PHARMACOLOGICAL EFFECTS OF *Boerrhavia diffusa* L. IN LABORATORY RODENTS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy

Faculty of Veterinary and Animal Sciences
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MANNUTHY, THRISSUR - 680651
KERALA, INDIA

2002

DECLARATION

I hereby declare that this thesis entitled **"Pharmacological effects of *Boerrhavia diffusa* L. in laboratory rodents"** 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, fellowship, associateship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Pharmacological effects of *Boerrhavia diffusa* L. in laboratory rodents"** is a record of research work done independently by **Smt. Mini Bharathan** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Dedicated to
my Mother

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ACKNOWLEDGEMENT

It is with immense pleasure that I record my unreserved gratitude and indebtedness to ***Dr. A.D. Joy,*** *the chairman of my advisory committee and Associate Professor, Department of Pharmacology and Toxicology for the inspiring guidance, infinite patience and invaluable support extended to me throughout the course of my study.*

My sincere thanks are due to ***Dr. N. Gopakumar,*** *Associate Professor and Head, Department of Pharmacology and Toxicology and member of the advisory committee, for his sincere help and guidance during the period of my study.*

I wish to express my deep sense of gratitude to ***Dr. A.M. Chandrasekharan Nair,*** *Associate Professor, Department of Pharmacology and Toxicology,* ***Dr. P.K. Ismail,*** *Professor, Department of Pathology and* ***Dr. G. Krishnan Nair,*** *Associate Professor, Department of Microbiology, the members of my advisory committee, for their timely assistance and constructive guidance.*

I also remember with gratitude ***Dr. M.K. Rajagopalan, Dr. Jacob. V. Cheeran, Dr. Zacharias Cherian*** *and* ***Dr. P. Marykutty,*** *former Professors of the Department of Pharmacology and Toxicology who were a great source of inspiration and encouragement.*

I also express my sincere gratitude to ***Dr. K. Venugopalan, Dr. C.M. Aravindakshan, Mr. V.R. Raghunandanan,*** *Associate Professors and* ***Dr. Usha P.T.A,*** *Assistance Professor, Department of Pharmacology and Toxicology, for their whole-hearted co-operation.*

I remain thankful to ***Dr. K.V. Valsala,*** *Professor and Head, Department of Pathology,* ***Mrs. Indirabai,*** *Assistant Professor,* ***Mrs. Santhabai,*** *Programmer, Department of Statistics,* ***Dr. P.T. Philomina,*** *Associate Professor and Head, Department of Physiology and* ***Dr. P. Nandakumar,*** *Associate Professor, College of Veterinary and Animal Sciences, for their unstinted support and assistance.*

I am deeply obliged to the ***Jawaharlal Nehru Memorial Fund,*** *New Delhi, for awarding me scholarship throughout the course of the study.*

I also wish to express my gratitude to the ***Dean****, College of Veterinary and Animal Sciences, Mannuthy, for the facilities provided for this research work.*

The generous help and constant encouragement given by my friends, ***Nisha, Suresh, Jyotsna*** *and* ***Padmaraj,*** *are also acknowledged thankfully.*

A special word of thanks to ***Mrs. Rema Nair*** *and* ***Miss. T.K. Simi*** *for their assistance in giving shape to this thesis.*

Last, but not the least, I acknowledge with love and gratitude, the unfailing patience, support and encouragement given to me by the members of my family during the period of study.

Mini Bharathan

Introduction

INTRODUCTION

Research on medicinal plants is an important facet of bio-medical research in India. Medicinal plants play a major role in human health care. Traditional medicines based on plant materials are used by about 80 per cent of human population (Subramoniam and Pushpangadan, 1999). Ancient indigenous medical systems like Ayurveda, Sidha, Unani etc. make use of plant derivatives or combinations for treatment of diseases which may produce additive or complementary effect on remedial measures. But they have been mostly based on practical experiences rather than on documented pharmacological basis. This accentuates the necessity for scientific validation of pharmacological effects of medicinal plants for their therapeutic application. However, the fact remains that each natural product is a combination of different plant principles, the great majority of which have not been validated scientifically.

The plant *Boerrhavia diffusa* L. known as 'punarnava' in sanskrit, 'spreading hogweed' in English and 'thazhuthama' in Malayalam, is a perennial, vigorously growing weedy vine indigenous to India and Brazil. The earliest mention of this plant is seen in 'Charaka samhita' (Charaka, 1949). In

this book, it has been recommended as an ingredient of various laxative, diuretic and emetic preparations in different dosage forms. A 'vitalization procedure' described in Charaka samhita includes consumption of preparation containing *B. diffusa* as the main ingredient. There is also reference to its use as an ointment in the treatment of leprosy and other skin disorders. The roots of the plant are employed for many curative purposes including liver, gallbladder, renal and urinary disorders. Earlier research has shown that an alkaloidal extract of the plant can produce a distinct and persistent rise in blood pressure and diuresis (Chopra *et al.*, 1958). The present study has been undertaken with a view to investigate into some pharmacological activities of *B. diffusa* (viz. anti-inflammatory, hepatoprotective and immunomodulatory).

Inflammation is the reaction of living tissues to injury and involves vascular, neurological, humoral and cellular responses at the site of the noxious stimulus. As a result of the inflammatory process, the injurious agent is either destroyed or diluted or contained at the site of injury and this is followed by the process of repair. The inflammatory and reparative processes essential to the organism can sometimes be harmful too. This is the case with diseases like rheumatoid arthritis, glomerulo

nephritis, lupus erythematosis etc. in which the inflammatory reactions produced by a hyperactive immune system inflict damage to various tissues instead of being beneficial. Methods of arresting such harmful inflammatory processes can bring about a halt to the degenerative changes taking place in the tissues as a result of inflammation. In this context, anti-inflammatory drugs with lesser side effects gain advantage over the routine non-steroidal anti-inflammatory drugs (NSAIDs) and steroids.

The present study has undertaken an assessment of the anti-inflammatory activity of four different extracts of *B.diffusa* and the different fractions of the most active extract of the above. A number of biochemical changes have been proposed to explain the mechanisms of action of anti-inflammatory drugs. They include stabilization of lysosomal membranes, release of steroidal hormones from adrenals, inhibition of synthesis and/or release of prostaglandins and other mediators of inflammation like the kinins, oxygen-derived free radicals etc. Hence the study also aims to investigate the biochemical basis of anti-inflammatory action of *B. diffusa*, if any.

Liver has a pivotal role in regulating the physiological functions in the body like metabolism,

secretion and storage. Moreover, it is in the liver that a number of drugs and xenobiotics are detoxified. It has a vital role in digestion also. Hence, any disease of liver can be fatal to life. Inspite of the tremendous advances made in recent years in pharmacological research, scientists have failed to evolve a 100 per cent safe and effective hepatoprotective allopathic medicine. Herbal drugs, on the other hand, are known to play a more important role in the management of liver diseases. *B. diffusa* has been used alone and in combination with other plants in Ayurvedic formulations for certain liver diseases. Thus, in the present study, the alcoholic extract of *B. diffusa* is investigated for the hepatoprotective activity against carbon-tetrachloride induced hepato toxicity in rats.

Immunomodulators have been widely used in recent years as supportive therapy in cancer chemotherapy, to reduce the myelosuppression induced by anti-neoplastic drugs. Similarly, they also play an important role in certain inflammatory diseases like rheumatoid arthritis, caused by a hyperactive immune system. Even though several natural compounds are used for their immuno-stimulant effect, the immuno-therapeutic value of most of the therapeutic regimen has not been scientifically validated. Hence, the present

study has been undertaken with a view to investigate into the immuno-modulatory activity of the alcoholic extract of *B. diffusa* in Balb/c mice also.

The objectives of the present study are as follows:-

1. to assess the anti-inflammatory activity of *B. diffusa*.
2. to elucidate the possible mechanism of action.
3. to assess the hepatoprotective activity of *B. diffusa*
4. to assess the immuno-modulatory activity of *B. diffusa*.

Review of Literature

REVIEW OF LITERATURE

2.1. Plants possessing anti-inflammatory activity

A number of plants have been said to possess anti-inflammatory activity in ancient literature. But only a few plants have undergone systematic screening trials and scientific validation.

Gujral *et al.* (1961) isolated glycyrrhizin the active principle from *Glycyrrhiza glabra*. They found that the compound at a dose of 20mg/100g b.w. administered orally in adrenalectamised rats possessed significant anti-inflammatory activity that was comparable to that of hydrocortisone.

A steroid obtained from *Commiphora mukul* showed significant anti-inflammatory activity on rat-paw oedema induced by carrageenan which was dose-dependant and more potent than the resin fraction of the same plant (Arora *et al.*, 1971).

Gossypin-a bioflavanoid obtained from *Hibiscus vitifolius* Linn. significantly reduced the rat paw oedema and the increased vascularity induced by various phlogistic agents (Parmar and Ghosh, 1978). It also produced significant inhibition of accumulation of pouch fluid,

granulation tissue formation, migration of leukocytes and formation of pleural exudates. It also possessed significant activity against adjuvant and formalin-induced arthritis in rats.

Joshi *et al.* (1984) compared the anti-inflammatory activity of the alcoholic extract of *Bougainvilla spectabilis* leaves with that of oxyphenbutazone. Oral administration of 0.5 and 1g/kg of the extract produced 26.39 per cent and 27.13 per cent inhibition of rat paw oedema respectively at fourth hour while oxyphenbutazone produced 32.55 per cent inhibition. In the chronic model of inflammation also, 1g/kg of the extract produced 33.76 per cent inhibition while oxyphenbutazone produced 41.67 per cent inhibition.

A comparative study on certain biochemical changes brought about by curcumin and ibuprofen during sub-acute inflammation in rats was done by Srivastava and Srimal (1985). Curcumin was found to be more potent than ibuprofen as a stabilizer of lysosomal membranes and as an uncoupler of oxidative phosphorylation. At higher doses curcumin was shown to act by stimulation of adrenals, resulting in the release of endogenous corticoids. Curcumin inhibited the synthesis of prostaglandins also.

Dihydrozingerone isolated from *Zingiber officinale* was found to inhibit the thermal denaturation of bovine serum albumin and possessed significant anti-inflammatory activity as tested by the carrageenan induced rat paw oedema method in rats (Elias and Rao, 1988).

Goel *et al.* (1988) reported the anti-inflammatory and anti-ulcer effects of Kaempferol, a flavone isolated from *Rhamnus procumbens* in rats. The drug @ 200 mg/kg i.p. possessed significant activity against carrageenan and serotonin, but not against histamine induced pedal oedema in rats. It also showed anti-ulcer activity against 4-hour pylorus-ligated and immobilization-stress induced gastric ulcers in rats and duodenal ulcers in male guinea pigs.

The anti-inflammatory, analgesic and anti-pyretic activities of a glycosidal fraction isolated from *Maesa chisia* was compared with known non steroidal anti-inflammatory drugs (NSAIDs) (Gomes *et al.*, 1989). The pharmacological activities @ 25mg/kg compared favourably with NSAIDs while the remarkable lack of toxicity was an added advantage.

Tandan *et al.* (1994) revealed that the alcoholic extract of the roots of *Ageratum conyzoids* possessed significant anti-inflammatory (carrageenan induced) and

analgesic (acetic acid induced writhing) activities @ 100 and 500mg/kg respectively in rats. The extract did not show anti-convulsant activity in electroshock test and protective activity against cardiazole-induced convulsions in mice at the above doses.

Alam *et al.* (1992) studied the anti-inflammatory and anti-pyretic activities of vicolides A, B, C and D isolated from *Vicoa indica*. It was observed that the vicolides exhibited significant anti-inflammatory activity against cotton-pellet granuloma in rats @ 100mg/kg b.w. and reduced the protein content, acid and alkaline phosphatase and alanine aminotransferase and aspartate aminotransferase levels in liver and serum. Significant reduction in the ascorbic acid content of the adrenals was also observed.

Emblica officinalis leaf extracts were reported to possess significant anti-inflammatory activity by the carrageenan induced rat-paw model of inflammation in rats (Asmawi *et al.*, 1993). Their effects on the synthesis of mediators of inflammation like Leukotriene B_4, Platelet Activating Factor, Thromboxane B_2 and formyl methionyl leucyl phenylalamine (f-MLP) induced migration of human polymorphonuclear leukocytes ((PMNLs) were also tested *in vitro*. They found that the drug inhibited migration of

human PMNLs, but did not inhibit the synthesis of these mediators suggesting that the mechanism of anti-inflammatory activity did not involve inhibition of synthesis of these mediators.

Saeed *et al.* (1993) examined the effects of ajmaline on human platelet aggregation, arachidonate metabolism and PAF induced lethality in rabbits. Platelet aggregation induced by several stimuli (ADP, collagen and PAF) was inhibited by increasing concentrations of ajmaline. The formation of thromboxane A_2 and 12-HETE was also inhibited by ajmaline. Ajmaline also inhibited carrageenan induced rat paw oedema formation.

Makwana *et al.* (1994) evaluated the pharmacological activity profile of the ethanolic extract (ETE) and cold aqeous infusion (CAI)of *Vitex leucoxylon* leaves in a battery of tests. They observed that ETE showed significant inhibition of carrageenan induced paw oedema and granulation tissue formation in rats. Suppression of acetic acid induced writhing was observed with both ETE and CAI. LD_{50} values of ETE and CAI were 73g/kg and 0.8-1.2g/kg respectively i.p.

Jain *et al.* (1994) screened the anti-inflammatory activity of an Ayurvedic preparation 'Brahmi rasayan'

containing *Bacopa monnieri* as the main component. The drug @ 1-10g/kg administered orally in rats was found to suppress various experimentally induced inflammatory reactions and did not show any gastric irritation. The anti-inflammatory activity might be mediated by interfering with the action and/or synthesis of prostaglandins and by stabilization of lysosomal membranes.

Lupeol, isolated from the petroleum ether fraction of the ethanolic extract of *Ixora coccinea* Linn. was found to possess significant anti-inflammatory activity in carrageenan induced paw oedema in rats and anti-mitotic activity in a preliminary cytotoxic study using the 'Allium' test of Levan (Zachariah *et al*, 1994).

Khobragade and Jangde (1996) reported that the aqeous extract of bulb of *Allium sativum* had significant anti-inflammatory activity comparable to that of ibuprofen and prednisolone when tested by the carrageenan induced paw oedema method @ 500mg/kg p.o. in rats.

Oil of *Psidium guajava* was given orally to rats to study its effects on the exudative and proliferative phases of inflammatory reaction using the acute and chronic models of inflammation and compared with ketorolac tromethamine (Kavimani *et al.*, 1996). The results showed that 0.8 ml/kg

of the volatile oil showed significant anti-inflammatory activity in both carrageenan-induced paw oedema and cotton pellet granuloma models.

Ahumada *et al.* (1997) studied the effects of a triterpene fraction - cycloarterol, isolated from *Crataegus monogyna* Jacq. on different acute inflammatory models in rats and mice, leukocyte migration and phospholipase A_2 (PLA_2) inhibition. In the carrageenan induced paw oedema model in rats, inhibition was 61.5 and 52.5 per cent at 3h and 5h respectively @40mg/kg b.w. In the mouse carrageenan peritonitis test, the fraction inhibited peritoneal leukocyote infiltration and PLA_2 *in vitro*.

Kavalali and Tuncel (1997) revealed that the petroleum ether extract of *Urtica pilulifera* (F. Urticaceae) showed significant anti-inflammatory activity comparable to that of indomethacin in carrageenan-induced rat paw oedema model of inflammation in rats @ 1g/kg i.p.

Krishnaveni *et al.* (1997) observed that 4, 5, 6-trihydroxy 3,7 dimethoxy flavone isolated from *Vicoa indica* DC possessed significant anti-inflammatory activity by the carrageenan induced rat paw oedema method and analgesic activity by the acetic acid induced writhing model in rats @ 50mg/kg b.w., which was comparable to that of morphine

Tandan *et al.* (1997) reported that the alcoholic extract of *Hedychium spicatum* possessed significant anti-inflammatory activity in carrageenan induced hind paw oedema and analgesic activity in acetic acid induced writhing in mice and Randall-Selitto assay in rats.

Anto *et al.* (1998) studied the anti-inflammatory activity of three natural and eight synthetic curcuminoids using the mouse paw oedema method. Among the natural curcuminoids, curcumin III and among the synthetic curcuminoids, salicyl, veratryl and piperonyl curcuminoids were found to possess significant anti-inflammatory activity.

The petroleum ether, benzene, chloroform, acetone and ethanolic extracts of *Abies pindrow* Royle leaves were tested at different dose rates for anti-inflammatory activity in both acute and sub-acute models of inflammation. It was observed that all the extracts possessed significant anti-inflammatory activity which was comparable to that of phenyl butazone (Singh *et al.*, 1998).

The ethanolic extract of *Curcuma amada* was found to possess significant anti-inflammatory activity in acute and chronic models of inflammation in rats @ 100 and 200 mg/kg p.o (Majumdar *et al.*, 2000). The anti-inflammatory activity

@ 200mg/kg was found to be comparable to that of standard drug indomethacin.

The methanolic extract of the flowers of *Michelia champaca* Linn., *Ixora brachiata* Roxb. and *Rhynchosia cana* Willd. were found to possess significant anti-inflammatory activity against cotton pellet induced subacute inflammation in rats. The latter two drugs showed higher activity and reduced the protein content, acid phosphatase, SGOT and SGPT activities in liver and serum (Dahanukar *et al.*, 2000).

2.2 Plants possessing hepatoprotective activity

Pushpangadan *et al.* (1988) described the ethnopharmacology of *Trichopus zeylanicus* as detected by a battery of tests. The alcoholic extract of the seeds of the plant was found to possess significant antihepatotoxic and immunomodulatory activity in rats @100mg/kg p.o. It also produced significant anti-stress and anti-fatigue activity in the same dose rate.

Extracts of certain food additives - turmeric, garlic and asafetida-were tested for their effect on aflatoxin production and aflatoxin induced liver damage in ducklings (Soni *et al.*, 1992). The extracts were found to inhibit aflatoxin production considerably at concentration of

5-10mg/ml of medium. Turmeric and curcumin, a synthetic derivative of it, were also found to reverse the aflatoxin induced liver damage produced by feeding aflatoxin B_1 (5mg/day for 14 days) to ducklings.

Patil *et al.* (1993) studied the effect of some hepatoprotective Ayurvedic drugs on lipolytic activities during carbontetrachloride (CCl_4) induced acute hepatic injury in albino rats. Administration of the drugs concomitant with CCl_4 was found to significantly decrease the levels of acid lipase, alkaline lipase and lipoprotein lipase of liver, kidney and adipose tissue and hormone sensitive lipase of adipose tissue exhibiting hepatoprotection.

Joy and Kuttan (1996) investigated the effect of lycovin syrup and *Picrorhiza kurroa* extract on acute and chronic hepatotoxicity induced by CCl_4 in rats. They observed that both lycovin syrup and the extract reduced the toxic effect of CCl_4 significantly in both models as shown by reduced lipid peroxide, alkaline phosphatase and aspartate transaminase levels.

Saraf *et al.* (1996) observed the anti-hepatotoxic activity of the petroleum ether and ethanolic extracts of *Euphorbia antisyphilitica* against thioacetamide and CCl_4

induced hepatotoxicity in rats. The active ethanolic extract was further fractionated into chloroform soluble and insoluble fractions of which the latter was found to be more active.

Karunakar *et al.* (1997) reported the antihepatotoxic activity of *Jigrine* (a unani herbal formulation) against alcohol and CCl_4 induced hepatotoxicity in rats. The drug was found to decrease significantly the elevated levels of serum AST, ALT, tissue γ-GTP, triglycerides and lipid peroxides, showing significant protective activity. The histopathological changes were also confirmatory.

The powdered roots, aerial parts and the aqueous extract of *Sida rhombifolia* were found to possess significant hepatoprotective activity against CCl_4, paracetamol and rifampin induced hepatotoxicities as revealed by a decrease in the elevated levels of SGOT, SGPT, SALP, serum total bilirubin and direct bilirubin. The methanolic extract of the aerial parts showed significant anti-inflammatory activity, against carrageenan induced paw oedema in rats (Rao and Mishra, 1997a).

The petroleum ether (PE), methanolic and total aqeous extracts of *Fumaria indica* were tested for hepatoprotective activity against CCl_4, rifampicin and paracetamol induced

hepatotoxicities in albino rats. The PE extract was found to be significantly active against CCl_4, aqueous extract against paracetamol and methanolic extract against rifampicin induced hepatotoxicity respectively (Rao and Mishra, 1997b).

