ANTI-ULCER EFFECT OF *Azadirachta indica* (NEEM) AND *Eupatorium triplinerve* (AYAPPANA) IN RATS

SANGEETHA SATHEESAN

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

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

DECLARATION

I hereby declare that this thesis entitled **"Anti-ulcer effect of *Azadirachta indica* (Neem) and *Eupatorium triplinerve* (Ayappana) in rats"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy

SANGEETHA SATHEESAN

CERTIFICATE

Certified that this thesis, entitled **"Anti-ulcer effect of *Azadirachta indica* (Neem) and *Eupatorium triplinerve* (Ayappana) in rats"** is a record of research work done independently by **Dr. Sangeetha Satheesan**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

Mannuthy
02-08-2005

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

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Sangeetha Satheesan,** a candidate for the degree of Master of Veterinary Science in Pharmacology and Toxicology, agree that the thesis entitled **"Anti-ulcer effect of *Azadirachta indica* (Neem) and *Eupatorium triplinerve* (Ayappana) in rats"** may be submitted by **Dr. Sangeetha Satheesan,** in partial fulfilment of the requirement for the degree.

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

Dr. N. Gopakumar
Associate Professor and Head
Department of Pharmacology and Toxicology
College of Veterinary and Animal Sciences,
Mannuthy.
(Member)

Dr. K. Venugopalan
Professor
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences,
Mannuthy.
(Member)

Dr. T. Sreekumaran
Professor and Head
Department of Pathology
College of Veterinary and Animal Sciences,
Mannuthy.
(Member)

External Examiner 07/10/05
(Dr. P. THOMOORTHY)

ACKNOWLEDGEMENT

To the greatest degree, I am indebted to my guide and Chairman of the Advisory Committee, ***Dr. A.D. Joy,*** *Associate Professor, Department of Pharmacology and Toxicology for his affectionate guidance, valuable suggestions, generous support and kindly help in all possible ways during the entire period of my study. Without his strong support and co-operation throughout the study, the successful completion of this work would not have been possible.*

I am greatly indebted to ***Dr.N. Gopakumar,*** *Associate Professor and Head, Department of Pharmacology and Toxicology for the valuable help and whole hearted co-operation in carrying out the study.*

I am very much obliged to ***Dr. K. Venugopalan,*** *Professor, Department of Pharmacology and Toxicology for his suggestions, earnest help, guidance and prompt review of thesis.*

I humbly place my respect and gratitude to ***Dr.T. Sreekumaran,*** *Professor and Head, Department of Pathology and member of the advisory committee for his valuable suggestions, forthright views, patient and constructive review of the manuscript.*

I am grateful to ***Dr.A.M. Chandrasekharan,*** *Associate Professor, Department of Pharmacology and Toxicology, for his continuous interest, valuable suggestions and support throughout the course of the thesis.*

I express my sincere thanks to ***Dr. P.T.A. Usha,*** *Assistant Professor,* ***Dr.C.M. Aravindakshan*** *and* ***Shri. V.R. Reghunandanan,*** *Associate Professors, Department of Pharmacology and Toxicology for their mental support and encouragement during this stud*

I greatly acknowledge the whole hearted help rendered by ***Mrs. K.S. Sujatha,*** *Assistant Professor and Head of the Department and* ***Mrs. Mercy,*** *Associate Professor, Department of Statistics for statistical analysis.*

I am indebted to ***Dr.N. Divakaran Nair****, Assistant Professor, Department of Pathology for his valuable advice and timely help.*

I remember with gratitude the help and co-operation offered by ***Shri. P.R. Chandrasekhar****, Instrumentation Engineer and Head, Central Instrumentation Lab* ***Mrs. Dhanya, C.R., Miss. Annie and Miss. Sandhya*** *for their Technical Assistance during the course of this study.*

I am grateful to ***Dr.E. Nanu****, Dean i/c, College of Veterinary and Animal Sciences, Mannuthy for providing the facilities to conduct the research.*

