mixed acid fraction. A study conducted by Matin *et al.* (1969) revealed the presence of a cardiac stimulant glycoside 'Paniculatin' in the tubers of *Ipomoea digitata.*

Among the naturally occurring phenolic compounds in plants, flavonoids form the largest group. Flavonoids are used as anti-inflammatory, antioxidants, stress modifiers, antiviral, anticarcinogenic and antiallergic agents (Onwukaeme, 1994). Some flavonoids may be found in association with sugars and are called flavonoid glycosides. These are demonstrated to have anti-inflammatory, antiallergic, antithrombotic and vasoprotective properties. Flavonoids are known to

inhibit prostaglandins, which are involved in inflammation and pain perception (Rajnarayana *et al.*, 2001). The present study revealed the presence of flavonoids and glycosides in Ipomoea root extract and hence the anti-inflammatory effect produced by Ipomoea roots might be due to the presence of flavonoids and glycosides. The inhibition of inflammatory mediators by flavonoids and glycosides were proved by Singh and Pandey (1996) through the anti-inflammatory activity of *Pongamia pinnata* seed extract in rats.

According to Safayhi and Sailer (1997) the anti-inflammatory effect is a common property of many triterpenes. Present study revealed the presence of triterpenes in Ipomoea roots and so triterpenes also might have contributed to the anti-inflammatory effect of Ipomoea root extract. Terpenoids form the largest group of plant principles, which are derived biosynthetically from the molecule of isoprene. They are categorized as monoterpenoids, diterpenoids, triterpenoids, and sesquiterpenoids and are used as antioxidants and anti-inflammatory agents (Greenwald, 1991 and Wood *et al.*, 1982). Ipomoea roots contains at least four sesquiterpene lactones. These along with other ingredients account for the healing medicinal properties of this herb.

5.2. EVALUATION OF THE ANTI-INFLAMMATORY EFFECT OF ETHANOLIC EXTRACT OF *Ipomoea mauritiana*

5.2.1. Effect on carrageenin induced paw oedema

Carrageenin has been widely used as a noxious agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min (Roch-Arveiller and Giroud, 1979). Carrageenin induced oedema is believed to be biphasic. The early phase (1–2 h) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings (Vinegar *et al.*, 1969). As the present study showed that the ethanolic extract of Ipomoea roots produced maximum reduction of paw volume during the first two hours after carrageenin injection, it can be inferred that the anti-inflammatory effect of root

extract of *Ipomoea mauritiana* might be mainly due to the inhibition of the synthesis and release of inflammatory mediators viz., histamine, serotonin and prostaglandin.

The late phase of carrageenin induced inflammation is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Vinegar *et al*., 1987; Brito and Antonio, 1998). In Group V, the inhibition of oedema by ethanolic extract of Ipomoea root was increased upto 3 hours after carrageenin injection. So the anti-inflammatory activity exerted by Ipomoea roots might also be due to the inhibition of late phase of carrageenin induced inflammation.

In the third group (Group-III-300 mg/kg) maximum inhibition of oedema was noticed at 60 minutes itself. Group IV fed with 600 mg/kg extract showed more anti-inflammatory activity than the other two groups and the maximum percentage of inhibition for Group IV was obtained at two hours after carrageenin injection. The present result indicated the efficacy of ethanolic extract of *Ipomoea mauritiana* at the dose rate of 600 mg/kg as an efficient therapeutic agent in acute anti-inflammatory conditions. These findings are in accordance with Ismail *et al.* (1997), who got the maximum percentage of inhibition of carrageenin induced acute inflammation at 2.5 hours after carrageenin injection for the group fed with 150 mg/kg of *Gmelina asiatica* root powder than the group fed with *Gmelina asiatica* root powder at the dose rate of 200 mg /kg.

The percentage of inhibition of oedema shown by all the three groups in this study were decreased after 3 hours of inflammation. Barros *et al.* (2006) observed that the maximal percentage of inhibition of carrageenin induced rat paw oedema was obtained at 60 minutes after administration of *Pluchea quitoc* extract and decreased thereafter. Asongalem *et al.* (2004) evaluated the anti-inflammatory activities of aqueous extract of *Erigeron floribundus*, where the maximal percentage of inhibition of rat paw oedema was obtained at 60 minutes for higher dose groups. Kumar *et al.* (1997) also got the maximum percentage of inhibition of carrageenin induced paw oedema at 60 minutes of observation while evaluating the anti-

inflammatory action of *Sida rhombifolia*. Borgi *et al.* (2007) observed significant reduction in paw volume during the third hour after carrageenin injection for *Zizyphus lotus* root barks. On the contrary, only a small and not significant activity was seen at the sixth hour after the injection of ethyl acetate and chloroform extracts of *Zizyphus lotus* root barks.