Darshan and Doreswamy (1998) reviewed the medicinal plant patent scenario of India. They enlisted three plants possessing hepatoprotective activity and for which patents have been filed in India namely *Picrorhiza kurroa, Plumbago zeylanica* and *Berberis vulgaris*.

Ahmad *et al*. (1999) evaluated the hepatoprotective activity of *jigrine*, a polyherbal formulation containing the aqeous extract of 14 medicinal plants, on thioacetamide induced liver damage in rats. The results showed that *jigrine* @ 0.5 and 1ml/kg/day p.o. reduced the thioacetamide induced increase in the levels of AST, ALT, Na^+ and K^+ in serum and thiobarbituric acid reacting substances (TBARS) in liver. It also restored the thioacetamide induced decrease in liver GSH (reduced glulathione). The histopathological findings also confirmed the hepatoprotective activity.

Joy and Kuttan (1999) showed that oral administration of *Picrorhiza kurroa* extract reduced the hepatotoxicity and

fibrosis induced by chronic administration of CCl_4 and thioacetamide in rats significantly. This was evident from the decreased values of serum and liver lipid peroxides, alkaline phosphatase, SGPT, and liver collagen hydroxyproline content.

Somkuwar and Pawar (2000) showed that the aqueous extract of *Emblica officinalis* possessed significant protective activity against CCl_4 induced hepatotoxicity in rats as revealed by a decrease in serum enzyme levels and histopathological findings. The extract was also found to possess significant anti-anaemic activity.

HD-03, a polyherbal formulation containing extracts of five plants was tested for its activity against paracetamol induced liver damage in rats. Reversal of Na^+ K^+ ATPase, glycogen, glutathione levels and restricted hepatic damage in HD-03 treated rats confirmed the hepatoprotective activity (Udupa *et al.*, 2000).

The petroleum ether and alcoholic extracts of *Moringa pterygosperma* Gaertn. leaves were screened for their hepatoprotective and haemopoetic activities in rats. The extracts were found to have significant hepatoprotective activity against CCl_4 and paracetamol induced hepatic damage, whereas the total aqueous extract was found to

possess significant haemopoetic activity (Mathew and Gopi, 2000).

Latha *et al.* (2001) studied the hepatoprotective activity of the hexane extract of *Ixora coccinea* flowers. The extract was found to possess significant protective activity against paracetamol induced hepatotoxicity in rats, as revealed by a decrease in serum marker enzyme levels, liver lipid peroxide levels and choleretic activity.

According to Suja *et al.* (2001), the ethanolic extract of *Spilanthes ciliata* significantly reduced the levels of liver marker enzymes like SGOT, SGPT and alkaline phosphatase and increased the choleretic activity of anaesthetised normal rats and decreased the hexobarbitone induced sleeping time, thus proving its hepatoprotective activity.

2.3. Plants possessing immunomodulatory activity

Dua *et al.* (1989) showed that the Indian pseudojinseng saponins possessed better adaptogenic activity and immunomodulatory activity than those of Korean ginseng saponins, using a battery of tests in rats and mice.

Kuttan and Kuttan (1992) have reported that a peptide isolated from *Viscum album* extract increased the Natural Killer (NK) cell activity, antibody dependant cellular cytotoxicity (ADCC), antibody forming cells in spleen and antibody titre in normal and tumour bearing animals. The activity of the crude plant extract simulated that of the peptide indicating that peptide was mainly responsible for the immunomodulatory activity of the extract.

Oral pretreatment with the leaf extract of *Azadiracta indica* reversed the inhibitory effect of restraint stress on formation of anti sheep RBC antibody titres in rats immunized with SRBC and also the increase in footpad thickness. It also reversed the DDT induced suppression of antibody response and leukocyte migration inhibition in tetanus toxoid immunized rats (Ray *et al.*, 1997).

Curcumin, an active ingredient present in *Curcuma longa*, when tested for its immunomodulatory activity, was found to increase the total WBC count, circulating antibody titre and plaque forming cells in spleen against sheep red blood cells (SRBC) in Balb/c mice. Bone marrow cellularity, α-esterase activity and macrophage phagocytic activity were also enhanced, showing immunostimulant activity of curcumin (Antony *et al.*, 1999).

Praveenkumar *et al.* (1999a) studied the effect of 'rasayanas', a herbal drug preparation on the immune response in mice when given orally. The drug was found to enhance the proliferation of spleen cells (especially in presence of mitogens), esterase activity in bone marrow, antibody forming cells in spleen and circulating antibody titre. The results confirmed the immunostimulant activity of 'rasayanas'.

2.4. Plants possessing antioxidant activity

Soudamini *et al.* (1992) reported the effect of oral administration of curcumin on lipid peroxidation in various organs of normal mice and those given CCl_4, paraquat and cyclophosphamide. It was found to decrease significantly the increased peroxidation of lipids in these organs produced by these chemicals, revealing its anti oxidant activity.

Piperine, isolated from *Piper nigrum* was studied for its activity against lipid peroxidation during experimental inflammation in rats (Dhuley *et al.*, 1993). Pretreatment with piperine or oxyphenbutazone reduced lipid peroxidation, acid phosphatase activity and oedema induced by carrageenan.

Pandey *et al.* (1994) described the antiperoxidative property of solvent free alcoholic extract of *Rubia cordifolia* in rat liver homogenate. The extract was found to prevent the cumine-hydroperoxide induced malondialdehyde formation and maintained reduced glulathione (GSH) levels even in the presence of the above toxin.

Jose and Kuttan (1995) observed that an aqeous extract of *Emblica officinalis* inhibited lipid peroxide formation and was a potent scavenger of hydroxyl and superoxide radicals *in vitro*. Chyavanaprash, an indigenous preparation containing Emblica mainly was also found to possess significant antioxidant activity.

Chatterjee (1996) investigated the antiperoxide and free radical scavenging activity of IH/AO-3-a polyherbal formulation on different *in vitro* systems and compared the results with known antioxidants. The drug was found to possess antioxidant action which was concentration dependant.

Active principles of *Withania somnifera* consisting of equimolar concentrations of sitoindosides VII - X and withaferin A induced a dose related increase in superoxide dismutase, catalase and glutathione peroxide activities in

rat brain frontal cortex and striatum, comparable to diprenyl, a known antioxidant (Bhattacharya *et al.*, 1997).

The alcoholic and hexane fractions of *Bacopa monniera* as an antioxidant against $FeSO_4$ and cumene hydroperoxide induced lipid peroxidation was studied by Thripathi *et al.* (1996). The alcoholic fraction showed greater and dose dependant protection against both.

Quercetin, a plant bioflavanoid, was found to possess significant cyto protective effect on cisplatin - induced renal tubular damage *in vivo* in rats @ 20mg/kg i.p. Cisplatin, which produced renal toxicity by increasing lipid peroxidaton, was found to markedly increase the levels of lipid peroxides (TBARS) and decrease the levels of antioxidant enzymes like SOD, catalase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase. Quercetin pretreatment was found to reverse all there changes (Devipriya and Shyamaladevi, 1999).

Chatterjee *et al.* (2001) reported the antioxidant effect of *Terminalia arjuna*. The plant bark effectively antagonized various reactive oxygen species (ROS) like superoxide, hydroxyl radicals, lipid peroxide formation, singlet oxygen and hydroxy peroxide induced membrane damage.

The hexanoic extract of fruits of *Hippophae rhamnoides* was found to possess significant protective activity against ethanol induced gastric injury which was proved to be due to its antioxidant activity, as shown by the increase in gastric tissue glutathione level in the drug treated group (Suleyman *et al.*, 2001). The drug was given @ 500mg/kg p.o. and ethanol was administered after 30min. The drug was found to be more potent than melatonine in this activity.

2.5. Pharmacological activities of the plant under study

Chopra *et al.* (1956) described the use of *Boerrhavia diffusa* in asthma, oedema, anemia, jaundice, anasarca and as an antidote to snake poisoning. The roots of the plant was reported to have diuretic, laxative and expectorant properties.

The leaves of the plant were found to be useful in inflammations, heart disease, liver diseases, dropsy and poisoning of various origin. The roots were said to have diuretic, anti-inflammatory and antipyretic properties (Priyavrat Sharma, 1956).

Chopra *et al.* (1958) reported that the active principle of *B. diffusa* was a compound of alkaloidal nature

called 'punarnavine'. Large quantities of potassium nitrate and other salts contained in the plant might contribute to its diuretic effect. Intravenous injection of the alkaloid in cats produced distinct and persistent rise in blood pressure and diuresis.

Abraham (1975) reported that the plant as a whole was effective in jaundice, oedema, and bloodpressure as a diuretic. In mild doses, it cured asthma, while in high doses, it acted as an emetic.

Kirtikar and Basu (1975) recommended the use of the plant in anaemia, inflammations, vatha and 'kapha'. The plant in combination with other drugs was prescribed for snake bite, dropsy, jaundice and ascitis.

Nadkarni (1976) opined that 1-4 drachms of the liquid extract from the plant produced diuresis in cases of oedema and ascites, especially due to early liver, peritoneal and kidney conditions. The diuresis was said to be mainly due to the action of 'punarnavine'- the alkaloid on renal epithelium though rise in blood pressure is also a contributing factor.

An extract obtained from the roots of *B. diffusa* plants, which inhibits the infection of several plant

viruses, was tested by agar diffusion hole method for its action on RNA containing bacterial viruses. Plaque formation of the phages was only partially and non uniformly influenced by the extract so that a uniform principle of action was not realized for the RNA viruses of prokaryotic and eukaryotic host organisms (Aswathy and Menzel, 1986).

B. diffusa was included as one of the extensively investigated medicinal plants in India (Vohora, 1989). He recommended detailed investigation on its anti-inflammatory and diuretic properties.

Barthwal and Srivastava (1990) studied the effect of anti-fibrinolytic agents, anti-inflammatory drugs and the root extract of *B. diffusa* on the endometrial histology of IUD-fitted menstruating monkeys. A high degree of stromal edema, heavy infiltration of inflammatory cells, long tortuous endometrial glands and thin - walled empty blood vessels were observed in IUD endometrium. *B. diffusa* was found to be most effective in reducing stromal edema, inflammation and tortuosity of glands and in increasing the degree of deposition of fibrin and platelets in the vessel lumen and thus could be recommended for the treatment of IUD associated menorrhagia.

In another study by Barthwal and Srivastava (1991) the above finding was confirmed by evaluating the effect of antifibrinolytic agents, anti-inflammatory drugs and the extract of *B. diffusa* on menstrual cycle length (MCL), duration of menstrual flow (DMF), menstrual iron loss (MIL) and activity of uterine tissue plasminogen activator (tPA) in IUD-fitted female rhesus monkeys. They concluded that the root extract of *B. diffusa* treated monkeys showed noticeable reduction in DMF, MIL and tPA activity and hence could be used effectively in the management of IUD-associated menorrhagia.

The alcoholic extract of *B. diffusa* @ 500mg/kg possessed significant protective activity against CCl_4 induced hepatic injury in rats as revealed by a decrease in the levels of SGOT, SGPT, serum bilirubin, plasma prothrombin and BSP clearance time (Chandan *et al.*, 1991). The extract did not show any signs of toxicity upto an oral dose of 2g/kg in mice.

Two known lignans-liriodendrin and syringaresinol mono-β-D-glucoside have been isolated from the methanolic extract of the roots of *B. diffusa* Linn. (Lami *et al.*, 1991). The former compound was found to exhibit a significant calcium channel antagonistic effect in frog

heart single cells using the whole cell voltage clamp method.

Singh *et al.* (1991) in an experimental evaluation of possible teratogenic potential of *B. diffusa* in albino rats, observed that the ethanolic extract of *B. diffusa* @ 250 mg/kg was devoid of any teratogenic effect. The litter size and survival rates of fetuses were also the same as that of the controls.

Sohni *et al.* (1995) reported the antiamoebic effect of a crude drug formulation containing extracts of five medicinal plants - *B. diffusa, Tinospora cordifolia, Berberis aristata, Terminalia chebula* and *Zinigiber officinale* as evidenced by a decrease in the average degree of infection (ADI) *in vivo* and *in vitro*. The formulation had a minimum inhibitory concentration (MIC) of 1000 µg/ml as compared 10 µg/ml for metronidazole.

The same formulation was found to reduce the average degree of infection (ADI) of experimental hepatic amoebiasis in golden hamsters significantly @ 800mg/kg/day (Sohni and Bhatt, 1996). The drug also enhanced the humoral immunity and cell-mediated immunity as evidenced by the hemagglutination titre and leukocyte migration inhibition test respectively.

Mugantiwar *et al.* (1997) studied the effect of the alkaloidal fraction of *B. diffusa* on stress induced changes in plasma and adrenal cortisol levels and immune responsiveness in rats. The drug was found to possess restorative activity against stress induced changes in plasma and adrenal cortisol levels and augmented antibody production.

Rawat *et al* (1997) investigated the effect of seasons, thickness of roots and form of dosage on the hepatoprotective activity of the roots of *B. diffusa* against thioacetamide induced hepatotoxicity in rats. The results showed that an aqueous extract (2 ml/kg) of roots of diameter 1 - 3 cm collected in the month of May (summer) exhibited maximum protection of the serum enzymes SGOT, SGPT and ALP. The aqueous form of drug administration had more activity than the powder form.

A polyherbal formulation containing *B. diffusa*, *Plumbago zeylanica*, and *Vitex peduncularis* was found to be effective in controlling kala-azar and in preventing its relapses (Varghese *et al.*, 1998).

Srivastava *et al.* (1998) reviewed the chemistry, pharmacology and botany of *B. diffusa* extracts and their isolates. They described various chemical constituents of

B. diffusa and reported that some of them possessed hepatoprotective, adaptogenic, antifibrinolytic, diuretic and antiviral properties.

Another polyherbal preparation (LIVP-7) containing extracts of 7 herbs including *B. diffusa* was found to provide significant protection against CCl_4 induced hepatopathy using brom sulfophthalein uptake model in rat liver slices (Chatterjee and Das, 1999).

Mugantiwar *et al.* (1999) observed that the oral administration of an alkaloidal fraction of *B. diffusa* significantly inhibited SRBC-induced delayed hypersensitivity reactions in mice, during the post-immunization drug treatment and increased the circulating antibody titre. But it failed to show any blastogenic response of murine spleenocytes to concanavaline and lipopolysaccharride.

Nadig and Rao (1999) studied the effect of Hepatogard - an indigenous polyherbal formulation containing *B. diffusa* on dexamethasone induced antihealing effects in male albino rats. The drug was reported to reverse dexamethasone induced decrease in breaking strength and hydroxyproline content in both granulation and incised tissue and hence had the potential for antagonizing the

antihealing effect of steroids in patients receiving steroid therapy.

In a study to investigate the antibacterial activity of aqueous extract of sixteen different medicinal plants against three gram +ve and seven gram -ve bacteria, by the filter paper disc diffusion method, maximum activity was observed in *Tridax procumbens, Cleome viscosa, Acalypha indica* acid *B. erecta* (PerumalSamy *et al*, 1999).

The effect of lyophilized decoction (DE) and juice (JE) of fresh leaves of *B. diffusa* on chemical model of hyperalgesia in mice were investigated by Hiruma-lima *et al*. (2000). They found that JE and DE produced a significant inhibition of acetic acid induced writhing comparable to that of dipyrone sodium @ 1000 mg/kg each.

Geetha and Sangeetha (2000) conducted a controlled experimental study to assess the effect of *B. diffusa* extract @ 2.4g/kg b.w. orally to ward off post surgical infection and mortality in albino rats. The results showed that the drug caused (1) better prevention of post surgical infection; (2) better expectancy of life after surgery; (3) normal maintenance of level of water intake and urine output after surgery; (4) maintenance of total and differential WBC count after surgery and infection and (5)

prevention of accumulation of peritoneal fluid and onset of gangrene.

The aqueous leaf extract of *B. diffusa* was found to possess significant hypoglycaemic activity against alloxan induced diabetes in rats @ 100, 200 and 400 mg/kg p.o., with the peak activity at 6 h. post administration (Chude *et al.*, 2001).

Materials and Methods

MATERIALS AND METHODS

The technical programme was carried out in IV phases:-

3.1. Phase I: - Anti-inflammatory screening of the plant extracts:-

This was performed in 2 steps:-

3.1.1. Taxonomical identification, collection of plant materials and extraction:-

The plant under study namely *B. diffusa* L. growing in the premises of College of Veterinary and Animal Sciences was identified and collected for the study. The plant materials (whole plant including leaves, stem and roots) were dried under shade at 30°C, pulverized and extracted with petroleum ether, methanol and chloroform separately in a soxhlet apparatus. The extracts were evaporated to dryness under reduced pressure. The aqueous extract was prepared by boiling 150 g of the extract in 1.5 litre of water. After filtering, the filtrate was again boiled continuously till its volume was reduced to one - tenth. The percentage yields of the four extracts were then calculated.

3.1.2. Anti-inflammatory screening of the plant extracts by the carrageenan induced rat paw oedema method:-

The anti-inflammatory activity of each extract was compared with that of diclofenac sodium and tested by the carrageenan induced paw oedema method in rats (Winter *et al.*, 1962) as described below:-

Eighty Wistar albino rats of either sex weighing 100-200g, procured from Small Animal Breeding Station (SABS), Mannuthy were used for the study. They were housed in well-ventilated cages (temperature 30 ± 2°C, humidity - 65 - 70 per cent and 12h light/dark cycle) and fed with standard rodent diet from SABS, Mannuthy and drinking water *ad libitum*. The test extracts and diclofenac sodium were suspended in distilled water using Tween 80 as the emulsant (2 drops for 1 ml) and was administered to rats orally as given in the table shown below using a Eustachian catheter.

Group No.	Treatment
I	Control-distilled water-1ml + 2 drops TWEEN 80
II	200 mg/kg dried alcoholic extract
III	400 mg/kg dried alcoholic extract
IV	200 mg/kg dried aqueous extract
V	400 mg/kg dried aqueous extract
VI	200mg/kg dried chloroform extract

VII	400 mg/kg dried chloroform extract
VIII	200 mg/kg dried petroleum ether extract
IX	400 mg/kg dried petroleum ether extract
X	Diclofenac sodium @ 3 mg/kg

Paw oedema was induced half an hour after drug administration by injecting 0.05 ml of a 1 per cent suspension of carrageenan in normal saline into the plantar aponeurosis of the left hind paw of the rat. The paw thickness was measured in three planes using vernier calipers and the average was recorded immediately after carrageenan injection and three hours later.

Percentage inhibition of oedema was calculated by using the formula $\left(1-\frac{T_t}{T_c}\right)\times 100$ where T_t is the mean increase in paw thickness of the treated group and T_c is the mean increase in paw thickness of the control group. The data were analysed statistically using analysis of variance (Snedecor and Cochran, 1967). The extract showing maximum activity was chosen for further studies.

3.2. Phase II. Selective anti-inflammatory studies:-

3.2.1 Effect on cotton-pellet induced granuloma formation

The method described by Meier *et al.* (1950) was followed. Thirty two adult apparently healthy Wistar albino

rats weighing 100-150 g were procured from SABS, Mannuthy and were randomly divided into four groups of eight each. Each group was kept in a single cage. They were maintained on identical diet (compounded feed from SABS, Mannuthy) and environmental conditions. Feed and water were provided *ad libitum*. They were kept under observation for one week before the experiment in order to familiarize laboratory handling and management.

Cotton pellets weighing 20 ± 1mg made of absorbent surgical cotton were used for implantation. The pellets were made by hand, dipped in 70 per cent alcohol and dried in hot air oven at 50°C for 2 hours. The weight of the pellets were found to be unchanged after the sterilization procedures.

The rats were anaesthetized using ether, and two sterile cotton pellets were implanted sub-cutaneously at the two symmetrical ventrolateral sides of abdomen by a single midline skin incision. The incision was closed by simple interrupted suture with cotton thread.

The treatment schedule adopted was as shown in the table given below:-

Group No.	Treatment
Ia	Kept as control - given distilled water 1ml + 2 drop Tween 80 orally
IIa	Diclofenac sodium @ 3 mg/kg orally
IIIa	Methanolic extract of *B. diffusa* @ 200 mg/kg orally
IVa	Methanolic extract of *B. diffusa* @ 400 mg/kg orally

The drugs were administered to the rats orally for seven consecutive days after the implantation of pellets. On the 8th day, blood was collected from the orbital sinus and kept for separation of serum. Then the rats were sacrificed using anaesthetic ether. The pellets of each rat were dissected out and kept in separate petri dishes. The liver samples and adrenals from each rat were also collected separately.