Nothing will be sufficient to show my deep sense of gratitude to my colleagues, ***Dr. Anu, Dr. Senthil, Dr. Deepa, and Dr. Vikrama Chakravarthy*** *for the priceless help, mental support and sustained encouragement rendered by them throughout the course of my research work.*

I am obliged, thankful and grateful to my respected and reverant seniors, ***Dr. Sujith, Dr. Seema, Dr. Archana, Dr. Jerald, Dr. Preethy, Dr. Suja, Dr.Deepa, Dr. Suresh, Dr. Fakrudeen*** *for their help, valuable advice and guidance rendered to me during the period of my study.*

I express my sincere and heartfelt gratitude to ***Dr. Jeyamurugan*** *for the support, guidance and help extended to me throughout the course of my research work. I gratefully acknowledge the whole hearted help and co-operation rendered by* ***Dr. Vandana, Dr. Sivanesan*** *and* ***Dr. Jagaveera Pandian*** *during the course of the research work.*

I remember with gratitude the warm friendship and affectionate encouragement of my friends ***Dr. Sreeja, Dr. Prasanna, Dr. Renju, Dr. Roshin, Dr. Raji,Dr. Surya, Dr. Smitha, Dr. Julie, Dr. Naicy, Dr. Durgga, Miss. Seena, Miss. Rebha and Dr. Misha.***

The help and co-operation rendered by ***Smt. Latha, Smt. Sumathi, Smt. Ramani, Miss. Saritha, Mr. Anush*** *are also greatly acknowledged.*

I thankfully remember all those who directly or indirectly helped me and contributed to finalise the work.

I am indebted to ***Kerala Agricultural University*** *for awarding me the fellowship for the post graduate study.*

No words can implicitly express the deep gratitude to my beloved ***Achan, Amma, Unni, Appooppan, Ammoomma*** *and the dear and near ones for their love, affection, encouragement, prayers and blessings. I owe very much to them.*

Above all, I bow before ***Almighty,*** *for all the blessings showered on me and leading me to the successful completion of this course.*

SANGEETHA SATHEESAN

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1. INTRODUCTION

Peptic ulcer has afflicted man since ancient times. Perhaps the first description of this malady is the one inscribed on the pillars of the Temple of Aesculapius at Epidaurus from around the fourth century B.C. Many prominent people have suffered from indigestion and ulcers, including the Roman Emperor Marcus Aurelius, whose death has been attributed to a perforated ulcer and whose physician was none other than Galen himself.

Peptic ulcer represents a major health problem both in terms of morbidity and mortality. It is considered as one of the major human sufferings today affecting nearly five per cent of the global population. A mucosal interruption in the stomach or duodenum is called a peptic ulcer, only if it is deep enough to penetrate through the entire mucosa. Any lesser penetration is called as erosion. The most frequent site of occurrence of peptic ulcer is the duodenum, as well as lesser curvature of the antral end of the stomach.

The peptic ulcer develops as a result of localized areas of necrosis and digestion of mucus lining of the digestive tract. The process is a penetrating one, beginning in the mucosa and gradually extending through the muscularis propria. In some cases, the ulcer penetrates into blood vessels resulting in haemorrhage or completely through the gut wall into adjacent organs or as a free perforation into the peritoneal cavity. Healing starts from below and extends upward with the growth of granulation tissue and fibroblast. In small superficial lesion, healing is complete. In chronic ulcers healing is slower, new glands are not formed and the tissue is replaced by fibrous and elastic tissue.

It is accepted that ulcer occur due to imbalance between offensive acid-pepsin secretion and defensive factors which include mucin-bicarbonate secretion, life span of cells, cell proliferation, mucosal blood flow, mucosal glycoproteins and sulfhydryl compounds (Dorababu *et al.*, 2004). It was observed that in 45-70 per cent patients with duodenal ulcer, acid secretion was within normal limits, whereas in gastric ulcer patients, acid secretion was either normal or subnormal. It was

apparent that peptic ulceration was not solely induced by the offensive factors of acid and pepsin, and breakdown of mucosal resistance was an important factor.