Diclofenac, the reference drug (Group II) showed much more significant anti-inflammatory activity during all the three hours of inflammation induced by carrageenin than Ipomoea root extracts. The percentage of inhibition of paw oedema observed in the diclofenac treated group was 57.69, 75.4 and 65.97 per cent during 1st, 2nd and 3rd hour after carrageenin injection. The maximum inhibition noticed was 75.4 per cent at two hours after carrageenin injection. This result of the present study is in agreement with the observation obtained by Bothara *et al.* (1998) who got 67 .24 per cent inhibition of oedema with diclofenac sodium.

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

Both transaminases are widely distributed. The serum transaminases activity increases during damage to organs like liver and muscle. According to Kaneko *et al.* (1997) in rats the liver has the highest ALT specific activity and an increase in serum ALT is an established sensitive indicator of hepatocyte damage.

In the present study, no significant change was noticed in the levels of AST and ALT in groups treated with Ipomoea root extract (Group III, IV and V) and the values were within the normal range. The diclofenac treated group (Group II) showed significant elevation of AST and ALT level in the present study. It may be a reversible elevation of enzyme as observed by Tripathi (1999) who reported a reversible elevation of serum aminotransferase activity occurred in patients treated with diclofenac sodium.

5.2.3. Effect of treatments on haematological parameters.

The result of haematological studies revealed no significant variation in any of the parameters like haemoglobin concentration, total leukocyte count,

differential leukocyte count, RBC count as well as PCV in any of the groups studied. Barros *et al.* (2005) also observed that the haematological parameters in rats treated with the *Pothomorphe umbellata* L. Miq. root did not show any significant variation.

Ramesh *et al*. (2001) also obtained similar observation when they studied the effect of diclofenac on haematological parameters in dogs. It revealed that the diclofenac did not produce any significant variation in RBC count, haemoglobin concentration, PCV, total leukocyte count and differential leukocyte count.

5.2.4 Evaluation of anti-inflammatory activity using croton oil induced skin inflammation

Croton oil is a commonly used cutaneous irritant. The mechanism of croton oil induced inflammation involves an increase in phospholipase A2 activity (Kondoh *et al.*, 1985; Mc coll *et al.*, 1986) which in turn leads to the release of arachidonic acid and subsequent biosynthesis of leukotrienes and prostaglandins (Ashendel and Boutwell, 1979; Furstenberger and Marks, 1980). Thus it involves both cyclo-oxygenase and lipoxygenase path ways. Mouse epidermis had been reported to possess δ-5, δ-8 and δ-15 lipoxygenase activity (Nakadate *et al.*, 1986; Fisher *et al.*, 1988). 12-O-tetradecanoyl phorbol acetate (TPA), a phorbol ester present in croton oil had been shown to act as an inducer of inflammation. The treatment with TPA had been reported to induce a variety of changes in murine skin, including dark basal keratinocytes and sustained epidermal hyperplasia, reactive oxygen species formation in epidermis and elevated epidermal cyclo-oxygenase, lipoxygenase activities (Saleem *et al*., 2001). Furthermore, TPA is believed to function, at least in part, by interacting with and activating protein kinase C, an important enzyme involved in the regulation of variety of biological processes, including cell growth and differentiation (Couturier *et al*., 1984; Cochet *et al.*, 1986).

According to the result obtained, applications of ethanolic extract of Ipomoea root 30 minutes before the application of croton oil reduced the inflammatory oedema on mouse skin. The croton oil induced skin inflammation was evident from skin punch weight of 109.5±2.109 mg/punch in the inflammatory

control group of animals (Group 2). Application of ethanolic extract of Ipomoea roots at the dose rate of 20 mg before the application of croton oil (Group 4) exhibited only 7.4 per cent inhibition of thickness induced by croton oil. Group V (30 mg extract + croton oil) exhibited slight anti-inflammatory effect where the percentage of inhibition of oedema was 14.91 per cent. The results obtained for Ipomoea root extract treated groups were not significantly different when compared with control (Group 1), which indicate the extract tested could not reduce the inflammatory mediators and inflammatory effects on the mice skin produced by croton oil (Pena *et al.*, 2006).

The reference drug diclofenac (Group 3) showed a reduction in skin thickness where the percentage of inhibition after croton oil application was 33.4. The result obtained is in agreement with Kyuki *et al.* (1982) who got 34.5 per cent inhibition of mice ear oedema induced by croton oil with 5 per cent diclofenac cream. According to them diclofenac cream had significant inhibitory effect on increased vascular permeability induced by histamine and oedema reaction secondary to the increased vascular permeability. It was examined in a carrageenin-induced reactions and in the croton oil induced reaction. Diclofenac cream produced a potent inhibitory effect on these two cases of oedema.