The pellets were dried at 60°C overnight. In the morning, they were weighed once and again dried at 60°C for one hour before being finally weighed. The increase in weight of the pellets from each rat was calculated and statistically analysed using Analysis of variance. The percentage of anti-inflammatory activity was also

calculated using the formula $\left(1-\frac{W_t}{W_c}\right)\times 100$ where W_t is the mean increase in weight of the granuloma of the treated group and W_c is the mean increase in weight of the granuloma of the control group.

Serum was used for the estimation of protein and acid phosphatase in an auto analyzer using kits from Merck and Agappe respectively. Similarly the protein content of 1 per cent liver homogenate was also estimated by the method described by Lowry *et al.* (1951) Adrenal ascorbic acid content was estimated by the spectrophotometric method described by Nino and Prasad (1980) as given below.

a. Reagents

1. Trichloroacetic acid - 10g/dl solution
2. Sulfuric acid, 4.5 M solution
3. Sulfuric acid, 12 M solution
4. 2, 4-Dinitrophenyl hydrazine reagent, 2 g/dl in 4.5 M H_2SO_4
5. Thiourea, 5 g/dl solution
6. Copper sulfate, 0.6 g/dl solution.
7. Dinitrophenyl hydrazine - thiourea-coppersulphate (DTC) reagent- containing 10ml 2,4 dinitrophenyl hydrazine reagent + 5 ml thiourea solution + 5 ml copper sulphate solution.

b. Ascorbic acid standards

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

c. Procedure

1. Taken 1.2 ml aliquots of each working standards.
2. Prepared blank by taking 1.2 ml aliquot of trichloroacetic acid solution.
3. Added 0.4 ml DTC reagent to each tube. Mixed.
4. Incubated the tubes at 37°C for 3 hours.
5. Transferred the tubes to ice-water bath for 10 min.
6. Added slowly, 2 ml cold 12 M H_2SO_4.
7. Allowed the tubes to stand at room temperature for 20 min.
8. Set spectrophotometer at 0 absorbance at 520 nm with blank.
9. Prepared calibration graph plotting concentration against absorbance.

d. Specimen processing

1. Taken 2ml trichloroacetic acid solution in all the tubes. Added 0.5 ml plasma.
2. Mixed manually for 30s. The tubes were allowed to stand for 3-4 min.
3. Centrifuged for 10 min. at 2000-2200 rpm.

4. Pipetted out 1.2 ml supernatent to another tube
5. It was processed in the same way as working standards.
6. Final concentration was read from the calibration graph.

3.2.2. Assessing the anti-oxidant activity of the methanolic extract *in vitro*

A. Effect on inhibition of superoxide radical formation:-

This was done by the NBT reduction method of McCord and Fridovich (1969) as given below:-

a. Reagents:-

1. Nitroblue tetrazolium (NBT)- 1.5 m M.
2. Riboflavin - 0.12 m M.
3. Phosphate buffer - M/15 (pH 7.8)
4. Sodium cyanide - 0.0015 per cent in 0.1 M EDTA solution

b. Procedure

1. Prepared 2mg/ml concentration solution of the methanolic extract using distilled water.
2. Added varying volumes (10 µl - 250 µl) of the extract to different test tubes.
3. Added NBT solution, 0.1 ml to all the tubes.
4. Added NaCN solution, 0.2 ml to all tubes.
5. Final volume was made 3 ml using phosphate buffer
6. Added riboflavin solution, 0.5 ml to all the tubes kept at 20°C.

7. Placed the tubes in a bright light box to get uniform illumination for 15 minutes.

8. Control was prepared by adding all the reagents except the extract.

9. Measured optical density at 560 nm.

10. The per cent inhibition was calculated using the formula

$$\frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

B. Effect on inhibition of lipid peroxide formation:-

This was performed by the method described by Okhawa *et al.* (1979) as given below.

a. Reagents

1. Tris buffer (pH 7.0) - 0.2 M

2. Ascorbic acid - 0.3 mM

3. Ferrous ammonium sulphate - 0.8 mM.

4. Potassium chloride - 150 mM.

5. Acetic acid - 20 per cent.

6. Thiobarbituric acid - 0.8 per cent

7. Sodium dodecyl sulphate - 8 per cent

8. Butanol : pyridine - 15 : 1

b. Reaction mixture

1. Mice liver homogenate(25 per cent in tris buffer)-100 µl
2. Ascorbic acid - 100 µl
3. Ferrous ammonium sulphate - 100 µl
4. Potassium chloride - 100 µl
5. Methanolic extract (0.5 mg per cent) - (50 µl - 1000 µl)

Made upto 1ml with tris buffer. Incubated at 37°C for 1 h.

c. Estimation of thiobarbituric acid reacting substances (TBARS)

1. Taken 0.2 ml of reaction mixture
2. Added 200 µl of sodium dodecyl sulphate solution to all the tubes.
3. Added 1.5 ml acetic acid to all the tubes.
4. Added 1.5 ml thiobarbituric acid solution to all the tubes.
5. Incubated at 100°C (Boiling water bath) for 1 h.
6. Cooled the reaction mixture
7. Carefully added 1 ml distilled water to all the tubes.
8. Added butanol : pyridine 5 ml to all the tubes.
9. Mixed well centrifuged at 3000 rpm for 15`.
10. Control was kept with all the reagents except the extract
11. Read the optical density at 560 nm

12. The percentage inhibition was calculated using the formula $\frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$

C. Effect on inhibition of nitric oxide radical formation:-

This was performed by the method described by Green *et al*. 1982) as given below.

a. Reagents

1. Sodium nitroprusside solution, 10 mM, in phosphate buffered saline
2. Sulphanilamide solution, 1 per cent
3. Phosphoric acid, 2 per cent
4. Naphthylethylene diamine dihydrochloride - 0.1 per cent.
5. Greens reagent - containing 10 ml sulphanilamide solution, 20ml phosphoric acid solution and 1 ml naphthylethylene diamine dihydrochloride solution.

b. Procedure

1. Various volumes of the extract (2mg/mlconcentration) were added to different tubes (250 µl - 4 ml).
2. Sodium nitroprusside solution was added to all the tubes to make up the volume to 4 ml.
3. All the tubes were incubated at 25°C for 150 minutes.
4. Control tube without the extract was also incubated.
5. 0.5 ml of the mixture from each tube was removed to other tubes.
6. 0.5 ml of Green's reagent was added to all the tubes.
7. The optical density of the chromophore was read at 546 nm in a spectrophotometer.
8. The percentage inhibition was calculated using the formula

$$\frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

3.2.3. Serial fractionation and assessing the anti-inflammatory activity of the fractions (Fig.1)

1. Ten grams of the methanolic extract was reconstituted with 20 ml of water to make a slurry, and was poured to a 50 ml separating funnel.

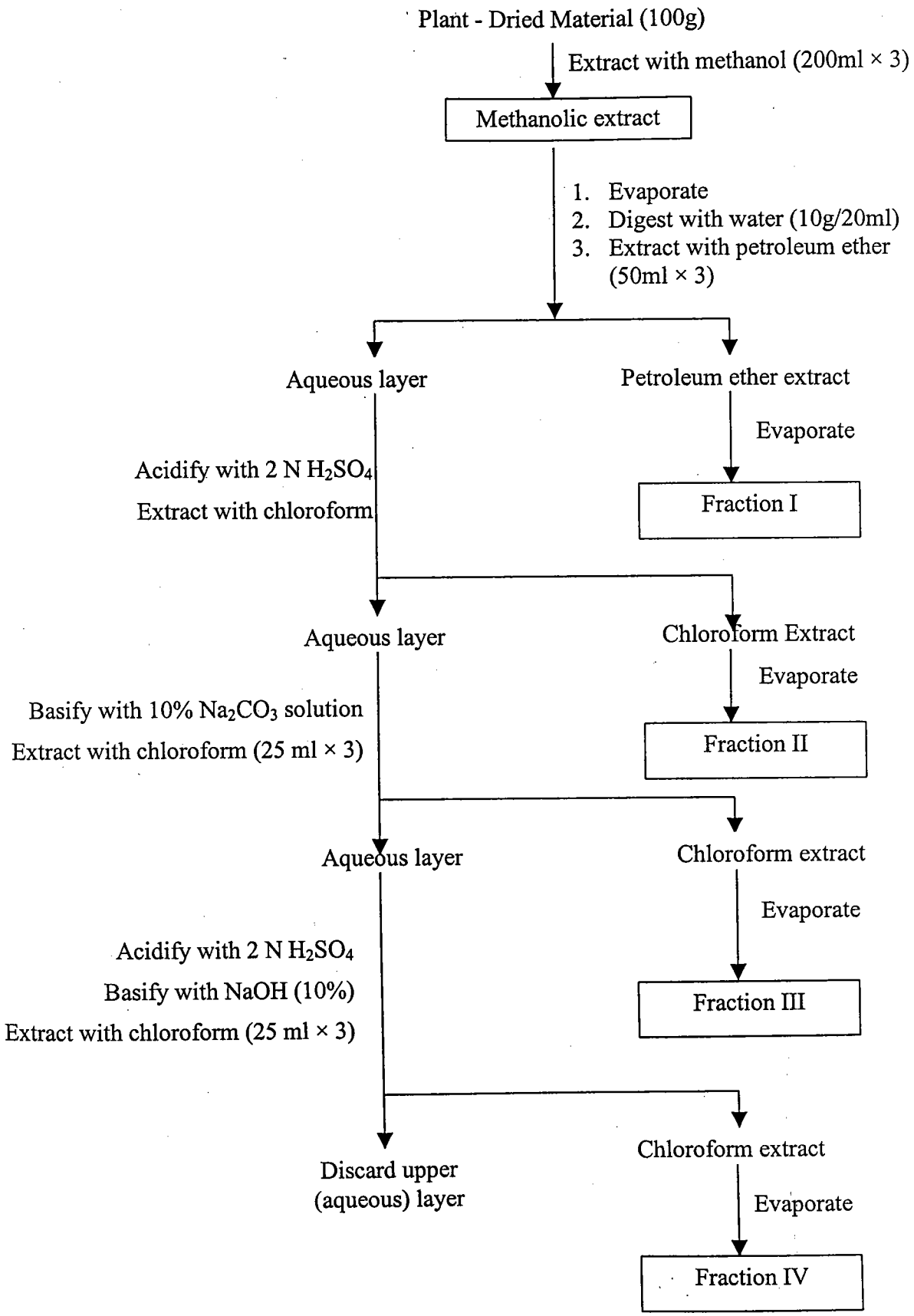
2. 20 ml of petroleum ether (PE) was added to it and was shaken 3 times and the lower fraction (aqueous) was separated. The upper PE fraction was poured off to a beaker and the procedure was repeated then thrice with the lower fraction. The separated PE soluble fraction was kept to test activity and marked as I.

3. The lower aqueous fraction was then acidified with 2N sulphuric acid and again extracted with chloroform in a similar way. The lower fraction was collected and kept to test activity and marked as II.

4. The upper aqueous layer was treated with 10 per cent sodium carbonate solution to make it alkaline and was extracted with chloroform. The lower fraction was again collected and marked III and kept to test activity.

5. The upper aqueous fraction was again made acidic with 2N sulphuric acid followed by basification with 2N NaOH and then extracted with chloroform. The lower fraction was

Fig. 1: Procedure for separation of methanolic extract



Fraction I – Neutral compounds
Fraction II – Organic acids
Fraction III – Phenolic alkaloids
Fraction IV – Strongly basic alkaloids

collected and marked as IV and kept to test activity. The upper aqueous layer was discarded.

6. The four fractions I, II, III and IV containing neutral, acidic, phenolic alkaloids, and strongly basic alkaloids respectively were then tested for anti-inflammatory activity using carrageenan - induced rat paw oedema method as described previously and compared with that of diclofenac sodium.

7. The fractions were dried and weighed, and were reconstituted with distilled water so as to make 200 mg/ml concentration. The fractions were administered to rats at the dose rate of 10 mg/kg b.w. p.o.Forty rats were randomly divided into 5 groups of 8 each and were used for the study. The treatment schedule followed was as given below:-

Group No.	Treatment
Ib	Fraction I (neutral fraction)
IIb	Fraction II (acidic fraction)
IIIb	Fraction III (Phenolic alkaloids)
IVb	Fraction IV (Strongly basic alkaloids)
Vb	Diclofenac sodium @ 3 mg/kg

3.3. Phase III: Assessing the hepato protective activity of the methanolic extract

Thirty two adult albino rats of either sex, divided into 4 groups of 8 each were used for the study. They were maintained in identical laboratory conditions and given standard compounded rat feed and water *ad libitum*. The protocol followed for the administration of carbon tetrachloride (CCl_4) and test samples was as given in the table shown below.

Group	0 h	12 h	24 h	36 h
Ic (control)	Veh. + OO	Veh.	Veh.	
IIc (Toxicant)	Veh. + CCl_4	Veh.	Veh.	Biochemical estimation in serum
IIIc	TSI + CCl_4	TS I	TS I	
IVc	TSII + CCl_4	TS II	TS II	

Veh.- Vehicle i.e. distilled water

OO - Olive oil

CCl_4 - Carbon tetrachloride suspension in olive oil in 1:1 proportion given @ 2.5 ml /kg b.w.

TS I- Extract in distilled water @ 200 mg/kg b.w.

TS II- Extract in distilled water @ 400 mg/kg b.w.

Thirty six hours after the initial dose, blood was collected and serum was separated. The estimation of levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (AP), Total Bilirubin (TB) and Direct Bilirubin (DB) in serum was done

using auto analyser kits from Merck. The rats were sacrificed and the liver samples were collected and examined for histopathological changes.

3.4. Phase IV: Assessing the immunomodulatory activity of the methanolic extract

This was performed using three different parameters.

3.4.1. Effect on haematological parameters

Eighteen albino rats of either sex, divided into three groups of six each were used for the study. The first group was given 1 ml distilled water, the second group was given the extract @ 200 mg/kg and the third group was given the extract @ 400 mg/kg respectively orally for five consecutive days. On the sixth day, blood was collected and the hematological parameters like total RBC count, total WBC count, differential count and Hemoglobin concentration were assessed by standard procedures described by Schalm *et al*, 1975.

3.4.2. Effect on plaque forming cells in the spleen

This was performed by the Jernes plaque assay (Jerne and Nordins, 1963) using a modified slide technique described by Mehrotra (1983) Thirty Balb/c mice of either sex divided into three groups of ten each were used for the

study. All the mice were immunized with 0.1 ml each of 20 per cent sheep red blood cells (SRBC) in phosphate - buffered saline given intra-peritonially using aseptic precaution. The first group was kept as the control and was given no drug. The second group was administered the extract @ 200 mg/kg orally daily till the mice were sacrificed. Similarly, the third group was administered the extract @ 400 mg/kg orally daily till the mice were sacrificed. On the 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} day after immunization, two mice from each group were sacrificed and spleen collected. Spleen cells were processed and plaque formation assay performed by the modified slide method, as given below:

The spleens were processed into single cell suspension (8×10^6 cells/ml) in Hanks Balanced Salt Solution (HBSS). To 0.5 ml of 5 per cent agarose in HBSS, 50 µl of 7 per cent sheep red blood cells (SRBC) and 50µl of spleen cell suspension were added, mixed well and poured over a glass slide. The slides were allowed to solidify and incubated with fresh rabbit serum as a source of complement for 1h. at 37°C. The plaques formed were counted over a light source and represented as plaque forming cells (PFCs) /million spleen cells (Lefkovits and Cosenza, 1979).

3.4.3. Effect on circulating antibody titre

This was done by the method described by Nelson and Davey (1992). Fifteen Balb/c mice were divided into three groups of five each. The first group was kept as the control. All the mice were immunized with 0.1 ml each of 20 per cent SRBC given intra-peritoneally. The second group was administered the extract @ 200 mg/kg orally daily till day 25 of immunization. The third group was administered the extract @ 400 mg/kg orally similarly. Blood was collected on day 5, 10, 15, 20 and 25 from all the mice. Sera were separated and heat inactivated at 56°C for 30 min. Two fold dilutions of sera samples were made using PBS (pH 7.2) in microtitre plates and mixed (1:1) with 1 per cent trypsinised suspension of SRBC in PBS. The plates were incubated at 37°C for 3h. The degree of agglutination was evaluated macroscopically. The hemagglutination titre was calculated as the reciprocal of highest dilution of serum which showed visible agglutination.

Results

RESULTS

4.1. Anti-inflammatory screening of the plant extract

4.1.1. Taxonomical identification, collection of plant materials and extraction

The plant was taxonomically identified (Fig. 2) and four extracts were prepared The w/w percentage yields of the extracts were (1) petroleum ether - 1.2 per cent (2) methanol - 26 per cent (3) chloroform - 2.9 per cent and (4) aqueous - 0.2 per cent

4.1.2. Anti-inflammatory screening of the plant extracts by the carrageenan - induced rat paw oedema method

The control group produced 2.025 ± 0.16mm increase in paw thickness after carrageenan injection (Table 1). The methanolic extract @ 200 mg/kg and 400 mg/kg produced 1.175 ± 0.17 mm and 0.876 ± 0.2 mm increase in paw thickness respectively with the per cent inhibition of oedema calculated as 42 per cent and 55 per cent respectively. The aqueous extract @ 200 mg/kg and 400 mg/kg produced 2.413 ± 0.28 mm and 1.6 ± 0.19 mm increase in paw oedema respectively with the per cent inhibition of oedema being -20 per cent and +20 per cent respectively. The chloroform extracts produced 1.638 ± 0.27 mm and 2.312 ± 0.21 mm

Fig. 2 *Boerrhavia diffusa* L.

Table 1: Anti-inflammatory screening of the plant extracts by the carrageenan – induced rat paw oedema method

Group No.	Treatment	Increase in paw thickness (mm) (Mean ± SE)	Per cent inhibition of paw oedema
1.	Control (no drug)	2.025 ± 0.16	--
2.	Alcoholic extract @ 200 mg/kg	1.175 ± 0.17	42
3.	Alcoholic extract @ 400 mg/kg	0.876 ± 0.2*	55
4.	Aqueous extract @ 200 mg/kg	2.413 ± 0.28	-20
5.	Aqueous extract @ 400 mg/kg	1.600 ± 0.19	20
6.	Chloroform extract @ 20 mg/kg	1.638 ± 0.27	20
7.	Chloroform extract @ 400 mg/kg	2.312 ± 0.21	-15
8.	Petroleum ether extract @ 200 mg/kg	2.338 ± 0.28	-20
9.	Petroleum ether extract @ 400 mg/kg	2.337 ± 0.21	-10
10.	Diclofenac sodium @ 3mg/kg	0.962 ± 0.07*	52.5

*Significant at 5% level

ANOVA

	Df	SS	MSS	F value	Probability
B/W	9	25.842	2.871	8.099	0.00
W/I	70	24.816	0.355		
Total	79	50.658			

increase in paw oedema at 200 and 400 mg/kg dose rate respectively with the per cent inhibition calculated as 20 per cent and -15 per cent respectively. Similarly the petroleum ether extract also produced an increase in paw thickness of 2.338 ± 0.26 and 2.337 ± 0.21 mm respectively @ 200 and 400 mg/kg and per cent inhibition of -20 per cent and -10 per cent respectively (Fig. 3).

Reference drug diclofenac sodium exhibited 0.962 ± 0.07 mm increase in paw thickness and 52.5 per cent inhibition of paw oedema. In this study, between diclofenac sodium and the methanolic extract @ 400 mg/kg there was no significant difference. Since the methanolic extract produced maximum anti-inflammatory activity, it was selected for further studies.