In the experiment conducted by Lakshmi *et al.* (2003), croton oil induced skin inflammation was evident from skin punch weight of 70.8±2.4 mg. Application of extract of medicinal mushroom *Ganoderma lucidum* 30 minutes before the application of croton oil exhibited 52.7 and 79.8 per cent inhibition of skin oedema respectively in a dose dependant manner. Paula *et al.* (2003) found that the topical application of *Ipomoea imperati* extract inhibited mouse ear oedema induced by croton oil significantly (89 per cent by lipid fraction and 57 per cent by aqueous fraction).

5.2.5 Lipid peroxides

Lipid peroxide levels measured by formation of thiobarbituric acid reactive substance was significantly increased in croton oil treated groups. This is in agreement with Lakshmi *et al.* (2003) who evaluated the antiperoxidative activity of *Ganoderma lucidum* using phorbol ester (croton oil) induced lipid peroxidation in mouse skin. They also got significantly high values of lipid peroxides in croton oil treated groups and obtained 37 per cent inhibition of croton oil induced peroxidation on the mouse skin by the application of 20 mg extract of *Ganoderma lucidum* 30 minutes before the application of croton oil.

In this study the Group 4 (treated with 20 mg of extract before croton oil application) and the Group 5 (treated with 30 mg of extract before croton oil application) also showed significant increase in lipid peroxide values which indicate the absence of antiperoxidative property of ethanolic extract of Ipomoea roots. Group 3 treated with diclofenac before application of croton oil produced slightly reduced values for lipid peroxides. But the reduction was not significant when compared to that of normal animals.

The treatment with12-O-tetradecanoyl phorbol acetate (TPA) had been reported to induce a variety of changes including reactive oxygen species formation in epidermis of skin (Saleem *et al*., 2001). When generation of the reactive oxygen species overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins, and nucleic acids) occurs, leading to various pathological conditions. Reactive oxygen species mediated lipid peroxidation, oxidation of proteins and DNA damage are well-known outcomes due to oxygen-derived free radicals, leading to cellular pathology and ultimately to cell death (Bandyopadhyay *et al.*, 1999). This experiment revealed that the Ipomoea root extract when applied topically over the skin were not able to remove the reactive oxygen species generated by TPA in croton oil.

Delazer *et al.* (2003) also observed that the application of croton oil increased the lipid peroxide values of mouse skin significantly, whereas application of tannic acid prior to croton oil treatment diminished cutaneous lipid peroxide level in mice skin compared to the untreated groups.

5.2.6. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is a metalloprotein complex that catalyzes the dismutation of superoxide anion radicals into hydrogen peroxide and oxygen.

The biosynthesis of SOD is mainly controlled by its substrate, superoxide radicals (O^-) (Fridovich, 1983). Induction of SOD by increased intracellular fluxes of superoxide radicals has been observed in numerous organisms (Bandyopadhyay *et al.*, 1999). In this study Group 2 (applied with croton oil alone) showed significant reduction of SOD values. The Group 4 (treated with 20 mg of extract + croton oil) and Group 5 (treated with 30 mg of extract + croton oil) also showed reduced values for SOD. The decreased values of cutaneous SOD after croton oil application might be due to the enzyme inactivating activity of reactive oxygen species generated by TPA. Application of Ipomoea root extracts could not regain skin SOD activity to normal which indicated the absence of antioxidant property of ethanolic extract of Ipomoea roots when applied on mouse skin. Group 3 treated with diclofenac before croton oil application produced slight increase in SOD values but not significant.

5.2.7. Reduced glutathione

The selenoenzyme glutathione peroxidase (GPx) could reduce and detoxify hydrogen peroxide as well as various organic hydroperoxides at the expense of glutathione (GSH) to form oxidized glutathione (GSSG). GSH is naturally regenerated from GSSG by the catalyst glutathione reductase (GR) (Bandyopadhyay *et al.* 1999). The Group 2 (applied with croton oil alone) showed significant inflammation characterized by decreased values of reduced glutathione. This result

obtained is in accordance with Saleem *et al.* (2001) who observed a significantly reduced value of reduced glutathione after treating with croton oil in murine skin. Group 4 (treated with 20 mg of extract with croton oil) and Group 5 (treated with 30 mg of extract with croton oil) also showed the same reduction in reduced glutathione values which indicate an increase in glutathione peroxidase activity due to the increased generation of reactive oxygen species, which catalyses the conversion of GSH to oxidized glutathione. The reference drug diclofenac produced a small increase in GSH value, but it was not significant when compared with inflammatory control.

5.3. EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF IPOMOEA ROOTS

The analgesic activity of *Ipomoea mauritiana* was tested in the present study by tail flick reaction.

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

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

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

In the present study diclofenac treated group (Group-II) showed a gradual increase in reaction time till 90 minutes of the observation, and it attained maximum at 90 minutes. The increase in reaction time was gradual for all the treated groups with a maximum at 90 minutes of observation. Diclofenac treated group showed significant increase in reaction time at 60 and 90 minutes of observation. Velankar *et al*. (1998) also got peak analgesic action at 90 minutes post administration of NSAIDs like diclofenac, flubiprofen and nimusulide, when they administered different combinations of above drugs in tail flick analgesic model in rats. The

analgesic activity of diclofenac has been traditionally related to the inhibition of prostaglandin synthesis (Menasse *et al.*, 1978), direct blockade of inflammatory sensitization by activation of NO-cGMP pathways (Tonussi and Ferreira, 1994) and involvement of opioid pathways in the central analgesic effect (Bjorkman, 1995).

In the present study all the groups treated with Ipomoea root extract showed a gradual increase in reaction time from 60 minutes of observation onwards and showed a maximum response at 90 minute and decreased thereafter. The results obtained are in agreement with Jha *et al.* (2006) who observed for the drug Tramadol, at the rate of 10 mg/kg it did not have any antinociceptive effect when tested by tail flick test. The antinociceptive effect was noted with 22.8 mg/kg with onset at 60 minutes, and reached peak at 90 minutes. The activity shown by the Ipomoea root extracts treated group at a dose rate of 300 mg/kg was significant as compared to that of control at 90 minutes of observation. But the groups fed with Ipomoea root extract (Group1V-600 mg/kg and Group V-1200 mg/kg) did not have significant influence in the analgesic activity. Flavonoids are known to inhibit the enzyme prostaglandin synthetase. Since, prostaglandins are also involved in the pain perception, inhibition of their synthesis might be the possible reason for the slight analgesic activity of the ethanolic extract of *Ipomoea mauritiana.*

Summary

6. SUMMARY

The present study was undertaken to assess the anti-inflammatory and analgesic effect of the root extract of *Ipomoea mauritiana* in rats. Diclofenac potassium was used as the reference drug for both anti-inflammatory and analgesic screening.

Anti-inflammatory effect was studied in carrageenin induced hind paw oedema model as well as croton oil induced skin inflammatory model. The analgesic action in acute pain models was studied by tail flick method.

Forty adult male Sprague Dawley rats of 150-200 g body weight were divided into five groups of eight animals each for the anti-inflammatory study using carrageenin induced hind paw oedema model. The experiment was conducted for a period of seven days. Five per cent gum acacia was fed to Group I and II in which the Group II received the diclofenac potassium at the dose rate of 3 mg/kg on the 7^{th} day before carrageenin administration. The ethanolic extract of Ipomoea roots were administered to group III, IV, V at the dose rate of 300 mg/kg, 600 mg/kg and 1200 mg/kg respectively for seven days.

Five groups of six female swiss albino mice each were used for anti inflammatory study using croton oil induced skin inflammation. Back of each animal was shaved over an area with 8 mm diameter using surgical clippers two days before the experiment and the animals with complete stoppage of hair growth were used for the particular experiment. Group without any treatment was kept as healthy control (Group1). Group 2 treated with croton oil alone was kept as inflammatory control. Alcoholic extract of *Ipomoea mauritiana* was applied topically to the shaved area of dorsal skin 30 minutes before each application of croton oil. After 24 hour, the extract and croton oil treatment was repeated on the same area. Five per cent diclofenac potassium cream was used as the reference drug. One hour after the

second treatment of croton oil, animals were sacrificed and the skin punches were removed using an 8 mm diameter cork borer. The skin punches were weighed in an analytical balance to find out the percentage inhibition of skin inflammation induced by croton oil. Then the skin samples were used for the estimation of lipid peroxides, superoxide dismutase, reduced glutathione as well as for histopathological studies.

Similarly five other groups of Sprague Dawley rats (eight animals each) were maintained for analgesic screening by tail flick method. Five per cent gum acacia were fed to Group I and II in which the Group II received the diclofenac potassium at the dose rate of 3 mg/kg on the 7^{th} day before screening for analgesic effect. Dose rates of 300 mg/kg, 600 mg/kg and 1200 mg/kg body weight of Ipomoea root extract was administered to the groups III, IV and V respectively for 7 days.