4.2. Selective anti-inflammatory studies using the methanolic extract

4.2.1. Effect on cotton pellet induced granuloma formation

The results are presented in table 2. The extract @ 200 mg/kg and 400 mg/kg produced 95.375 ± 12.27 mg and 75.5 ± 3.09 mg increase in weight of the pellets respectively. Their per cent anti-inflammatory activity was calculated as 24.31 and 43.25 per cent respectively. Diclofenac sodium

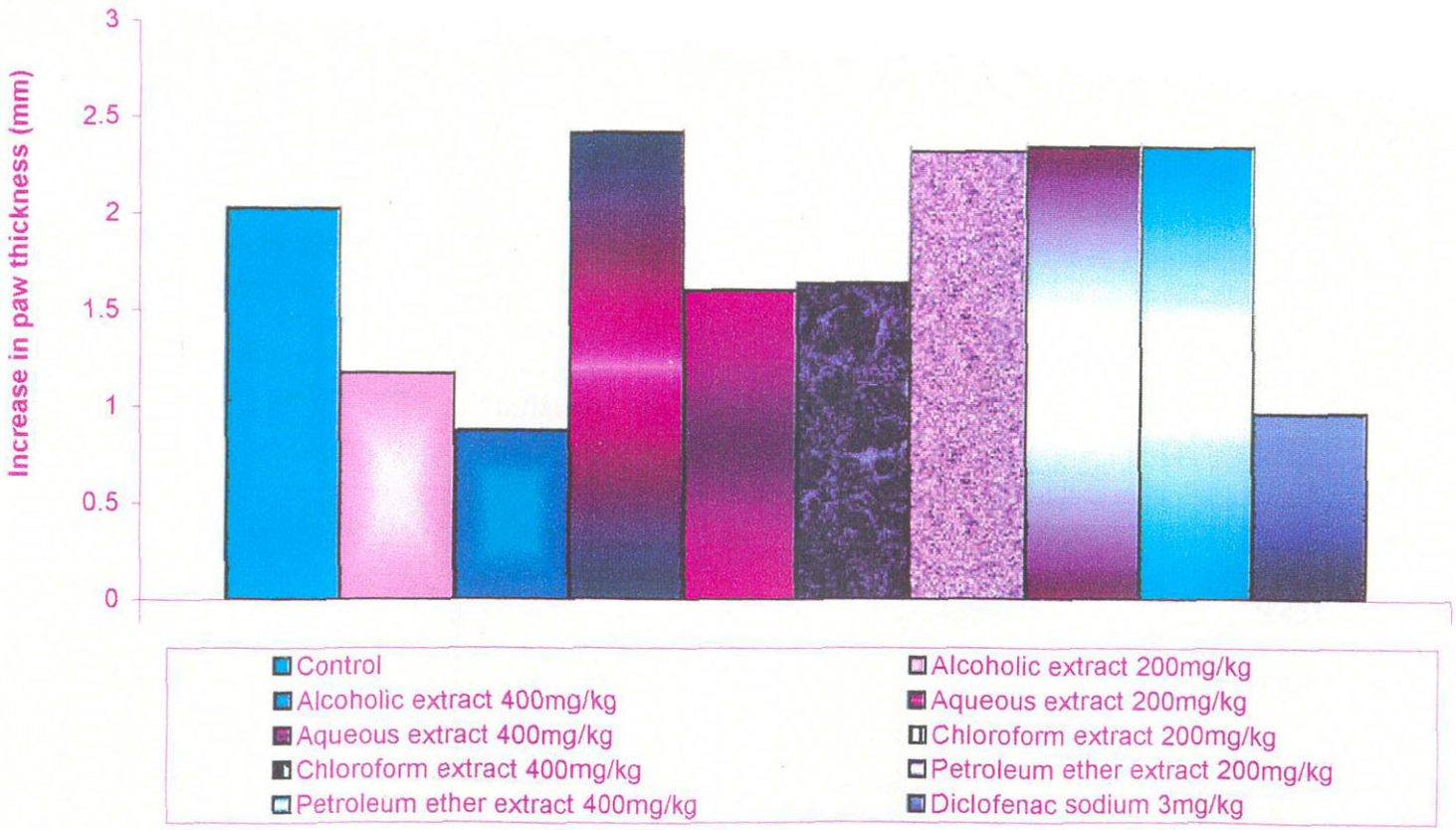


Fig. 3 Antiinflammatory screening of the plant extracts by the carrageenan induced rat paw oedema method

Table 2: Anti-inflammatory activity of the methanolic extract using cotton pellet induced granuloma formation in rats

Groups	Increase in weight of cotton pellets (mg) (Mean ± SE)	Per cent of anti-inflammatory activity	Serum acid phosphatase KA units (Mean ± SE)	Adrenal ascorbic acid mg/g (Mean ± SE)	Serum protein g/dl (Mean ± SE)	Liver protein mg/g (Mean ± SE)
I (Control)	126.00 ± 13.12	--	13.61 ± 3.55	3.15 ± 0.189	8.391 ± 0.52	0.204 ± 0.06
II (Diclofenae)	44.857 ± 6.35*	64.4	11.98 ± 1.93	2.641 ± 0.23	6.061 ± 0.45*	0.201 ± 0.01
III (Ext. @ 200 mg/kg)	95.375 ± 12.27	24.31	16.16 ± 5.25	3.624 ± 0.35	5.399 ± 0.43*	0.212 ± 0.03
IV (Ext. @ 400 mg/kg)	75.5 ± 3.09*	43.25	11.23 ± 7.26	3.172 ± 0.31	5.485 ± 0.43*	0.150 ± 0.04

* Significant at 5% level

produced 64.4 per cent anti-inflammatory activity with the mean increase in weight of the cotton pellets being 44.87 ± 6.35 mg. Thus the extract had lesser activity than diclofenac sodium though it produced dose dependant anti-inflammatory activity (Fig.4).

The serum acid phosphatase value for the group Ia was 13.61 ± 3.55 KA units and for the group IIa it was 11.98 ± 1.93 KA units. The extract @ 200 mg/kg and 400 mg/kg showed mean acid phosphatase values of 16.16 ± 5.25 and 11.23 ± 7.26 KA units respectively. Thus the decrease in serum acid phosphatase values for the extract @ 400 mg/kg was found to be similar to that of diclofenac sodium.

The adrenal ascorbic acid values for the groups Ia, IIa, IIIa and IVa were 3.15 ± 0.189, 2.641 ± 0.23, 3.624 ± 0.35 and 3.172 ± 0.31 mg/g respectively. These values showed no significant change between the groups Ia, IIIa and IVa, whereas the value for group IIa alone showed a significant decrease.

The mean serum protein levels for the groups Ia, IIa, IIIa and IVa were 8.391 ± 0.52, 6.061 ± 0.45, 5.399 ± 0.43 and 5.485 ± 0.43 respectively. These values for groups IIa, IIIa and IVa were found to be significantly different from

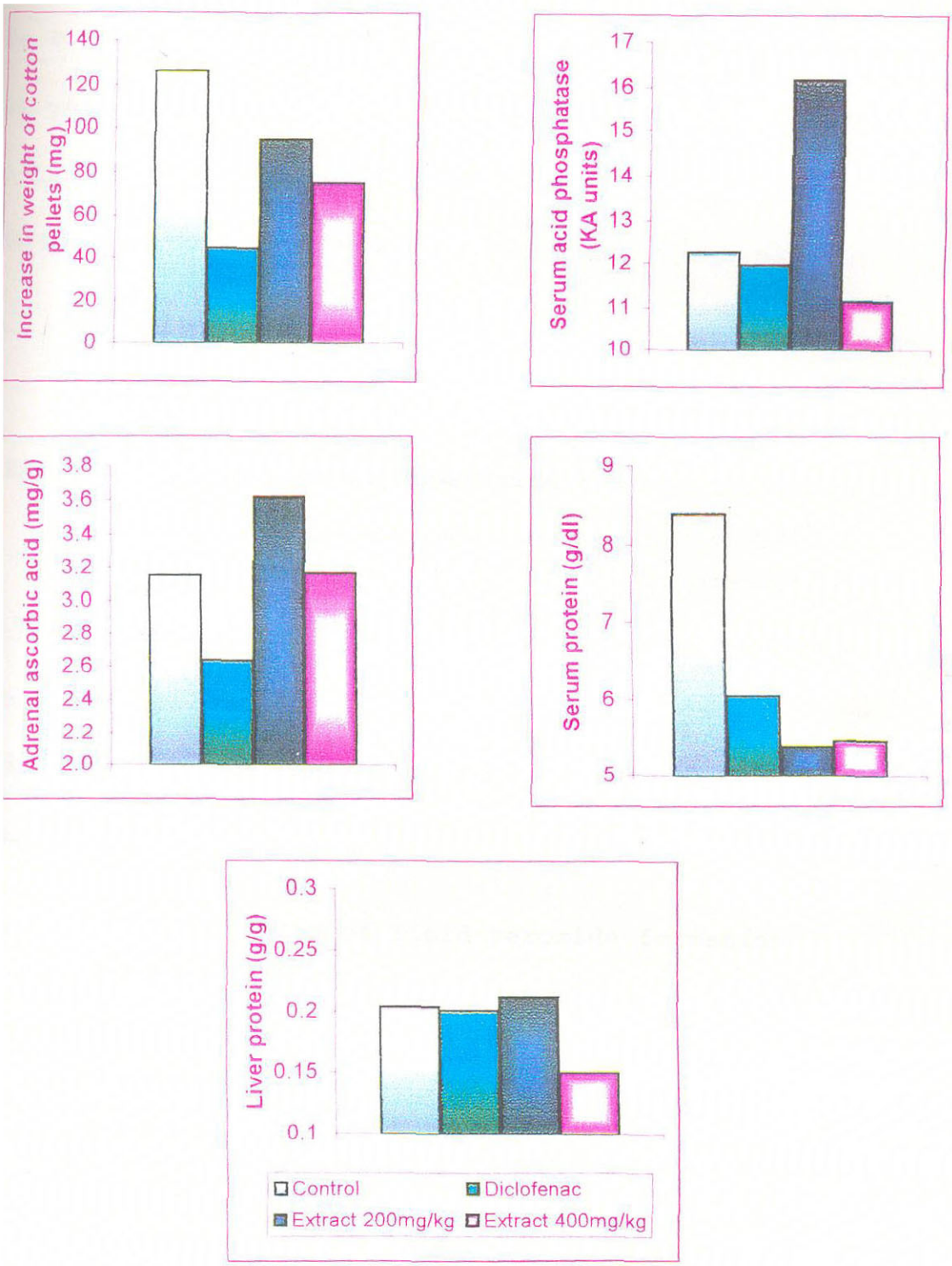


Fig. 4 Anti-inflammatory activity of the methanolic extract using cottonpellet induced granuloma formation in rats

that of the control groups (group Ia). Similarly, the values for liver protein concentration for the 4 groups were 0.204 ± 0.06, 0.201 ± 0.01, 0.212 ± 0.03 and 0.150 ± 0.04 g/g respectively. These values were found to be lesser for the groups IIa and IVa when compared to that of the control, but not significant.

4.2.2. Assessing the antioxidant activity of the extract *in vitro*

A. Effect on inhibition of superoxide radical formation

The superoxides generated by photoreduction of riboflavin reduced the NBT salt, the reduced form of which gave a blue colour, that was measured at 530nm. The amount needed for 50 per cent inhibition (IC 50 value) was 400 µg/ml (Table 3a, Fig.5a).

B. Effect on inhibition of lipid peroxide formation

The ferrous ascorbate system induced Thio Barbituric Acid Reacting Substances (TBARS) in mice liver homogenate *in vitro* which is a measure of lipid peroxidation. Lipid peroxide formation in the system was effectively inhibited by the methanolic extract. The IC 50 value was 200 µg/ml (Table 3b, Fig. 5b).

Table 3a: Effect on inhibition of superoxide radicals formation *in vitro*

Vol. of drug (µl)	Concentration (µg)	Per cent inhibition
10	20	-1.9
50	100	39.01
100	200	42.99
200	400	50.38
250	500	56.08
C	No drug	--

Table 3b: Effect on inhibition of lipid peroxide formation *in vitro*

Vol. of drug (µl)	Concentration (µg)	Per cent inhibition
50	25	-17.65
100	50	7.4
150	75	23.53
200	100	26.47
300	150	30.88
400	200	48.53
500	250	86.76
1000	500	94.12
C	No drug	--

Table 3c: Effect on inhibition of nitric oxide radical formation *in vitro*

Vol. of drug (µl)	Concentration (µg)	Per cent inhibition
250	500	-4.95
500	1 mg	3.47
1 ml	2 mg	24.75
2 ml	4 mg	45.05
4 ml	8 mg	51.0
C	No drug	--

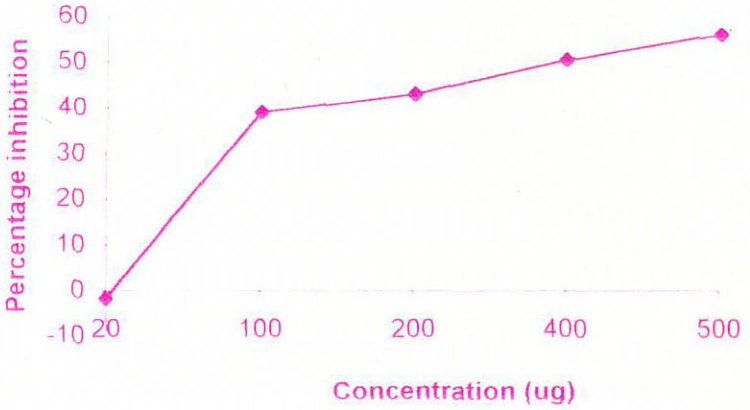


Fig. 5a Effect on inhibition of superoxide radical formation *in vitro*

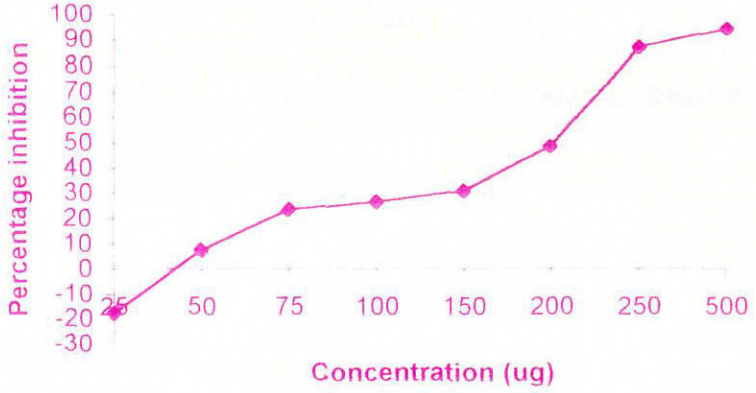


Fig. 5b Effect on inhibition of lipid peroxide formation *in vitro*

Fig. 5c Effect on inhibition of nitric oxide radical formation *in vitro*

C. Effect on inhibition of nitric oxide radical formation

Nitric oxide radicals generated from sodium nitroprusside at physiological pH was found to be inhibited by the extract (Table 3c, Fig. 5c). The IC 50 value was 8 mg/ml.

4.2.3. Serial fractionation and assessing the anti-inflammatory activity of the fractions

Four different fractions namely neutral, acidic, phenolic alkaloidal and the strongly basic alkaloidal fractions were obtained. They were then tested for anti-inflammatory activity by the carrageenan induced paw oedema method in rats.

The neutral, phenolic alkaloidal and the strongly base alkaloidal fractions produced 6.67 per cent, 10 per cent and 23 per cent inhibition of rat paw oedema, while diclofenac sodium produced 60.4 per cent inhibition (Table 4, Fig.6).

4.3. Assessing the hepato protective activity of the methanolic extract

The levels of all the serum parameters studied were increased significantly in the toxicant group ie group IIc (Table 5). The levels of AST, ALT and direct bilirubin

Table 4: Assessing the anti-inflammatory activity of the fractions

Group	Treatment	Increase in paw thickness (mm) (Mean ± SE)	Per cent inhibition
Control	Distilled water	2.7 ± 0.28	--
I	Neutral	2.52 ± 0.07	6.67
II	Acidic	3.2 ± 0.29	--
III	Phenolic alkaloids	2.43 ± 0.02	10
IV	Strongly basic alkaloids	2.03 ± 0.17	23
+ve control	Diclofenac sodium	1.07 ± 0.03	60.4

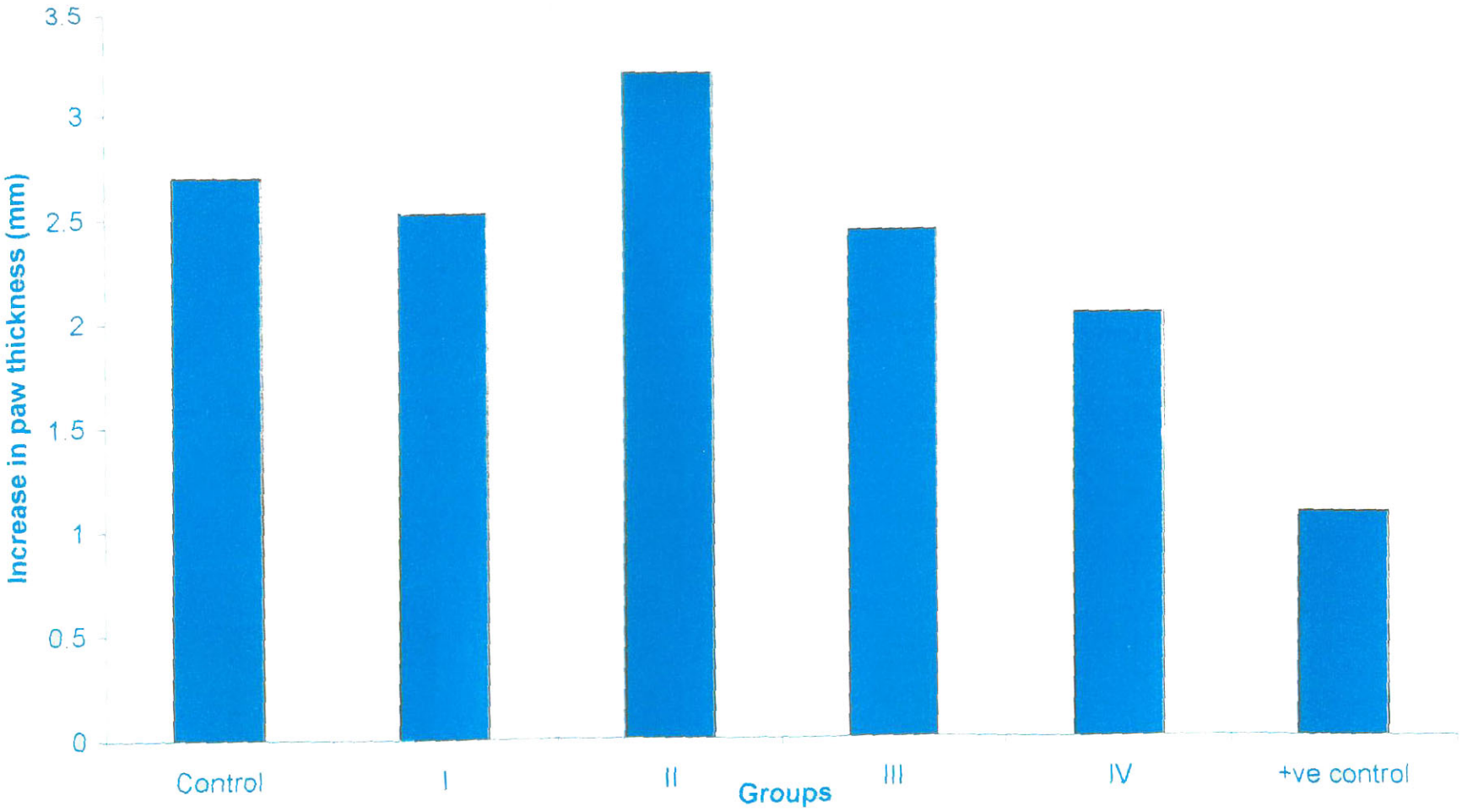


Fig. 6 Assessing the anti-inflammatory activity of the fractions

showed significant decrease in the group IVc. The levels of AP and TB were also decreased, but it was not significant. Similarly the levels of all the parameters except AP were also decreased in the IIIc group, but it was not significant (Fig.7).

The results of histopathological study can be read from the Fig. 8, 9, 10 and 11. Fig. 8 shows the histopathological picture of the liver of group Ic rats. The hepatic cells are radially placed and each cell has a large spherical nucleus with prominent nucleolus and granular cytoplasm.

Fig. 9 is the photomicrograph of the liver section of group IIc rats. It can be seen that there is heavy destruction of the overall arrangement of liver cells because most of the cells are in a ruptured state and without cytoplasm. Space formation and high degree of vacuolation are also seen.

Fig. 10 and 11 show the photomicrograph of liver slices of groups IIIc and IVc respectively. Hepatic cells have become more distinct in Fig. 10 with prominent nucleus and are arranged in the form of cords. Vacuolation also is lessened. In Fig. 11, the liver section is almost normal, with minimum vacuolation and clear hepatic cells.