In the anti-inflammatory screening, Ipomoea roots showed significant inhibition of carrageenin induced oedema on the second and third hour after carrageenin injection. Group IV with 600 mg/kg extract showed more activity than the other two groups (Group III-300 mg/kg and Group V-1200 mg/kg) in all the three hours of inflammation.

In anti-inflammatory screening using croton oil, application of Ipomoea root extract 30 minutes before the application of croton oil reduced the inflammatory oedema slightly in a dose dependant manner. But the reduction in skin thickness was not significant when compared to the healthy control (Group 1).

Administration of croton oil produced significant increase in lipid peroxidation and significant decrease in superoxide dismutase as well as a reduction in glutathione levels. Administration of herbal extracts at various dose levels brought about a slight reduction in lipid peroxide level and slight increase in the activities of

antioxidant enzymes namely superoxide dismutase and reduced glutathione. But the change in values were not significant when compared to normal, which suggested that the Ipomoea root extract was not efficient in alleviating the free radical induced damage on mouse skin.

In case of analgesic screening, all the treated groups showed effect in tail flick method (slight increase in reaction time). But the results were not significant except for group III, treated with Ipomoea root extract at a dose rate of 300 mg/kg, which showed significant increase in reaction time only at 90 minutes of observation.

Serum enzymes like ALT and AST and haematological parameters like total and differential leukocyte count, RBC count, haemoglobin and PCV were recorded in both anti-inflammatory and analgesic screening on the 7^{th} day of the experiment. The serum transaminase enzymes AST and ALT were within the normal level in both the studies. Even though there was a slight variation in neutrophil count, all the values were within the normal range. The total leukocyte counts showed reduction in both anti-inflammatory and analgesic studies, however, the value were within the normal range.

From the present study it can be concluded that the ethanolic extract of Ipomoea roots have significant anti-inflammatory effect in rats and hence it can be recommended for the treatment of various inflammatory conditions.

References

REFERENCES

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

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

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

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

Ashendel, G.L. and Boutwell, R.K. 1979. Prostaglandin E and F levels in mouse epidermis are increased by tumor promoting phorbol esters. *Biochem. Biophys. Res. Commun*. 90: 623-627.

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

Asongalem, E.A., Foyet, H.S., Ngogang, J., Folefoc, G.N., Dimo, T. and Kamtchouing, P. 2004. Analgesic and Anti-inflammatory activities of *Erigeron floribundus*. *J. Ethnopharmacol.* 91: 301-308.

Bandyopadhyay, U., Das, D. and Banerjee, R.K. 1999. Reactive oxygen species: Oxidative damage and pathogenesis. *Current Science*. 77(5): 658-666.

Barros, I.M.C., Lopes, L.D.G., Borges, M.O.R., Borges, A.C.R., Ribeiro, M.N.S. and Freire, S.M.F. 2006. Anti-inflammatory and analgesic activities of *Pluchea quitoc* (DC) ethanolic extract. *J. Ethnopharmacol.* 106: 317-320.

Barros, S., Ropke, C.D., Sawada, T.C.H., Silva, V.V.D., Pereira, S.M.M., Barros, S.B.D.M. 2005. Assessment of acute and subchronic oral toxicity of ethanolic extract of *Pothomorphe umbellata* L. Miq (Pariparoba). *Braz. J. Pharm. Sci*. 41: 53-60.

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

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

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

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

Borgi, W., Ghedira, K. and Chouchane, N. 2007. Anti-inflammatory and analgesic activities of *Zizyphus lotus* root barks. *Fitoterapia*. 78: 16-19.

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

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

Brito, A.R.M.S. and Antonio, M.A. 1998. Oral anti-inflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). *J. Ethnopharmacol.* 61: 215–228.

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

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

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

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

Cochet, C., Keramides, M., Souvignet, C., Chambaz, E.M. 1986. Phorbol ester induced alteration of proteinkinase C catalytic properties occurs at the membrane level and is not reproduced by physiological stimuli. *Biochem. Biophys. Res. Commun.* 138: 1283-1290.

Costa, V.B., Coube, C.S., Marinho, B.G., Matheus, M.E., Leita, S.G. and Fernandes, V.B. 2003. Anti-inflammatory and analgesic activity of *Bouchea fluminensis. Fitoterapia.* 74 (2003) 364–371.

Couturier, A., Bazgar, S. and Castagna, M. 1984. Further characterization of tumor promoter mediated activities of protein kinase C. *Biochem. Biophys. Res. Commun.* 121: 448–455.

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

Delazer, A., Babaei, H. and Rezazadeh, H. 2003. Inhibitory effect of tannic acid from nutgall on iron-dextran augmented 7, 12-dimethyl benz (A) anthracene-inhibited and croton oil promoted skin carcinogenesis. *DARU*. 11: 21-26.

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

Ellman, G.L. 1959. Tissue sulfhydril groups. *Arch. Biochem. Biophys*. 82: 70-77.

Ferreira, A.A., Amaral, F.A., Duarte, I.D.G., Oliveira, P.M., Alves, R.B., Silveira, D., Azevedo, A.O., Raslan, D.S. and Castro, M.S.A. 2006. Antinociceptive effect from *Ipomoea cairica* extract. *J. Ethnopharmacol*. 105:148-153.

Fisher, S.M., Baldwin, J.K., Patrick, K.E. and Cameron, G.S. 1988. Phorbol ester induction of 8-lipoxygenase in inbred SECAR (SSIN) but not C57Bl/6J mice correlated with hyperplasia, oedema and oxidant generation but not ornithine decarboxylase induction. *Cancer Res*. 48: 658-664.

Fraga, C.G., Leiboritz, B.E. and Toppel, A.L.1988. Lipid peroxidation measured as TBARS in tissue slices. Characterization and comparison with homogenates and microsomes. *Free Radic. Biol. Med*. 4:155-161.

Fridovich, I. 1983. Superoxide radical: an endogenous toxicant. *Annu. Rev. Pharmacol. Toxicol*. 23: 29–257.

Furstenberger, G. and Marks, F. 1980. Early prostaglandin E synthesis is an obligatory event in the induction of cell proliferation in mouse epidermis *in vivo* by phorbol ester TPA. *Biochem. Biophys. Res. Commun.* 92: 749-756.

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

Goun, E., Cunningham, G., Chu, D., Nguyen, C. and Miles, D. 2003. Antibacterial and antifungal activity of Indonesian ethnomedical plants. *Fitoterapia.* 76: 592-596.

Greenwald, R.A. 1991. Animal model for evaluation of arthritic drugs. *Methods find. Clin. Pharmacol*. 13: 75.

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

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

Harborne, J.B. 1991. *Phytochemical Methods*. *Guide to Modern Techniques of Plant Analysis*. Second edition. Chapman and Hall, India. 653 p.

Huang, D.J., Lin, C.D., Chen, H.J. and Lin, Y.H. 2004. Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.] Lam `Tainong 57') constituents. *Bot. Bull. Acad. Sin*. 45: 179-186.

Hueza, I.M., Fonseca, B.S.M., Paulino, C.A., Haraguchi, M. and Gorniak, S.L. 2003. Evaluation of immuno modulatory activity of *Ipomoea carnea* on peritoneal cells of rats. *J. Ethnopharmacol.* 87: 181-186.

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

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

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

Jagetia, G.C. and Balgia, M.S. 2004. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. *J. Med*. *Food*. 7(3): 343-8.

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

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

Jha, P.K., Mazumdar, B. and Bhatt, J.D. 2006. Analgesic activity of venlafaxine and its interactions with tramadol, celecoxib and amlodipine in mice. *Indian J Pharmacol.* 3: 181-84.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. 1997. *Clinical Biochemistry of Domestic Animals.* Fifth edition. Academic press, California, 932p.

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

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

Kirtikar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants*. Second Edition. Periodical Experts, Delhi, pp 116-120.

Kondoh, H., Sato, Y. and Kanoh, H. 1985. Arachidonic acid metabolism in cultured mouse keratinocytes. *J. Investigative Dermatol*. 85: 64-69.

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

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

Kyuki, K., Shibuya, T., Tsurami, K. and Fujimura, H. 1982. Anti-inflammatory effect of diclofenac sodium ointment in topical application. *Japan. J. Pharmacol*.33: 121-132.

Lakshmi, B., Ajith, T.A., Sheena, N., Gunapalan, N. and Janardhanan, K.K. 2003. Antiperoxidative, anti-inflammatory and antimutagenic activities of ethanol extract of the mycelium of *Ganoderma lucidum* occurring in South India. *Teratogenesis, carcinogenesis and mutagenesis*. 23(1): 85-97.

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

Lowry, D.H., Rosenbrough, N.J., Farr, A.I. and Randal, R.J. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem*.193: 265-275.

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

Malalavidhane, T.S., Wickramasinghe, S.M.D.N., Perera. M.S.A. and Jansz, E.R. 2003. Oral hypoglycemic activity of *Ipomoea aquatica* in streptozocin induced Diabetic wistar rats and Type 11 Diabetics. *Phytother. Res*. 17: 1098-1100.

Matin, M.A., Tewari, J.P. and Kalani, D.K. 1969. Pharmacological effects of paniculatin--a glycoside isolated from *Ipomoea digitata* Linn. *J. Pharm. Sci*. 58(6): 757-9.