Table 5: Assessing the hepatoprotective activity of the methanolic extract- results of biochemical study

Group No.	Treatment	ALT (U/l) (Mean ± SE)	AST (U/l) (Mean ± SE)	AP (U/l) (Mean ± SE)	TB (mg/dl) (Mean ± SE)	DB (mg/dl) (Mean ± SE)
I	Vehicle	209.5 ± 21.85	73.25 ± 11.97	310 ± 46.99	0.34 ± 0.5	0.28 ± 0.5
II	Toxicant	1150.86 ± 281.68	3405.86 ± 739.72	1375.25 ± 281.0	1.81 ± 0.37	1.2 ± 0.4
III	Ext. @ 200 mg/kg	696.375 ± 41.067	607.125 ± 198.827	1392.25 ± 226.53	1.8 ± 2.8	0.963 ± 0.18
IV	Ext. @ 400 mg/kg*	78.375 ± 10.48*	425.5 ± 70.22*	1025.85 ± 140.99	0.713 ± 1.2	0.5125 ± 0.16*

*Significant at 5% level

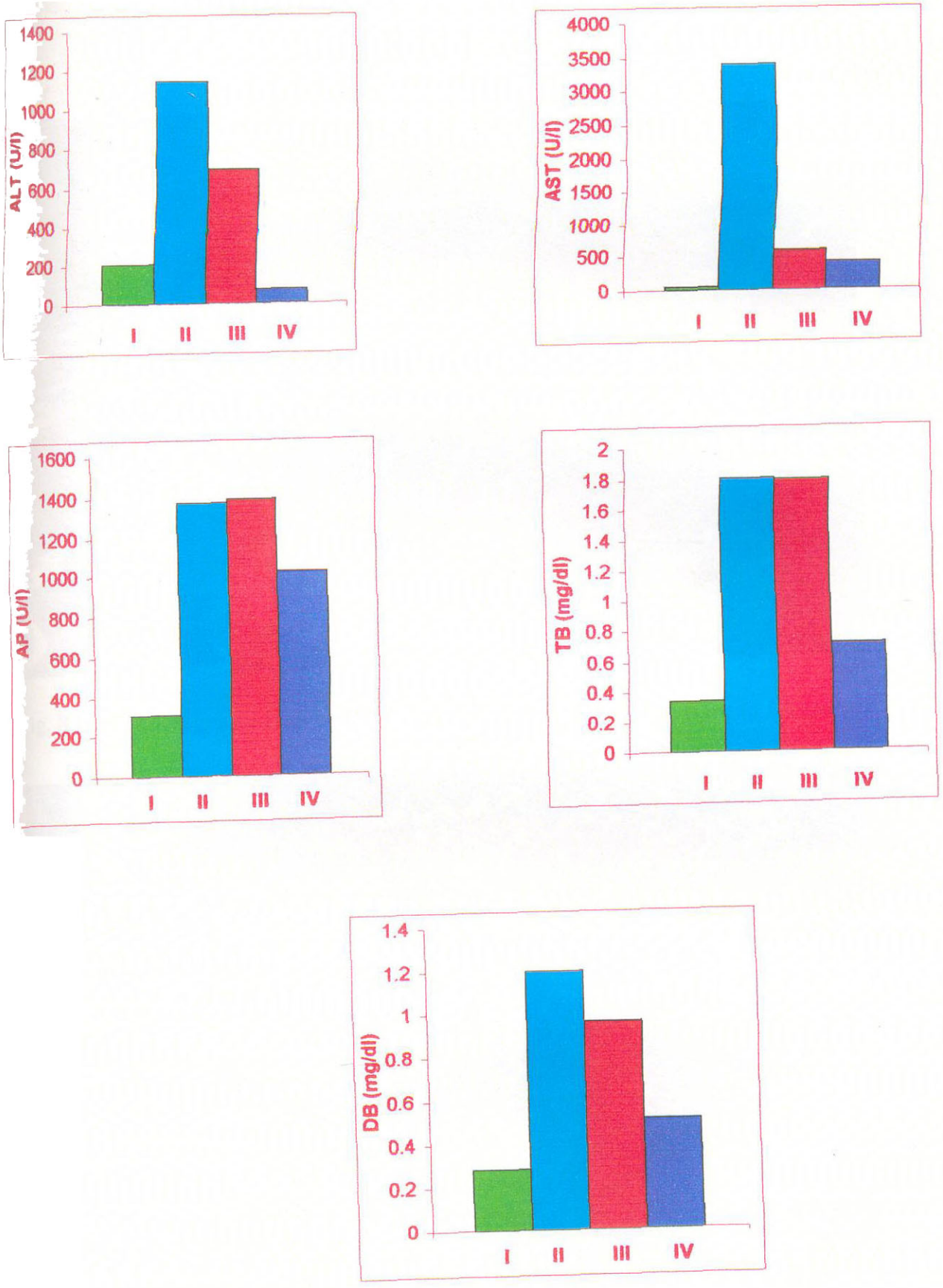


Fig. 7 Assessing the hepatoprotective activity of the methanolic extract - results of biochemical study

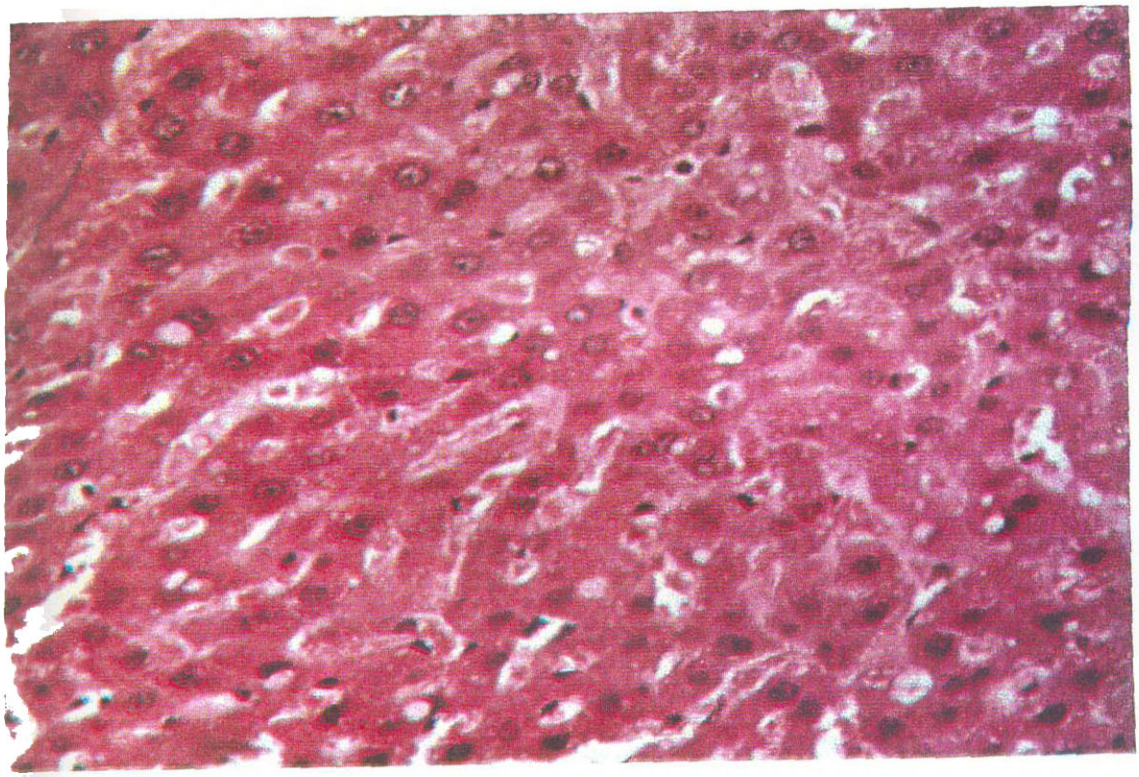


Fig. 8 Photomicrograph of liver of normal rat given vehicle alone (Group Ic) (H&E)

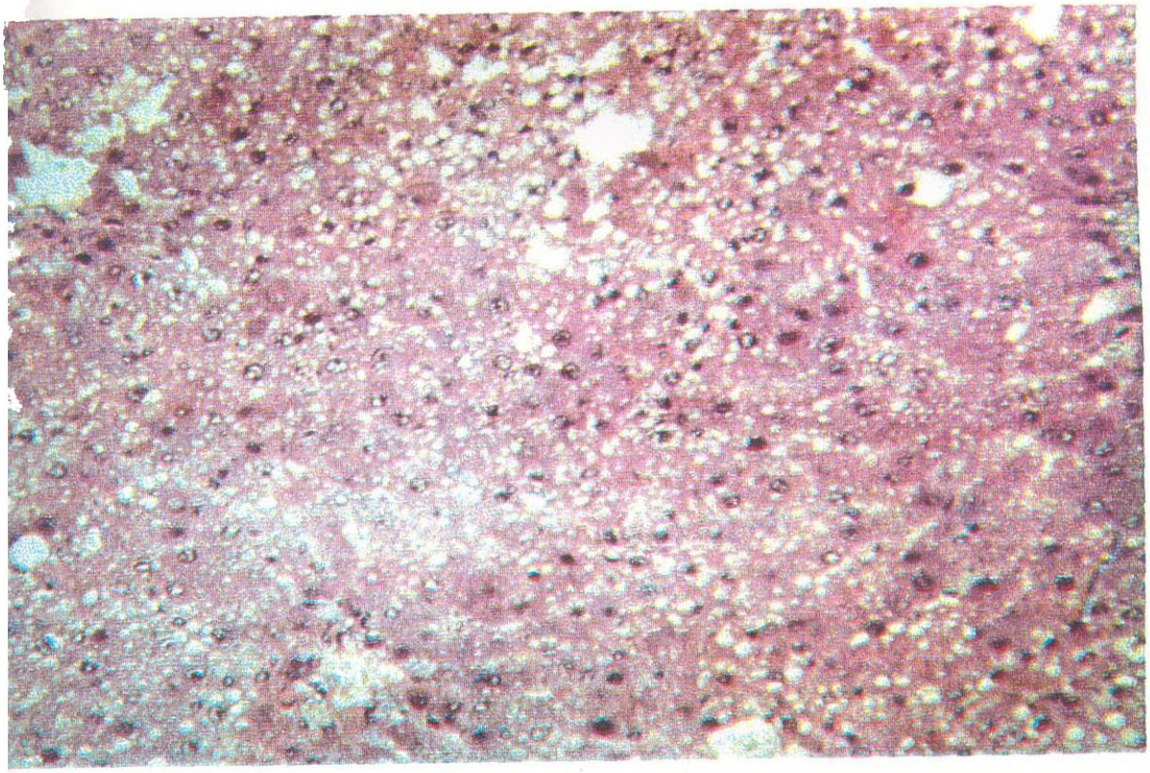


Fig. 9 Photomicrograph of liver of rat given CCl_4 alone (Group IIc) (H&E)

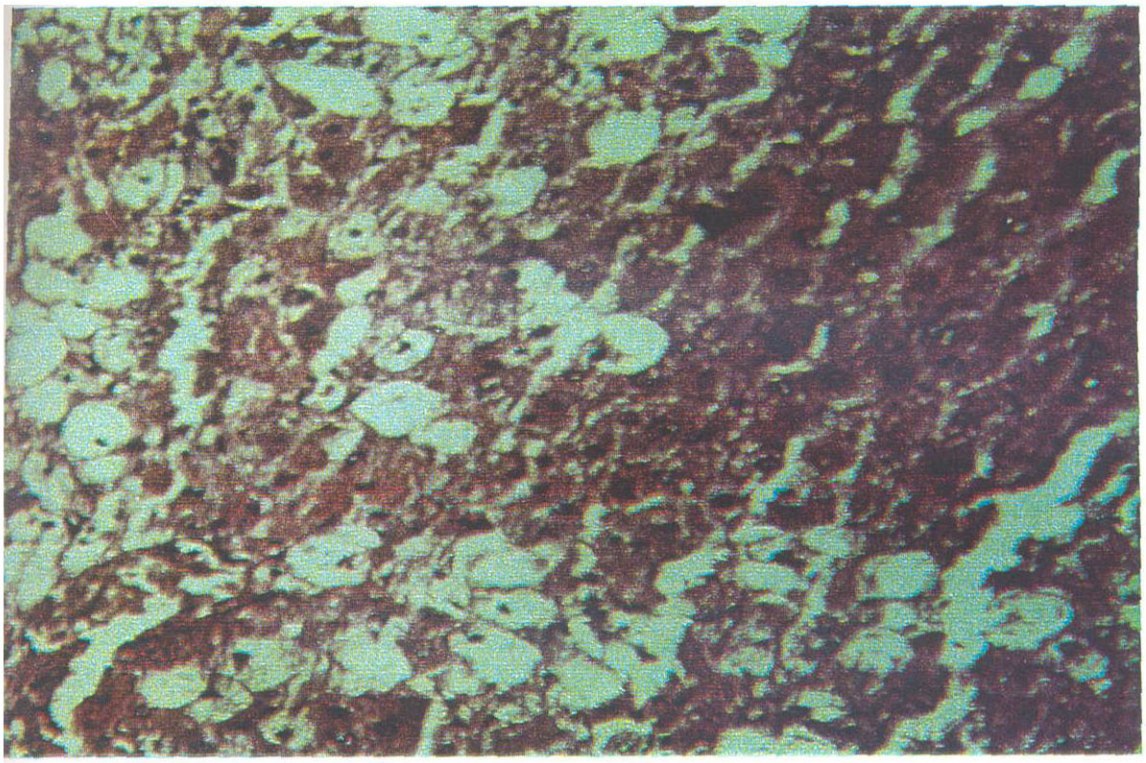


Fig. 10 Photomicrograph of liver of rat given CCl_4 + *B. diffusa* methanolic extract @ 200mg/kg. (Group IIIc) (H&E)

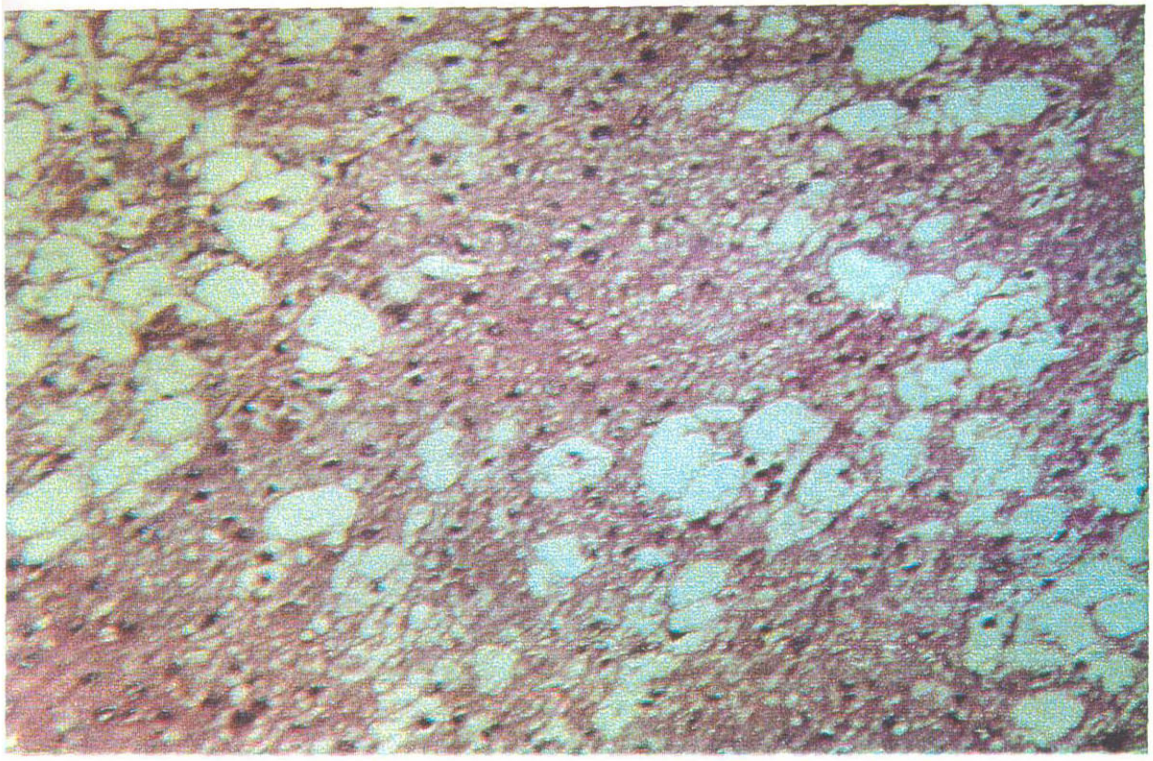


Fig. 11 Photomicrograph of liver of rat given CCl_4 + *B. diffusa* methanolic extract @ 400mg/kg. (Group IVc) (H&E)

4.4. Assessing the immuno modulatory activity of the methanolic extract

4.4.1. Effect on haematological parameters

There were no significant differences between the 3 groups in haemoglobin concentration, RBC count, total and differential WBC counts (Table 6).

4.4.2. Effect on plaque forming cells (PFCs) in the spleen

Administration of the extract at both dose rates produced increase in antibody forming cells in the spleen (as shown by the number of plaque forming cells) when compared with that of the control (Table 7). The number of PFCs started increasing from day 3 and reached a peak level on day 6. The number gradually decreased in the higher dose group while it suddenly returned to near normal values at day 7 for the group given the extract @ 200 mg/kg (Fig.12,13).

4.4.3. Effect on circulating antibody titre

The extract at both dose rates produced increase in circulating antibody titre when compared to that of the control group (Table 8). The peak titre was observed at day 15 for the three groups but it was maintained upto day 20 in the third group ie. the group given the extract @ 400 mg/kg (Fig.14,15).

Table 6: Effect of methanolic extract of *B. diffusa* on haematological parameters

Groups	Hb-g (%)	RBC-(millions /cu.mm.)	WBC – (10^3/cu.mm.)	DC		
				Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)
Control	15.1 ± 1	8.28 ± 0.59	5.076 ± 1.328	61.9 ± 6.4	31.3 ± 6.4	1.3 ± 0.9
Ext. @ 200 mg/kg	11.22 ± 0.41	8.68 ± 1.11	6.480 ± 2423	67.8 ± 1.52	26.4 ± 1.13	4.8 ± 0.82
Ext @ 400 mg/kg	12.52 ± 0.17	8.09 ± 1.64	5.450 ± 987	63 ± 3.32	33 ± 2.52	4 ± 1.16

Table 7: Effect of methanolic extract of *B. diffusa* on plaque – forming cells

Group	No. of PFCs/10^6 spleen cells (days after immunization)				
	3	4	5	6	7
Control	40	112	200	280	96
Ext. @ 200 mg/kg	56	152	192	416	104
Ext. @ 400 mg/kg	32	136	120	304	194

Table 8: Effect of methanolic extract of *B. diffusa* on circulating antibody titre

Group	Antibody titre (days after immunization)				
	5	10	15	20	25
Control	16	128	512	256	256
Ext. @ 200 mg/kg	32	256	1024	512	512
Ext. @ 400 mg/kg	32	512	1024	1024	512