McColl, S.R., Hurst, N.P. and Cleland, L.G. 1986. Modulation by phorbol myristate acetate of arachidonic acid release and leukotriene synthesis by human polymorphonuclear leukocytes stimulated with A23187. *Biochem. Biophys. Res. Commun.* 141: 399-404.

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

Medeiros, R.M.T., Barbosa, R.C., Correa, R.F., Lima, E.F., Tabosa, I.M., Barros, D.S.S., Gardner, D.R. and Molyneux, R.J. 2003. Tremorgenic syndrome in Goats caused by *Ipomoea asarifolia* in north eastern Brazil. *Toxicon*. 41: 933-935.

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

Mishra, S.S., Tewari, J.P. and Matin, M.A. 1964. Investigations of the fixed oil from *Ipomoea digitata* tubers. *J. Pharm. Sci*. 54: 471-72.

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

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

Nadkarni, A.K. 1976. *Indian Materia Medica*. VOL: 1. Popular book Depot, Bombay. pp. 624.

Nakadate, T., Aizu, E., Yamamoto, S. and Kato, R. 1986. Some properties of lipoxygenase activities in cytosol and microsomal fraction of mice epidermal homogenate. *Prostaglandins leukotrienes Med*. 21: 305-319.

Narayana, K., Kundur, P.S., Usha, N. and Sridhar, N.B. 2003. *Poisonous and Medicinal Plants*. First Edition. Jayashri publications, Bangalore. p.376.

Onwukaeme, N.D. 1994. Anti-inflammatory activities of flavonoids of *Baphia nitida* Lodd. (Leguminosae) on mice and rats. *J. Ethnopharmacol*. 46(2): 121-124.

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

Paula, A.C.B., Hayashi, L.S.S. and Freitas, J.C. 2003. Anti-inflammatory and Antispasmodic activity of *Ipomoea imperati* (Vahl) Griseb (Convolvulaceae). *Braz. j. med. Boil. Res.* 36:105-112.

Pena, D., Montes de Oca, N., Rojas, S., Parra, A. and García, G. 2006. Anti-inflammatory and antidiarrhoeic activity of *Isocarpha cubana* Blake. *Pharmacologyonline*. 3: 744-749.

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

Pongprayoon, U., Baeckstro¨m, P., Jacobsson, U., Lindstrom, M and Bohlin, L., 1992. Inhibition of ethyl phenylpropionate-induced rat ear oedema by compounds isolated from *Ipomoea pes-caprae. Phytother. Res*. 6,104–107.

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

Rabanal, R.M., Bonkanka, C.X., Perez, M.H. and Mateo, C.S.S. 2005. Analgesic and anti-inflammatory activity of *Hypericum canariense* L. and *Hypericum glandulosum Ait. J. Ethnopharmacol*. 96: 591-596.

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

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

Ramesh, M., Roy, A.A., Rao, V.N., Prabhakar, M.C., Rao, C., Muralidhar, N. and Reddy, B.M. 1998. Anti-nociceptive and anti-inflammatory activity of flavonoid isolated from *Caramulla attenuata. J. Ethnopharmacol*. 62: 63-66.

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

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

Rao, P.N. 2000. Problems with basic science education: policy and practice. *Current science.* 78: 1181.

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

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

Roch-Arveiller, M and Giroud, J.P. 1979. Biological and pharmacological effects of carrageenan. *Pathol. Biol.* 27(10): 615-626.

Safayhi, H. and Sailer, E.R. 1997. Anti-inflammatory actions of pentacyclic triterpenes. *Planta Med*. 63: 487-493.

Saleem, M., Alam, A. and Sultana, S. 2001. *Cheiranthus cheiri* Abrogates phorbol ester induced tumor promotion response in Murine Skin. *Pharmaceutical Biol*. 39: 25-34.

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

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

Sasthri, G.A. 1998. *Veterinary clinical pathology*. Third edition. CBS Publishers and Distributers. Pvt. Ltd., New Delhi, 207p.

Schalm, O.W., Jain, N.C. and Carroll, E.J. 1986. *Veterinary Haematology*. Fourth edition. Lea and Febiger, Philadelphia. P: 45-48.

Schawarz, A., Gorniat, S. L., Bernardi, M. M., Dagli, M.L. and Spinosa, H.S. 2003. Effects of *Ipomoea carnea* aqueous fraction intake by dams during pregnancy on the physical and neurobehavioural development of rat offspring. *Neurotoxicology and Teratology*. 25: 615-62.

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

Shindo, Y., Witt, E., Han, D., Tzenq, B., Aziz, T., Nguven, L. and Packer, L. 1994. Recovery of antioxidant and reduction of Lipid hydroperoxides in murine epidermis and dermis after acute ultra violet radiation exposure. *Photodermatol Photoimmunol Photomed*. 1994. 10(5): 183-91.