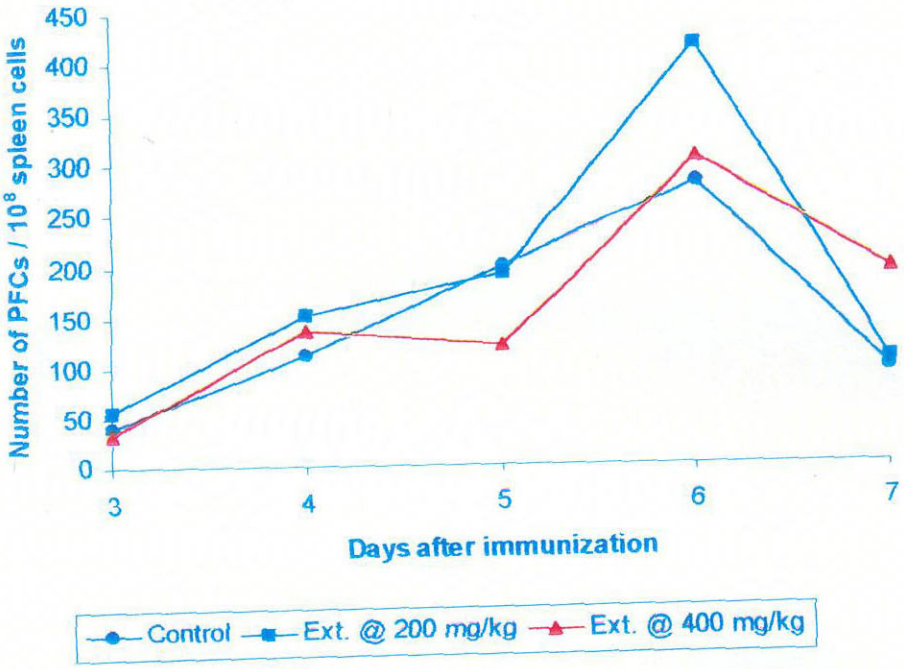


Fig. 12 Effect of methanolic extract of *B. diffusa* on plaque forming cells

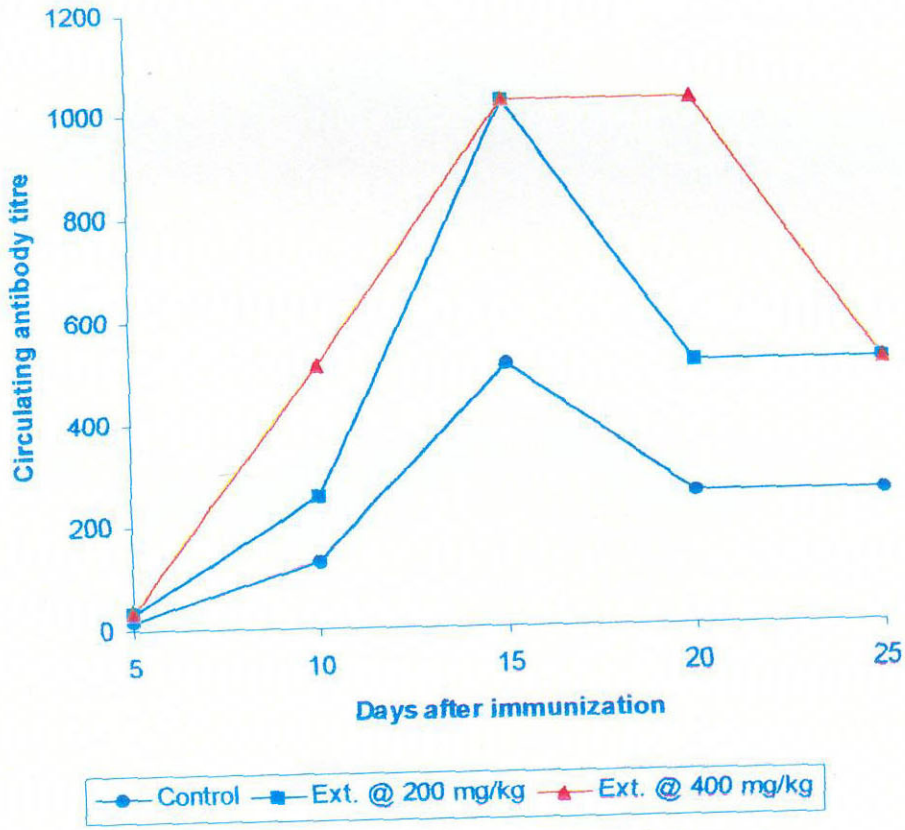


Fig. 14 Effect of methanolic extract of *B. diffusa* on circulating antibody titre

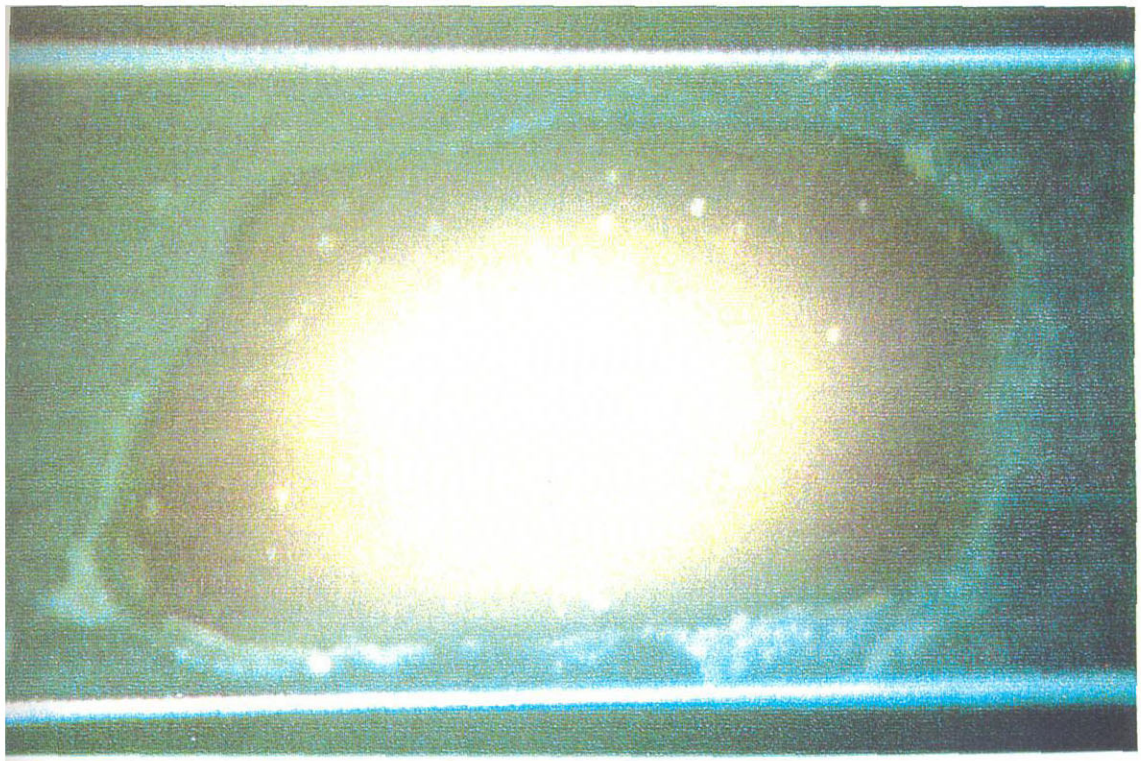


Fig. 13 Plaque formation by spleenocytes of mice immunized with SRBC - modified slide method.

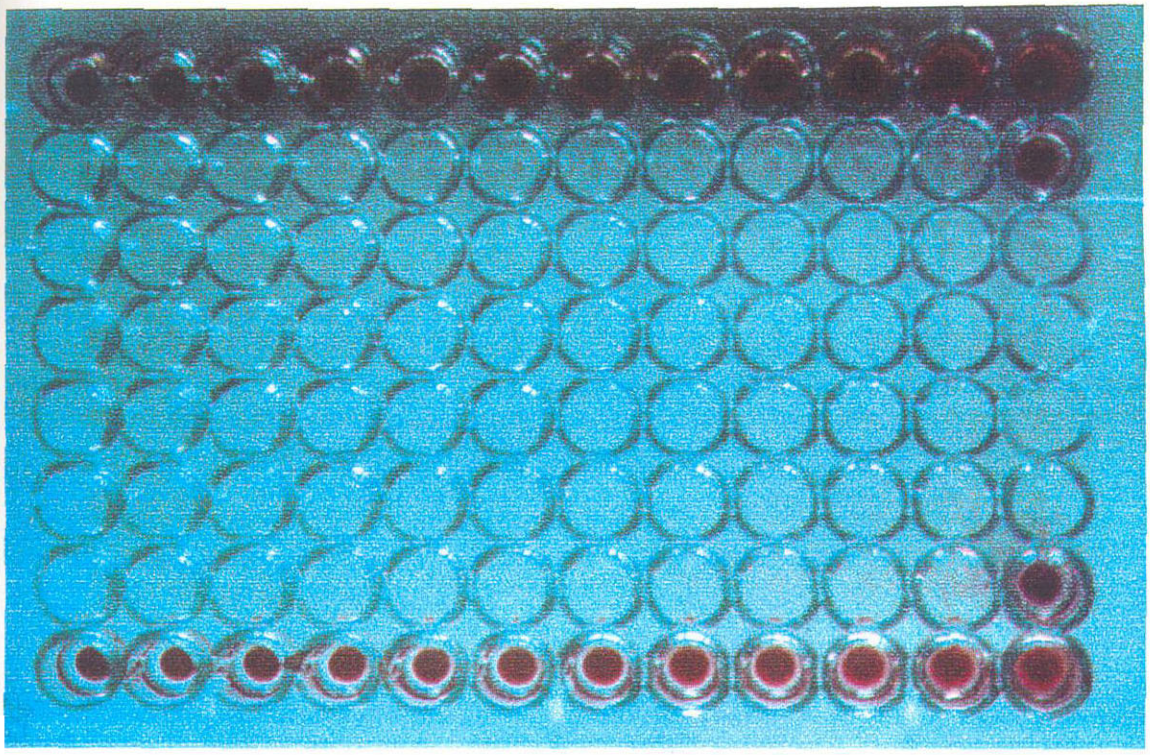


Fig. 15 Hemagglutination of SRBC by sera of mice immunized with SRBC (day 10) (Group I)

Discussion

DISCUSSION

5.1. Anti-inflammatory screening of the plant extracts

5.1.1. Taxonomical identification, collection of plant materials and extraction

The results show the percentage yields of the four extracts namely the methanolic, aqueous, petroleum ether and chloroform extracts. Extraction of the plant by using an organic solvent gave a higher amount of the crude extract compared to aqueous extraction as evident from the high yield of crude extract by methanol (26 per cent) and the low yield by the aqueous extraction (0.2 per cent).

5.1.2. Anti inflammatory screening of the plant extracts by the carrageenan induced rat paw oedema method

The methanolic extract @ 400 mg/kg given orally was found to produce maximum inhibition of paw oedema formation (55 per cent) which was comparable to that produced by the standard drug diclofenac sodium (52.5 per cent). Similarly the increase in paw thickness produced by the same dose of the extract was also significantly lesser when compared with that of other groups (Table 1, Fig. 3). Though the aqueous and petroleum ether extracts also produced some inhibition of paw oedema formation, the mean increase in

paw thickness was not significant, when compared with that of the control group. The chloroform extract was found to have pro-inflammatory activity.

Zachariah *et al.*(1994) reported that lupeol isolated from the PE fraction of ethanol extract of the leaves of *Ixora coccinea* produced percentage inhibition of paw oedema of 80.35 per cent and 86.98 per cent at 100 and 200 mg/kg dose rates respectively, while the standard NSAID indomethacin produced only 71.07 per cent inhibition of paw oedema in carrageenan induced paw oedema model of inflammation in rats. Similarly the PE extract of *Urtica pilulifera* seeds was also found to produce more percentage inhibition of paw oedema than the standard drug indomethacin at one hour and three hours after injection of carrageenan. The per cent inhibition at three hours was 20 per cent for indomethacin and 27 per cent for the seed extract (Kavalali and Tuncel, 1997).

The carrageenan-induced rat paw oedema has been recognized and widely used as a model of acute inflammation in rats. The development of oedema in the paw of rat after injection of carrageenan had been described as a biphasic event (Dirosa *et al.*, 1971). The initial phase (the first hour) had been attributed to the release of histamine and

serotonine, the oedema maintained during the plateaux phase, to kinin-like substances and the second accelerating phase (developing by 3 hours) to the release of prostaglandin-like substances. Hence, in the present study, the significant anti-inflammatory effect of the methanolic extract of the plant could be attributed to its histamine, serotonin, kinins and endoperoxide - inhibitory activity.

The research findings of Rao and Mishra (1997a) have also interpreted the above aspects. They suggested that the significant oedema suppressant activity of the aerial parts of *Sida rhombifolia* L. might be due to the inhibitory action on the release of mediators of inflammation like histamine, serotonin, brady kinin etc.

The anti-inflammatory activity of diclofenac sodium is well documented. It is an inhibitor of cyclooxygenase with a potency substantially greater than that of indomethacin, naproxen and many others. In addition, it decreases the intra-cellular concentration of free arachidonate in the leukocytes, perhaps by altering the release or uptake of the fatty acid (Insel, 1996).

The plant *B. diffusa* has been found to contain a variety of phytochemicals viz. flavones like 5-7 - dihydroxy -3`-4`-dimethoxy-6-8-dimethyl flavone, reducing

sugars, triterpenes like β-sitosterol, alkaloids like punarnavine, tannins, aminoacids like alanine aspartic acid, methionine, threonine and histidine, lignins like liriodendrin etc. (Asolkar *et al.*, 1992). Flavanoids are potent inhibitors of arachidonic acid metabolism and inhibit the synthesis of prostaglandins. They also selectively inhibit 5-lipoxygenase. According to Heinrich *et al.* (1998) flavones are known to function by atleast 4 physiological mechanism. They can bind to enzymes and cell membranes and complex heavy metal ions, participate in the electron transfer of enzyme systems, and exhibit free radical scavenging activity.Similarly, several triterpenes have been reported to have anti-inflammatory activity. β-sitosterol, one among them, has been reported to inhibit carrageenan induced oedema by 54 per cent, when administered @ 320mg/kg p.o., (Duwiejua and Zeithin, 1993). β-sitosterol showed a significant cortisone like action on arachidonic acid release, resulting in a decrease in cycloxygenase and lipooxygenase products. Thus, the anti-inflammatory activity of the methanolic extract could be attributed to its flavones, triterpenes and other chemical constituents.

Dahanukar *et al.* (2000) have demonstrated that all extracts of *Abies pindrow* Royle leaves showed anti-inflammatory effect in various animal models of inflammation like carrageenan-induced paw oedema, granuloma pouch and Freunds' adjuvant arthritis. Chemical analysis indicated the presence of glycosides, steroids, terpenoids and flavanoids. They opined that flavanoids and terpenoids were polar substances effective in acute inflammation while glycosides and steroids were non-polar substances effective in chronic inflammation. Similarly, according to Singh and Pandey (1996) the anti-inflammatory activity of *Pongamia pinnata* seed extract could be attributed to the presence of flavanoids and glycosides which were known to inhibit inflammatory mediators. They showed that the petroleum ether and chloroform extracts of *Pongamia pinnata* seeds had significant acute anti-inflammatory effect while the aqueous extract had pro-inflammatory effect.

Since the methanolic extract showed the maximum inhibitory activity against the carrageenan-induced paw oedema formation, it was chosen for further studies.

5.2. Selective anti-inflammatory studies using the methanolic extract

5.2.1. Effect on cottonpellet induced granuloma formation

The cotton-pellet granuloma inhibition assay introduced by Meier *et al.* (1950) is perhaps the most widely used assay method for assessing the activity of anti-inflammatory drugs on the proliferative component of the inflammatory response.

Swingle (1974) have described three phases of inflammatory response to sub-cutaneous implantation of cotton pellets in rats. The first transudative phase (within first 3 hours) represented the soaking of cotton with a fluid of low protein content. The second exudative phase occurred between 3 and 72 hours after implantation and the 3rd proliferative phase, 72 hours after implantation corresponded to the appearance of collagen in the granuloma.

In the present study, the mean increase in the weight of cotton pellets were 126.00 ± 13.12, 44.857 ± 6.35, 95.375 ± 12.27 and 75.5 ± 3.09 mg for the groups Ia, IIa, IIIa and IVa respectively (Table 2, Fig. 4). The increase in weight of the pellets was found to be significantly lesser for the group IVa when compared with that of the

control. The percentage of anti-inflammatory activity was 64.4 per cent, 24.31 per cent and 43.25 per cent for the groups IIa, IIIa and IVa respectively. Thus the extract at the dose rate of 400 mg/kg was found to possess significant anti-inflammatory activity in the subacute model of inflammation also.

The mean serum acid phosphatase value for the group Ia was 13.61 KA units and for the group IIa was 11.98 KA units. The extract @ 400 mg/kg decreased the value to near normal values. Lysosomal enzymes are important mediators of acute and chronic inflammatory reactions. Acid phosphatase is one among the different lysosomal enzymes used for studying lysosomal membrane stabilization. The release of the lysosomal enzymes into cytoplasm stimulates inflammatory mediators like reactive oxygen radicals, prostaglandins etc. The membrane stabilization of lysosomes is one of the mechanism of anti-inflammatory action of corticoids and some NSAIDs (Bhaskar Rao *et al.*, 1989).

The mean adrenal ascorbic acid content for the groups Ia, IIa, IIIa and IVa were 3.15 ± 0.189, 2.641 ± 0.23, 3.624 ± 0.35 and 3.172 ± 0.31 respectively. These values showed no significant change between the groups Ia, IIIa and IVa, whereas the value for group IIa alone showed a

significant decrease. This showed that there was increase in ACTH (adreno corticotropic hormones) and increased release of adrenal corticoids into the serum which might also contribute to its anti-inflammatory activity, whereas diclofenac sodium had no such effect.

Srivastava and Shrimal (1985), in an attempt to investigate into the effect of curcumin on some biochemical changes produced during subacute inflammation in rats, have found that curcumin was more potent than ibuprofen as a stabilizer of lysosomal membranes. At higher doses, curcumin was also shown to act by stimulation of adrenals resulting in the release of endogenous corticoids.

The serum protein concentration also was significantly decreased in the groups IIa and IVa when compared with that of the control group ie. group Ia. The increase in the serum protein level in the control ie. group Ia could be attributed to the increased release of polypeptides like bradykinin and other kinin like substances during the inflammatory process, which was seen inhibited in the groups IIa and IVa significantly.

The liver protein levels were also found to be lesser in the groups IIa and IVa, but the difference was not significant. There is increased protein synthesis by the

liver during inflammation in group Ia, which was inhibited by the extract at the dose rate of 400 mg/kg and diclofenac sodium, p.o.

Alam *et al.* (1992) have shown that vicolides A, B, C and D, the sesquiterpene lactones from *Vicoa indica* exhibited significant anti-inflammatory activity against cotton-pellet granuloma in rats at the dose rate of 10mg/kg bw. subcutaneously. They were shown to reduce the protein content, acid and alkaline phosphatase and SGOT and SGPT values in liver and serum and ascorbic acid content in adrenals.

5.2.2. Assessing the anti-oxidant activity of the extract *in vitro*

The results showed that the alcoholic extract of *B. diffusa* possessed inhibitory activity against superoxide radicals, lipid peroxide formation and nitric oxide radicals *in vitro* (Table 3a, 3b and 3c; Fig. 5a, 5b and 5c).

Reactive Oxygen Species (ROS) such as oxygen derived free radicals (superoxide and hydroxyl radicals) and high-energy oxidants (peroxy nitrite radicals) have been implicated in the pathophysiology of various states like inflammation, shock and ischemia/reperfusion injury

(Cuzzocrea *et al.*, 2001). The biologically relevant free radicals derived from O_2 are the superoxide anion ($\dot{\overline{O}}_2$), the perhydroxyl radical (protonated superoxide ($H\dot{\overline{O}}_2$), the hydroxyl radical ($H\dot{O}$) and nitric oxide radical ($N\dot{O}$). Simultaneous generation of $N\dot{O}$ and $\dot{\overline{O}}_2$ favours the production of a toxic reaction produced, the peroxy nitrite anion ($ONOO^-$). A potential mechanism of oxidative damage is the nitration of tyrosine residues of proteins, peroxidation of lipids and degradation of DNA and oligo nucleosomal fragments. Antioxidants that act by scavenging the excess free radicals can be helpful in reversing the ROS-induced damages (Hemnani and Parihar, 1998).

Arora *et al.* (2000) have shown that monocytes from patients with rheumatoid arthritis released increased amount of ROS and had high oxidative burst capacity which had an important role in the pathogenesis of the disease. Similarly, according to Hayaishi and Shimuzu (1982), the TBARS (Thiobarbituric acid reacting substances) values representing the lipid peroxide radicals were an indirect indication of prostaglandin endoperoxides and their derivatives in the serum.

From the results of this study, it could be concluded that the *in vitro* antioxidant activity of the alcoholic

extract of *B. diffusa* was contributing to its anti-inflammatory activity also.

A similar work to assess the *in vitro* antioxidant activity of *Emblica officinalis* extract was undertaken by Jose and Kuttan (1995). They showed that the aqueous extract of the plant seeds inhibited the superoxide radicals, hydroxyl radicals acid lipid peroxide radicals in *in vitro* system, thus proving its antioxidant activity, which contributed to many of its medicinal properties.

Similarly, the anti-peroxidative property of *Nardostachys jatamansi* was tested *in vitro* by using iron-induced lipid peroxidation in rat liver homogenate. Both the hexane and alcoholic extracts provided protection against lipid peroxidation (Thripathi *et al.*, 1996).

5.2.3. Serial fractionation and assessing the anti-inflammatory activity of the fractions

Bioactivity guided fractionation is an important step in the standardization of crude drugs and polyherbal formulations (Tylor *et al.*, 1976). It involves pharmacological evaluation followed by separation by means of extraction using solvents of increasing polarity. Each of the successive extracts is then tested for biological activity and the inactive ones are discarded. Active

extracts are further separated into fractions and again tested for activity.

In the present study, the crude methanolic extract could be fractionated into four fractions, each one representing neutral, acidic, phenolic and basic compounds respectively. The first fraction obtained through petroleum ether was mostly composed of neutral compounds. Fraction II was composed of acidic compounds got by precipitation with mineral acid and subsequent chloroform extraction. Phenolic compounds were isolated from the crude extract by precipitation with Na_2 CO_3. The fourth fraction obtained by sodium hydroxide treatment was mainly composed of alkaloids and other basic compounds.

The results of the present study also showed that the neutral, phenolic alkaloidal and strongly basic alkaloidal fractions produced 6.67 per cent, 10 per cent and 23 per cent inhibition respectively of carrageenan-induced paw oedema (Table 4, Fig. 6).