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

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

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

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

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

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

Singh, S. and Majumdar, D.K. 1997. Evaluation of anti-inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J. Exp. Biol*. 35: 380-383.

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

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

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

Souza, M.M.D., Madeira, A., Berti, C., Krogh, R., Yunes, R.A. and Filho, V.C. 2000. Antinociceptive properties of the methanolic extract obtained from *Ipomoea pes caprae* (L.) R. Br. *J. Ethnopharmacol.* 69: 85-90.

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

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

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

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

Tripathi, K.D. 1999. *Essentials of medical pharmacology*. Fourth edition. Jaypee brother's medical publishers. New Delhi. 935 p.

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

Velankar, S.S.; Sharma, R.K and Reddy, A.G. 1998. Comparative studies on analgesic activity of individual and combinations of certain non-steroidal anti-inflammatory drugs in rats. *Indian Vet. Med. J.* 22 (3), p. 199-202.

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

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

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

Vinegar, R., Truax, J.F., Selph, J.L., Johnston, P.R., Venable, A.L. and McKenzie, K.K., 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Federation Proceedings*. 46, 118-126.

Vishnudass, N.T.S. 2007. Ayurvedic Herbs for Male Reproductive Problems. *J. Health.* 33:12-15.

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

Wood, A.W., Huang, M.T., Chang, R.L., Newmark, H.L., Lehr, R.E., Yagi, H., Sayer, J.M., Jerina, D.M. and Conney, A.H. 1982. Inhibition of mutagenicity of bay region diol epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenols: exceptional activity of ellagic acid. *Proc Natl Acad Sci of the USA*. 79: 5513-5517.

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

ASSESSMENT OF ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF *Ipomoea mauritiana* (Palmuthukku) IN RATS

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ABSTRACT

The present study was undertaken to assess the anti-inflammatory and analgesic effect of the root extract of *Ipomoea mauritiana* in rats. Diclofenac potassium was used as the reference drug for both anti-inflammatory and analgesic screening.

Forty adult male Sprague Dawley rats of 150-200 g body weight were divided into five groups of eight animals each for the anti-inflammatory study using carrageenin induced hind paw oedema model as well as for analgesic screening by tail flick method. The experiment was conducted for a period of seven days. Five per cent gum acacia was fed to Group I and II in which the Group II received the diclofenac potassium at the dose rate of 3 mg/kg on the 7^{th} day before carrageenin administration. The ethanolic extract of Ipomoea roots were administered to group III, IV, V at the dose rate of 300 mg/kg, 600 mg/kg and 1200 mg/kg respectively for seven days.

Five groups of six female swiss albino mice each were used for anti inflammatory study using croton oil induced skin inflammation. Group without any treatment was kept as healthy control (Group1). Group 2 treated with croton oil alone was kept as inflammatory control. Alcoholic extract of *Ipomoea mauritiana* was applied topically to the shaved area of dorsal skin 30 minutes before each application of croton oil. After 24 hour, the extract and croton oil treatment was repeated on the same area. Five per cent diclofenac potassium cream was used as the reference drug. One hour after the second treatment of croton oil, animals were sacrificed and the skin punches were removed and weighed in an analytical balance to find out the percentage inhibition of skin inflammation induced by croton oil. Then the skin samples were used for the estimation of lipid peroxides, superoxide dismutase, reduced glutathione as well as for histopathological studies.

In the anti-inflammatory screening, Ipomoea roots showed significant inhibition of carrageenin induced oedema in all the three hours of inflammation. In anti-inflammatory screening using croton oil, the reduction in skin thickness was not significant when compared to the healthy control for all the groups treated with Ipomoea root. Administration of croton oil produced significant increase in lipid peroxides and significant decrease in superoxide dismutase as well as a reduction in reduced glutathione levels. Ipomoea root treated groups produced slight changes, but the change in values were not significant when compared to normal, which suggested that the Ipomoea root extract was not efficient in alleviating the free radical induced damage on mouse skin.

In case of analgesic screening, group III, treated with Ipomoea root extract at a dose rate of 300 mg/kg, showed significant increase in reaction time only at 90 minutes of observation.

Serum enzymes like ALT and AST and haematological parameters like total and differential leukocyte count, RBC count, haemoglobin and PCV were recorded in both anti-inflammatory and analgesic screening on the 7^{th} day of the experiment. All the paameters were within the normal level in both the studies.

From the present study it can be concluded that the ethanolic extract of Ipomoea roots have significant anti-inflammatory effect in rats and hence it can be recommended for the treatment of various inflammatory conditions.