Vohora (1989) had stated that the active principle in the whole plant and roots of *B. diffusa* was an alkaloid 'punarnavine' and that it had anti-inflammatory and diuretic properties. The results of the present study agree with his findings in that the maximum anti-inflammatory activity was

obtained in the alkaloidal fractions, especially the strongly basic alkaloidal fraction, but this requires further indepth study.

5.3. Assessing the hepatoprotective activity of the methanolic extract

The levels of all serum parameters were increased significantly in the toxicant group i.e. group IIc. The levels of ALT, AST and DB showed a significant decrease in the group IVc, when compared with that of group IIc. The levels of AP and TB were also decreased, but it was not significant. Similarly, the levels of all the parameters except AP were decreased in the group IIIc also, when compared with that of the toxicant group, but it was not significant. The level of AP increased to almost 5 times of the normal values in the group IIc and group IIIc animals, which might be explained by the fact that AP being an obstructive enzyme would be released in large amounts from liver following severe hepatocellular damage and would not be eliminated from the blood through the normal biliary route, due to local cholestasis (Mc Neely, M.D.D., 1980).

The hepatotoxic effect of carbon tetrachloride was largely due to its active metabolite, the free radicals. These activated radicals bound covalently to the

macromolecules and induced peroxidative degradation of membrane lipids of endoplasmic reticulum leading to hepatocellular necrosis (Karunakar *et al.*, 1997). They demonstrated that pretreatment with '*jigrine*', a polyherbal formulation decreased the elevated levels of serum ALT, AST, tissue γ-GTP (gamma-glutamyl transpeptidase), triglycerides and lipid peroxide in alcohol and carbontetrachloride treated rats. The results showed that *B. diffusa* had significant hepatoprotective activity which might be due to its membrane stabilizing and antioxidant effect.

Flavanoids in plants have been reported to be potent antioxidants and have free radical scavenging abilities. Many flavanoids have also been found to possess hepatoprotective effect (Rajnarayana *et al.*, 2001). Hence the flavanoids of *B. diffusa* along with other principles may be contributing to the hepatoprotective activity of the plant.

Rao and Mishra (1997a) had studied the effect of different parts of *Sida rhombifolia* on chemical and drug induced hepatotoxicities. The powdered roots, aerial parts and their aqueous extracts showed significant hepatoprotective activity, as shown by a decrease in the

levels of serum ALT, AST, AP, TB and DB and by histopathological findings. They had concluded that the hepatoprotective effect might be due to the stimulating effect on hepatic regeneration or free-radical scavenging effect.

Chandan *et al.* (1991) had reported that the alcoholic extract of *B. diffusa* had significant hepatoprotective and choleretic activity against CCl_4 induced hepatotoxicity in rats and mice. Similarly, Rawat *et al.* (1997) had also reported that the roots of *B. diffusa* possessed significant protective activity against thioacetamide-induced hepatotoxicity in rats. The results of the present study also showed that the alcoholic extract of *B. diffusa* at the dose of 400 mg/kg p.o. possessed significant protective activity against CCl_4 induced hepatotoxicity in rats.

5.4. Assessing the immuno-modulatory activity of the alcoholic extract

The results showed that the extract at both the dose rates tested stimulated the humoral immune response, as shown by a significant increase in antibody forming cells in the spleen and circulating antibody titre against Sheep Red Blood Cells (SRBC) in Balb/c mice. The normal haematological values for rats are a) RBC count - 7-10

millions/cu.mm. b) Hb-11-18 g/dl. C) WBC count - 6-17 thousands/cu.mm. d)Neutrophil - 9-34%. E) lymphocyte 65-85% and f) Eosinophil - 0-6% (Hrapkiewicz *et al.* 1998). Since the mean values for all the groups fall within these ranges, there is no significant change in the hematological parameters.

Praveenkumar *et al.* (1999b) have found that 'rasayana', a herbal drug preparation could enhance humoral immune responses as seen from increased number of antibody forming cells and circulating antibody titre and hence had immuno stimulant properties. Similarly Pushpangadan *et al.* (1988), in an effort to study the ethnopharmacology of *Trichopus zeylanicus* found that the plant extract possessed immuno modulatory activity as shown by an increase in HA titre and antibody forming cells in the spleen against SRBC.

Mugantiwar *et al.* (1997) have earlier reported that the alkaloidal fraction of *B. diffusa* restored the suppressed humoral immune response in stressed rats as observed by an increase in antibody titre following immunization with SRBC in rats subjected to restraint stress. It also significantly reversed the depleted adrenal cortisol level and the elevated plasma cortisol level in stressed rats, thus appearing to have a corticosteroid

sparing effect in experimental stress. The results of the present study were also complementary to these findings supporting the immuno stimulant activity of *B. diffusa* extract.

The following are the conclusions that can be obtained from the whole study.

1. the methanolic extract of *B. diffusa* at the dose of 400 mg/kg is having significant anti-inflammatory activity.

2. the methanolic extract has multiple sites of action accounting to its anti-inflammatory property, which is not surprising, in view of the complex nature of phenomenon of inflammation.

3. the methanolic extract at the dose 400 mg/kg p.o. also has significant hepatoprotective activity.

4. the methanolic extract is having immuno stimulant activity also.

Summary & Conclusion

SUMMARY AND CONCLUSION

Boerrhavia diffusa L. has a long history of use in Ayurvedic medicine in India and has been used for the treatment of many ailments including liver, gall-bladder, renal and urinary disorders. The objectives of the present study were (1) to assess the anti-inflammatory activity of four extracts of *B. diffusa* (2) to find out the possible mechanism of anti-inflammatory activity. (3) to assess the hepatoprotective activity of the methanolic extract and (4) to assess the immunomodulatory activity of the methanolic extract.

The anti-inflammatory activity of the methanolic, aqueous, chloroform and petroleum ether extracts was assessed by the carrageenan induced rat paw oedema method in rats and compared with that of standard drug diclofenac sodium. Eighty rats divided into ten groups of eight each were used for the study. Each extract was given at two dose rates ie. 200 and 400 mg/kg orally. Half an hour after administration of the drug, oedema was induced by injecting 0.05 ml of carrageenan in NSS to the hind paw of rat. The thickness of hind paw was measured immediately after the injection and after 3 hours. The increase in thickness and the percentage inhibition of oedema was calculated.

The control group produced 2.025 ± 0.16 mm increase in paw thickness while diclofenac sodium produced 0.962 ± 0.07 mm increase in paw thickness. The methanolic extract @ 200 mg/kg and 400 mg/kg produced 1.175 ± 0.17 mm and 0.876 ± 0.2 mm increase in paw thickness respectively with the per cent inhibition of oedema calculated as 42 per cent and 55 per cent respectively. The aqueous extract @ 200 mg/kg and 400 mg/kg produced 2.413 ± 0.28 mm and 1.6 ± 0.19 mm increase in paw oedema respectively with the per cent inhibition of oedema being -20 per cent and +20 per cent respectively. The chloroform extracts produced 1.638 ± 0.27 and 2.312 ± 0.21 mm increase in paw oedema at 200 and 400 mg/kg dose rate respectively with the per cent inhibition calculated as 20 and -15 per cent respectively. Similarly, the petroleum ether extract also produced an increase in paw thickness of 2.338 ± 0.28 and 2.337 ± 0.21 mm respectively @ 200 and 400 mg/kg and per cent inhibition of -20 per cent and -10 per cent respectively. Between diclofenac sodium and the methanolic extract @ 400 mg/kg, there was no significant difference. Since the methanolic extract produced maximum anti-inflammatory activity, it was selected for further studies.

To assess the effect of the methanolic extract on cotton-pellet induced granuloma formation, forty rats were divided into five groups of eight each. All the rats were implanted with sterile cotton pellets subcutaneously. The first group served as the control and was given only the vehicle. The second group was given diclofenac sodium @ 3 mg/kg orally. The third and fourth groups received the extract @ 200 and 400 mg/kg daily orally. On the eight day, the difference in weight of the pellets was noted. The serum and 1% liver homogenate collected on the 8^{th} day were used for the estimation of acid phosphatase, serum and liver protein and adrenal ascorbic acid.

The extract @ 200 mg/kg and 400 mg/kg produced 95.375 ± 12.27 mg and 75.5 ± 3.09 mg increase in weight of the pellets respectively. Their per cent anti-inflammatory activity was calculated as 24.31 and 43.25 per cent respectively. Diclofenac sodium produced 64.4 per cent anti-inflammatory activity with the mean increase in the weight of the cotton pellet being 44.857 ± 6.35 mg. Thus the extract had lesser activity than diclofenac sodium though it produced dose-dependant anti-inflammatory activity. The serum acid phosphatase value for the first group was 13.61 ± 3.55 KA units and for the second group

was 11.98 ± 1.93 KA units. The extract @ 200 mg/kg and 400 mg/kg showed mean acid phosphatase values of 16.16 ± 5.25 and 11.23 ± 7.26 KA units respectively. Thus the decrease in serum acid phosphatase value for the extract @ 400 mg/kg was found to be similar to that of diclofenac sodium.

The adrenal ascorbic acid values for the groups Ia, IIa, IIIa and IVa were 3.15 ± 0.189, 2.641 ± 0.23, 3.624 ± 0.35 and 3.172 ± 0.31 mg/g respectively. These values showed no significant change between the groups Ia, IIIa and IVa, whereas the value for group IIa alone showed significant decrease. The mean serum protein levels for the group Ia, IIa, IIIa and IVa were 8.391 ± 0.52, 6.061 ± 0.45, 5.399 ± 0.43 and 5.485 ± 0.43 respectively. These values for groups IIa, IIIa and IVa were found be significantly different from that of group Ia. The values for liver protein concentration for the 4 groups were 0.204 ± 0.06, 0.201 ± 0.01, 0.212 ± 0.03 and 0.150 ± 0.04 g/g respectively. These values were found to be lesser for the groups IIa and IVa, when compared with that of the control, but not significant. These results show that the extract @ 400 mg/kg p.o. has significant anti-inflammatory activity in the subacute model of inflammation also.

To assess the antioxidant activity of the extract *in vitro*, the effect on inhibition of superoxide radical formation, lipid peroxide formation and nitric oxide radical formation were assessed. The results showed that the extract possessed significant inhibitory activity against all these radicals *in vitro*. The amount needed for 50 per cent inhibition (IC 50 value) was 400 mg/ml for the superoxide radicals, 200 mg/ml for the lipid peroxide radicals and 8 mg/ml for the nitric oxide radicals *in vitro*.

Serial fractionation of the methanolic extract using solvents of varying polarity yielded neutral, acidic, phenolic alkaloidal and strongly basic alkaloidal fractions, which were then tested for anti-inflammatory activity by the carrageenan induced paw oedema method in rats. The neutral, phenolic alkaloidal and the strongly basic alkaloidal fractions produced 6.67, 10 and 23 per cent inhibition, while diclofenac sodium produced 60.4 per cent inhibition.

In order to assess the hepatoprotective activity of the methanolic extract against CCl_4 induced hepatotoxicity , thirty two rats divided into four groups of eight each were used. The first group was kept as the control and was

given no drug. The group IIc was the toxicant group and was given CCl_4 dissolved in olive oil @ 2.5 ml/kg bw p.o. The groups IIIc and IVc were administered the extract @ 200 and 400 mg/kg bw orally along with CCl_4. The extract was given later at 12 h. intervals. Thirty six hours after CCl_4 administration, the rats were sacrificed and the liver and blood were collected. The levels of ALT, AST, AP, TB and DB in the serum were estimated using autoanalyser kits (Merck). The liver samples were used for histopathological study.

The levels of all the serum parameters were increased significantly in the group IIc. The levels of AST, ALT and DB showed significant decrease in the group IVc. The levels of AP and TB were also decreased, but it was not significant. Similarly, the levels of all the parameters except AP were decreased in the group IIIc also, but it was not significant. These results show that the methanolic extract @ 400 mg/kg has significant hepatoprotective activity against CCl_4 induced hepatotoxicity in rats. The histopathological findings were also confirmatory to this.

The immunomodulatory activity of the methanolic extract was also carried out, by assessing the effect on hematological parameters, circulating antibody titre and

plaque forming cells in the spleen of Balb/c mice. The results showed that the extract @ 200 and 400 mg/kg stimulated the humoral immune response, as shown by a significant increase in antibody forming cells in the spleen and circulating antibody titre against sheep red blood cells, though there was no significant change in the hematological parameters. Thus the results of this study suggested that the methanolic extract @ 400 mg/kg has significant anti-inflammatory activity with multiple facets of action, hepatoprotective activity and immuno stimulant activity and may be used as an additive drug to the existing Ayurvedic drugs armamentarium.

References

REFERENCES

Abraham, K.M. (1975). Oushadha sasyangal 1st Edn. Publ. By State Institute of Languages, Kerala, TVM. pp.92-93.

Ahmad, A., Pillai, K.K., Ahmed, S.J., Balani, D.K. Najmi, A.K., Marwah, R. and Hameed, A. (1999). Evaluation of the hepatoprotective potential of *jigrine* pretreatment on thioacetamide induced liver damage in rats. *Indian J. Pharmacol.* **31** (6): 416-421.

Ahumada, C., Saeuz, T., Garcia, D., RocioDeLa Peurta, Fernandez, A. and Martinez, E. (1997). The effects of a Triterpene fraction isolated from *Crataegus monogyna* Jacq. on Different Acute Inflammation. Models in Rats and Mice: Leukocyte Migration and Phospholipase A_2 inhibition. *J. Pharm. Pharmacol.* **49**: 329-331.

Alam, M., Susan, T., Joy, S. and Kundu, A.B. (1992). Anti-inflammatory and antipyretic activity of vicolides of *Vicoa indica* DC. *Indian. J. Expt. Biol.* **30** (1): 38-41.

Anto, R.J., Kuttan, G., Dinesh Babu, K.V., Rajasekharan, K.N. and Kuttan, R. (1998). Anti-inflammatory activity of natural and synthetic curcuminodis. *Pharm. Pharmacol. Commun.* **4**: 103-106.

Antony, S., Kuttan, R. and Kuttan, G. (1999). Immuno-modulatory activity of curcumin. *Immunol. Investigations.* **28** (5&6), 291-303.

Arora, M., Arora, R.,Ashokkumar, Das, N. and Srivastava, L.M. (2000). Monocytes from patients with rheumatoid arthritis release increased amount of reactive oxygen intermediates. *Curr. Sci.* **78** (8): 989-991.

Arora, R.B., Kapoor, V. Gupta, S.K. and Sharma, R.C. (1971). Effect of *Commiphora mukul* on carrageenan induced paw oedema method. *Indian J. Exp. Biol.* 9 (4): 403-407.

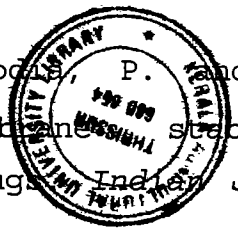
Asmawi, M.Z., Kankaanranta, H., Moilanen, E. and Vapaatalo, H. (1993). Anti-inflammatory activities of *Emblica officinalis* Gaertn. leaf extracts. *J. Pharm. Pharmacol.* **45**: 581-584.

Asolkar, L.V., Kakkar, K.K. and Chakre, O.J. (1992) in Glossary of Indian Medicinal Plants with Active Principles (Part I A-K) 1st Edn. National Institute of Science Communication (CSIR), New Delhi pp. 131-132.

Awasthi, L.P. and Menzel, G. (1986). Effect of root extract from *Boerrhavia diffusa* L., containing an antiviral principle upon plaque formation of RNA bacterio phages. *Zentralbl. Microbiol.* **141** (5): 415-419.

Barthwal, M. and Srivastava, K. (1990). Histologic studies on endometrium of menstruating monkeys wearing IUDS: Comparative evaluation of drugs. *Adv. Contracept.* 6 (2): 113-124.

Barthwal, M. and Srivastava, K. (1991). Management of IUD associated menorrhagia in female rhesus monkeys (*Macaca mullata*). *Adv. Contracept.* 7 (1): 67-76.



Bhaskar Rao, A., Sisodia, P. and Sattur, P.B. (1989). Lysosomal membrane stabilization by anti-inflammatory drugs. *Indian J. Expt. Biol.* **27** (12): 1097-1098.

Bhattacharya, S.K. Satyam, K.S. and Ghosal, S. (1997). Anxiolytic activity of glycowithanolides from *Withania somnifera*. *Indian J. Expt. Biol.* **35**:236-239.

Chandan, B.K., Sharma, A.K. and Anand, K.K. (1991). *Boerhaavia diffusa*: a study of its hepatoprotective activity. *J. Ethnopharmacol.* **30** (3): 299-307.

Charaka (1949) Charaka samhita (Vol V). Edited & published by Shree Gulabkunverba Ayurvedic Society, Jamnagar. p. 495, 950, 951, 955.

Chatterjee, S. (1996). Antiperoxide and radical scavenging action of IH/AO-3: A polyherbal formulation. *Indian J. Indigenous Medicines*. **18** (1): 11-20.

Chatterjee, S. and Das, S.N. (1999). Uptake of bromsulphalein by liver slices of rats treated with carbon tetrachloride and a herbal formulation. *Indian drugs* **36** (2): 140-141.

Chatterjee, S., Telang, R.S. and Varshneya, C. (2001). Antioxidative effect of *Terminalia arjuna*: Chemical and Pharmacological studies. *J. Vet. Pharmacol. Toxicol.* **1** (1&2): 45-50.

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapur, L.D. (1958) Chopras' Indigenous drugs of India. 2nd Edn, U.N. Dhur & Sons Pvt. Ltd, Calcutta. p. 297-300.

Chopra, R.N., Nair, S.L. and Chopra, I.C. (1956). Glossary of Indian Medicinal Plants Publ. By CSIR, New Delhi. pp. 39.

Chude, M.A., Orisakwe, O.E., Afonne, O.J., Gamaniel, K.S. Vongtau, O.H. and Obi, E. (2001). Hypoglycaemic effect of the aqueous extract of *Boerhaavia diffusa* leaves. *Indian J. Pharmacol.* **33**: 215-216.

Cuzzocrea, S., Reley, D.P., Caputic, A.P. and Salvemini, D. (2001). Antioxidant therapy: A new pharmacological Approach in Shock, Inflammation and Ischemia/reperfusion injury. *Pharmacol. Rev.* **53**: 135-159.

Dahanukar, S.A., Kulkarni, R.A. and Rege, N.N. (2000). Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* **32**: s81-s118.

Darshan, S. and Doreswamy, R. (1998). Medicinal plant patents scenario in the new era of drug development. *Indian Drugs.* **35** (2): 55-66.

Devipriya, S. and Shyamala Devi, C.S. (1999). Protective effect of quercetin in cisplatin induced cell injury in the rat kidney. *Indian J. Pharmacol.* **31** (6): 422-426.

Dhuley, J.N., Raman, P.H., Majundar, A.M. and Naik, S.R. (1993). Inhibition of lipid peroxidation by piperine during experimental inflammation in rats. *Indian J. Expt. Biol.* **31** (5): 443-445.

Dirosa, M., Giroud, J.P. and Willoughby, D.A. (1971). Studies on the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. Pathology*. **104** (4): 1529.

Dua, P.R., Shanker, G., Srimal, R.C., Saxena, K.C., Saxena, R.P., Puri, A. and Dhawan, B.N. (1989). Adaptogenic activity of Indian *Panax pseudoginseng Indian J. Expt. Biol*. **27** (7): 631-634.

Duwiejua, M. and Zeithin, I.J. (1993). Drugs from Natural products - Pharmaceuticals and Agro chemicals. Ellis Horwood, New York. pp. 152-167.

Elias, G. and Rao, M.N.A (1988). Inhibition of Albumin Denaturation and Anti-inflammatory activity of Dehydrozingerone and its analogs *Indian J. Expt. Biol*. **26** (7): 540-542.

Geetha, K. and Sangeetha, G. (2000). A study on *Boerhaavia diffusa* Linn with special reference to immuno-modulatory effect. Proceedings of 12th Kerala Science Congress, Kumily pp. 321-326.

Goel, R.K., Pandey, V.B., Dwivedi, S.P.D. and Rao, Y.V. (1988). Anti-inflammatory and antiulcer effects of kaempferol, a flavone, isolated from *Rhamnus procumbens*. *Indian J. Expt. Biol*. **26**: 121-124.

Gomes, A., Sharma, A.M. and Ghatak, B.J.R. (1989). Comparative study of known non-steroidal anti-inflammatory drugs with glycosidal fraction isolated from *Maesa chisia* D. don var. *augustifolia* Hook F and Thorn, *Indian J. Expt. Biol*. **27** (10): 874-876.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite and (15N) nitrate in biological fluids. *Anal. Biochem.* **126**: p. 131.

Gujral, M., Pal, V.S. and Saxena, P.W. (1961). Preliminary studies on glycyrrhizin - active principle from the plant Glycyrhiza glabra. *Indian J. Med. Res.* **44** (3): 657.

Hayaishi, O. and Shimizu, T. (1982). Metabolic and functional significance of prostaglandins in lipid peroxide research. Lipid Peroxides in Biology and medicine. 1st Edn. Academic Press, Inc. pp. 41-53.

Heinrich, M., Robles, M., West, J.E., *Ortiz de Montellano*, B.R. and Rodriguez, E. (1998). Ethnopharmacology of Mexican Asteraceae (Compositae). *Ann. Rev. Pharmacol. Toxicol.* **38:** 539-565.

Hemnani, T. and Parihar, M.S. (1998). Reactive oxygen species and oxidative DNA damage *Indian J. Physiol. Pharmacol.* **42** (4): 440-452.

Hiruma-Lima, C.A., Gracioso, J.S., Bighetti, E.J., Germonsen, R.L. and Souza, B.A.R. (2000). The juice of fresh leaves of *Boerrhavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. *J. Ethnopharmacol.* **71** (1-2): 267-274.

Hrapkiewicz, K., Medina, L. and Holmes, D.D. (1998). Clinical Laboratory Animal Medicine-an introduction. 2nd Edn. Iowa State University Press, lowa.

Insel, P.A. (1996). Goodman & Gilman's The Pharmacological Basis of Therapeutics. 9th Edn. Edited by Hardman J.G. and Limbird, L.K. The Mc graw Hill Co. NewYork. p-637.

Jain, P., Khanna, N.K., Trehan, N., Pendse, V.K. and Godhwani, J.L. (1994). Anti-inflammatory effects of an Ayurvedic preparation, *Brahmi Rasayan*, in rodents. *Indian J. Expt. Biol.* **32**: 633-636.

Jerne, N.K. and Nordins, A.A. (1963). Plaque formation in agar by single antibody producing cells. *Science* (140): 405-408.

Jose, J.K. and Kuttan, R. (1995). Inhibition of Oxygen free radicals by *Emblica officinalis* extract and chyavanaprasha. *Amala Cancer Research Bulletin*. **15:** pp. 46-52.

Joshi, D.D., Majumdar, A.M. and Narayanan, C.R. (1984). Anti - inflammatory activity of *Bougainvilla spectabilis* leaves. *Indian J. Pharm. Sci.* **69** (7): 187-188.

Joy, K.L. and Kuttan, R. (1996). Protective effect of Lycovin and *Picrorhiza kurroa* extract against acute as well as chronic hepatotoxicity induced by CCl_4 in rats. *Amala Cancer Research Bulletin* 16: pp. 67-72.

Joy, K.L. and Kuttan, R. (1999). Inhibition by *Picrorhiza kurroa* extract of oxygen free radical reactions and hepatic fibrosis in rats. *J. Clin. Biochem. Nutr.* **27**: 9-17.

Karunakar, N., Pillai, K.K., Hussain, S.Z., Rao, M. and Balani, D.K. (1997). Further studies on the antihepatotoxic activity of *jigrine*. *Indian. J. Pharmacol* **29**: 222-227.

Kavalali, G. and Tuncel, H. (1997). Anti-inflammatory activities of *Urtica pilulifera*. *J. Ethnopharmacol*. **35** (2): 138-140.

Kavimani, S., Karpagam, R.I. and Jaykar, B. (1996). Anti-inflammatory activity of volatile oil of *Psidium guajava Indian J. Pharm. Sci*. **59** (3): 142-144.

Khobragade, V.R. and Jangde, C.R. (1996). Anti-inflammatory activity of bulb of *Allium sativum* Linn. *Indian Vet. J*. **73**:349-351.

Kirtikar, K.R. and Basu, B.D. (1975). Indian Medicinal Plants. 2nd Edn. (Vol. III) Bishan Singh, New connaught Place, Dehradun. pp. 2045-2047.

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

Kuttan, G. and Kuttan, R. (1992). Immuno-modulatory activity of a peptide isolated from *Viscum album* extract (NSC 635089). *Immunol. Investigations*. **21** (4): 285-296.

Lami, N., Kadota, S., Kikuchi, T. and Momose, Y. (1991). Constituents of the roots of *Boerhaavia diffusa* L. Identification of Ca^2 channel antagonistic compound from the methanol extract. *Chem. Pharm. Bull.* **39** (6): 1551-1555.

Latha, P.G., Suja, S.R. and Rajasekharan, S. (2001). Hepatoprotective effects of *Ixora coccinea* flowers. 4-11. Proceedings of 13th Kerala Science Congress, Thrissur pp.245-246.

Lefkovits, I. And Cosenza, H. (1979). Assay for plaque forming cells. Immunological Methods. Ist Edn, Academic Press, New York pp. 277-285.

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

Majumdar, A.M., Naik, D.G., Dandge C.N. and Puntambekar, H.M. (2000). Anti-inflammatory activity of *Curcuma amada* Roxb. in albino rats. *Indian J. Pharmacol.* **32** (6): 375-377.

Makwana, H.G., Ravisankar, B. and Bhatt, V.S. (1994). General pharmacology of *Vitex leukoxylon* leaves. *Indian J. Physiol. Pharmacol.* **38** (2): 95-100.

Mathew, M. and Gopi, T.V. (2000). Hepatoprotective and Haemopoeitic activities of leaves of *Moringa pterygosperma* Gaertn. Proceedings of 12th Kerala Science Congress, January 2000, pp. 305-307.

McCord, J.M. and Fridovich, I. (1969). Superoxide dismutase: An enzymatic function of erythrocuprein. *J. Biol Chem.* **244**: 6049-6055.

McNeely, M.D.D. (1980). Gradwohls' Clinical Laboratory Methods and Diagnosis. Edited by Sonnenwirth, A.C. and Jarett, L. 8th Edn, The CV Mosby Co. St Louis. pp. 544-545.

Mehrotra, N.N. (1983). A hand book of practical immunology Edited by Talwar, G.P., 1st Edn. Vikas Publishing House Pvt, Ltd, N. Delhi pp.302-306.

Meier, R. Schuler, W. and Dasaulles, P. (1950). The mechanism of inhibition of connective tissue growth by cortisone. *Experimentia* **6** (12): 469.

Mugantiwar, A.A., Nair, A.M., Shinde, U.A. and Saraf, M.N. (1997). Effect of stress on plasma and adrenal cortisol level and immune responsiveness in rats - Modulation by alkaloidal fraction of *Boerhaavia diffusa*. *Fitoterapia* **68**(6): 498-500.

Mugantiwar, A.A., Nair, A.M., Shinde, V.A., Dikshit, V.J., Saraf, M.N., Thakur, V.S. and Sainis, K.B. (1999). Studies on the immuno modulatory effect of *Boerhaavia diffusa* alkaloidal fraction. *J. ethnopharmacol*. **65** (2): 125-131.

Nadig, S.S. and Rao, S.G. (1999). Effect of Hepatogard - an indigenous formulation on dexamethasone induced anti-healing effects in male albino rats. *Indian J. Physiol Pharmacol*. **43** (2): 230-234.

Nadkarni, A.K. (1976). *Indian Materia Medica* 3rd Edn. (Vol. II) Popular Book Depot, Bombay. pp.203-207.

Nelson, D.A. and Davey, F.R. (1992) *Hematology,* Edited by Bentler, E. Lichtman, M.A; Coller, B.S. and Kims, Mcgraw Hill, New York L-70.

Nino, H.V. and Prasad, A.S. (1980). Gradwohl's Clinical Laboratory Methods and Diagnosis. Edited by Sonnenwirth, A.C. and Jarett, L 8th Edn, The C.V. Mosby Company, St. Louis. pp. 370-372.

Okhawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351-358.

Pandey, S., Sharma, M., Chaturvedi, P. & Thripathi, Y.B. (1994). Protective effect of *Rubia cordifolia* on lipid peroxide formation in isolated rat liver homogenate. *Indian J. Expt. Biol.* **32** (3): 180-183.

Parmar, N.S. and Ghosh, M.N. (1978). Anti-inflammatory activity of Gossypin - a bioflavanoid isolated from *Hibiscus vitifolius* Linn. *Indian J. Pharmacol.* **16** (4): 277-293.

Patil, S., Kanase, A. and Varute, A.T. (1993). Effect of hepatoprotective Ayurvedic drugs on lipolytic activities during CCl_4 induced acute hepatic injury in albino rats. *Indian J. Expt. Biol.* **31** (3): 265-269.

Perumal Samy, R., Ignacimuthu, S. and Pastric Raja, D. (1999). Preliminary screening of ethnomedicinal plants from India. *J. Ethnopharmacol.* **66** (2): 235-240.

Praveenkumar, V., Kuttan, R. and Kuttan, G. (1999a). Effect of 'Rasayanas' a herbal drug preparation on cell mediated immune responses in tumour bearing mice. *Indian J. Expt. Biol.* **37** (1): 23-26.

Praveenkumar, V., Kuttan, R. and Kuttan, G. (1999b). Effect of `Rasayanas', a herbal drug preparation on immune response and its significance in cancer treatment. *Indian J. Expt. Biol.* **37** (1): 27-31.

Priyavrat Sharma (1956). Dravyaguna vignana Vol. II (Vegetable drugs) 1st Edn. Chaukhambha Bharathi Academy, Varanasi pp. 630-633.

Pushpangadan, P., Sharma, A.K. and Rajasekharan, S. (1988). Ethnopharmacology of *Trichopus Zeylanicus*. Glimpses of Indian Ethnopharmacology. *Publ.* By Tropical Botanical Garden and Research Institute. pp. 137-146

Rajnarayana, K., Reddy, M.S. and Chaluvadi, M.R. (2001). Bioflavanoids - classification, pharmacological, biochemical effects and therapeutic potential. *Indian J. Pharmacol.* **33** (1): 2-16.

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

Rao, K.S. and Mishra, S.H. (1997b). Hepatoprotective activity of the whole plant of *Fumaria indica*. *Indian J. Pharm. Sci.* **59** (4): 165-170.

Rawat, A.K., Mehrotra, S., Tripathi, S.C. and Shome, U. (1997). Hepatoprotective activity of *Boerhaavia diffusa* L. roots - a popular Indian ethnomedicine. *J. ethnopharmacol.* **56** : 61-66.

Ray. A. Banerji, B.D., Koner, B.C. and Sen, P. (1997). Influence of *Azadiracta indica* (Neem) extract on the immunotoxicity of stress and xenobiotics in experimental animals. *Indian J. pharmacol.* **29**: 38.

Saeed, S.A., Simjee, R.U., Mahmood, F. and Rahman, N.N. (1993). Dual inhibition of Platelet Activating Factor and Arachidonic acid metabolism by ajmaline and effect on carrageenan induced rat paw oedema. *J. Pharm. Pharmacol.* **45**: 715-719.

Saraf, S., Dixit, V.K., Patnaik, G.K. and Thripathi, S.C. (1996). Antihepatotoxic activity of *Euphorbia antisyphilitica. Indian J. Pharm. Sci.* **58** (4): 137-141.

Schalm, O.W., Jain, N.C. and Carrol, E.J. (1975). Veterinary Hematology, 3rd Edn. Lea & Febiger, Philadelphia p. 54.

Srivastava, R., Shukla, Y.N. and Sushilkumar (1998). Chemistry, pharmacology and botany of Boerhaavia diffusa - A review. *J. Med. and Aromatic Plant Sci.* **20** (3): 762-767.

Singh, A., Singh, R.G., Singh, R.H., Mishra, N. and Singh, N. (1991). An experimental evaluation of possible teratogenic potential of *Boerhaavia diffusa* in albino rats. *Planta Med.* **57** (4): 315-316.

Singh, R.K. and Pandey, B.L. (1996). Antiinflammatory of seed extracts of *Pongamia pinnata* in rat. *Indian J. Physiol. Pharmacol.* **40**: 355-358.

Singh, R.K., Gopal Nath, Goel, R.K. and Bhattacharya, S.K. (1998). Pharmacological actions of *Abies pindrow* Royle leaf. *Indian J. Expt. Biol.* **36**: 187-191.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods 6th Edn. Oxford and IBH Publishing Company, Calcutta. p. 59.

Sohni, Y.R. and Bhatt, R.M. (1996). Activity of a crude extract formulation in experimental hepatic amoebiasis and in immunomodulation studies. *J. Ethnopharmacol.* **54** (2) : 119-124.

Sohni, Y.R., Kaimal, P. and Bhatt, R.M. (1995). The antiamoebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica in vitro* and *in vivo*. *J. Ethnopharmacol.* **45** (1): 43-52.

Somkuwar, A.P. and Pawar, S.D. (2000). Hepatoprotective effect of *Emblica officinalis* in rats and cattle. Compendium of Abstract of I st annual conference of Indian Society of Veterinary Pharmacology and Toxicology. P.31.

Soni, K.B., Rajan, A. and Kuttan, R. (1992). Reversal of aflatoxin induced liver damage by turmeric and curcumin. *Cancer letters.* **66**: 115-121.

Soudamini, K.K., Unnikrishnan, M.C., Soni, K.B. and Kuttan, R. (1992). Inhibition of lipid peroxidation and cholesterol levels in mice by curcumin. *Indian J. Physiol. Pharmacol.* **36** (4): 239-243.

Srivastava, R. and Srimal, R.C. (1985). Modification of certain inflammation-induced biochemical changes by curcumin. *Indian J. Med. Res.* **81**: 215-223.

Srivastava, R., Shukla, Y.N. and Sushil kumar (1998). Chemistry, Pharmacology and Botany of *Boerhaavia diffusa*-a review. *J. Med. Aromatic Plant Sci.* **20** (3): 762-767.

Subramoniam, A. and Pushpangadan R. (1999). Development of phytomedicines for liver disease *Indian J. Pharmacol.* **31** (3): 166-175.

Suja, S.R., Latha, P.G. and Rajasekharan, S. (2001). Hepatoprotective properties of *Spilanthes ciliata* H.B.K. 4-16. Proceedings of 13th Kerala Science Congress, Thrissur pp. 258-260.

Suleyman, H., Buyukokuroglu, M.E. Koruk, M., Akcay, F., Kiziltune, A. and Gepdiremen, A. (2001). The effects of *Hippophae rhamnoides* L. extract on ethanol-induced gastric lesion and gastric tissue glutathione level in rats- A comparative study with melatonin and omeprazole. *Indian J. Pharmacol.* **33** (2): 72-76.

Swingle, K.F. (1974). Anti-inflammatory agents - chemistry and pharmacology (Vol. II). Edited by Scherrer, R.A. and Whitehouse, M.W. 1st Edn. Academic Press New York. Pp. 3-77.

Tandan, S.K., Chandra, S., Gupta, S. and Lal, J. (1997). Analgesic and anti-inflammatory effects of *Hedychium spicatum*. *Indian J. Pharm. Sci.* **59** : 148-150.

Tandan, S.K., Chandra, S., Thripathi, H.C. and Lal, J. (1994). Pharmacological effects of *Agaratum conyzoides* roots. *Indian J. Pharm. Sci.* **54** : 162-163.

Thripathi, Y.B., Chaurasia, S., Thripathi, E., Upadhyay, A. and Dubey, G.P. (1996). *Bacopa monnieri* L. as an anti-oxidant - mechanism of action. *Indian J. Expt. Biol.* **34**: 523-526.

Thripathi, Y.B., Thripathi, E. and Upadhyay, A. (1996). Antilipid peroxidation property of *Nardostachys jatamansi*. *Indian J. Expt. Biol.* **34**: 1150-1151.

Tylor, V.E., Brady, L.R. and Robbers, J.E. (1976). Pharmacognosy 7th Edn. Lea & Febiger, Philadelphia. pp. 21-23.

Udupa, V., Kulkarni, K.S. Rafiq. M., Gopumadhavan, S., Vengataranganna, M.V. and Mitra, S.K. (2000). Effect of HD-03 on levels of various enzymes in paracetamol induced liver damage in rats. *Indian J. Pharmacol.* **32** (6): 361-364.

Varghese, E.S.V.D., Hembrom, P.P. and Melookunnel, S.S.J. (1998). An ethno-medical alternative in treatment of Kala-azar. *Ethnobotany* **10**: 50-55.

Vohora, S.B. (1989). Research on Medicinal Plants in India. A review on reviews. *Indian Drugs* **26** (10): 526-532.

Winter, C.A., Risley, E.A. and Nuss, G.W. (1962). Carrageenan induced oedema in the hindpaw of rat as an assay for anti-inflammatory drugs. *Biol. Proc. Soc. Expt. Biol.* **111** (2): 544.

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

PHARMACOLOGICAL EFFECTS OF *Boerrhavia diffusa* L. IN LABORATORY RODENTS

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

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2002

ABSTRACT

Boerrhavia diffusa L. (F. Nyctaginaceae) has been used in Ayurvedic medicine for the treatment of liver, gall-bladder, urinary and many other diseases for ages. The present study was aimed (1) to assess the anti-inflammatory activity of different extracts of *B. diffusa*, (2) to find out the possible mechanism of anti-inflammatory activity, if any, (3) to assess the hepatoprotective activity of the methanolic extract and (4) to assess the immunodulatory activity of the methanolic extract.

The methanolic, aqueous, chloroform and petroleum ether extracts were tested for anti-inflammatory activity by the carrageenan induced rat paw oedema method at 2 dose rates 200 and 400mg/kg bw p.o., and compared with that of diclofenac sodium given @ 3 mg/kg p.o. The percentage inhibition of paw oedema was 42 and 55 per cent respectively for the methanolic extract @ 200 and 400 mg/kg, while it was -20 per cent and +20 per cent for the aqueous extract at both dose rates tested. The percentage inhibition of oedema was 20 and -15 per cent for the chloroform extract and -20 and -10 per cent for the petroleum ether extract at 200 and 400 mg/kg dose rates respectively. Diclofenac sodium produced a percentage

inhibition of 52.5 per cent. Since the methanolic extracts @ 400 mg/kg produced significant anti-inflammatory activity comparable to that of diclofenac sodium, the methanolic extract was selected for further studies.

The methanolic extract was further tested for anti-inflammatory activity by the cotton pellet induced granuloma formation method also. The percentage anti-inflammatory activity was found to be 24.31 and 43.25 per cent for the extract @ 200 and 400 mg/kg respectively, while it was 64.4 per cent for diclofenac sodium. The levels of serum acid phosphatase, adrenal ascorbic acid, serum and liver proteins of the rats given the extract @ 400 mg/kg were near to that of the rats given diclofenac sodium, thus showing that the methanolic extract of *B. diffusa* had significant anti-inflammatory activity in the chronic model of inflammation also.

To assess the antioxidant activity of the extract *in vitro*, the effect on inhibition of superoxide radical formation, lipid peroxide formation and nitric oxide radical formation were assessed. The results showed that the extract possessed significant inhibitory activity against all there radicals *in vitro*.

Serial fractionation of the extract using different solvents yielded neutral, acidic, phenolic alkaloidal and strongly basic alkaloidal fractions which were then tested for anti-inflammatory activity by the carrageenan induced rat paw oedema formation method. The neutral, phenolic alkaloidal and strongly basic alkaloidal fractions produced 6.67, 10 and 23 per cent inhibition, while diclofenac sodium produced 60.4 per cent inhibition. In order to assess the hepatoprotective activity of the extract against CCl_4 induced hepatotoxicity in rats, the levels of Alanine amino transferase, aspartate amino transferase, alkaline phosphatase, total bilirubin and direct bilirubin were estimated in the serum 36 hours after the administration of CCl_4. The levels of all the parameters (which were elevated significantly in the group given CCl_4 alone) were decreased in a dose dependent manner, in the groups given the extract at the dose of 200 and 400 mg/kg, with a significant decrease seen in the latter group. The histopathological findings were also confirmatory proving the hepatoprotective activity of the extract.

The immuno modulatory activity of the methanolic extract was also evaluated by testing its effect on the hematological parameters, circulating antibody titre and antibody forming cells in the spleen of Balb/c mice. The

result at both dose rates tested, stimulated the humoral immune response, as shown by an increase in antibody forming cells in the spleen and circulating antibody titre against sheep red blood cells (SRBC).

Thus from the present study, it could be concluded that

(1) the methanolic extract of *B. diffusa* @ 400 mg/kg has significant anti-inflammatory activity in rats.

(2) It has multiple facets of action including antioxidant effect.

(3) It has significant hepatoprotective activity.

(4) It has immunostimulant activity also.