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (Bafna and Balaraman, 2004a). Lipid peroxidation mediated by oxygen free radicals cause destruction of cell membranes and have been demonstrated in the pathogenesis of gastric mucosal injury.

The commonly prescribed drugs such as the non-steroidal anti-inflammatory drugs (NSAIDs) have ulcerogenic effect on the gastrointestinal tract. These drugs act by blocking the cytoprotective prostaglandin synthesis by inhibiting the cyclooxygenase activity. Corticosteroids appear to play a causative role in gastric ulceration. They inhibit phospholipase A_2 activity, which is the first enzyme in the synthesis of prostaglandins. Corticosteroids also decrease the mucosal cell turnover and mucus proliferation. However, recent reports indicate that most patients who develop gastrointestinal bleeding while receiving corticosteroids also received NSAIDS, which are known to promote ulceration (Schimmer and Parker, 2001).

Conditions of stress can result in acute erosion of gastric mucosa. Stress causes oxidative damage of the cellular constituents and the lipid peroxidation finally brought about loss of cellular functions. Stress also causes inactivation of prostaglandin synthetase leading to decreased biosynthesis of the cytoprotective prostaglandins (Tandon *et al.*, 2004). Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Sairam *et al.*, 2001).

Metabolic disease frequently results in secondary gastric ulceration. In renal failure, uraemic toxins directly injure the gastric mucosa and vessels of the gastric wall. The gastrointestinal hormone, gastrin is metabolized in kidney, in turn increases gastric acid output resulting in mucosal damage. Gastric ulceration secondary to liver failure occur due to decreased mucosal blood flow associated with

portal hypotension, altered mucosal renewal due to hypoproteinemia and elevated histamine concentration causing excess acid production. Gastric lesions have also been observed in some animals with adrenocortical insufficiency possibly resulting from hypotension and abnormal vascular tone (Strombeck and Guilford, 1991).

Tumours such as mastocytomas and gastrinomas are frequently associated with gastric ulceration.

Seventy five to eighty five per cent of chronic ulcer in human is due to *Helicobacter pylori* infection and there is accumulating evidence to incriminate this microbe in the etiology of duodenal ulcer and gastric carcinoma. It potentiates the polymorphic nuclear leucocyte oxidative burst leading to considerable production of reactive oxygen metabolites which degenerate the tissue causing ulcer.

Many of the pharmaceutical agents employed for the treatment of gastroduodenal ulcers can produce many adverse effects like arrythmias, impotence, gynaecomastia and haematopoeitic changes (Akhtar *et al.*, 1992). Moreover, these pharmaceutical agents are too expensive for people in the lower sections of the society. About 60 per cent of the world's population relies on natural products for their primary medication and 60-80 per cent of the newly emerging drugs in the market are derived from natural sources such as plants, animals, and microorganisms (Vineesh *et al.*, 2004).

The reduction of gastric acid secretion as well as re-inforcement of gastric mucosal protection has been the major approaches for therapy of peptic ulcer (Hoogerwerf and Pasricha, 2001).

Approaches for the treatment of peptic ulcers are:

1. Reduction of gastric acid secretion by H_2 antagonists, proton pump inhibitors, anticholinergics and prostaglandin analogues.

2. Neutralisation of gastric acid by systemic and non-systemic antacids.

3. Ulcer protectives.

4. Ulcer healing drugs.

5. Anti *Helicobacter pylori* drugs.

India is well known for the use of medicinal plants for the treatment of various ailments. Besides the Vedas, the ancient scholars like Charaka, Susruta, Vaghbatta and others brought out texts containing the description of various plants used in several preparations for the treatment of various diseases including ulcers. Many indigenous drugs are known to possess anti-ulcer activity like *Piper betle, Musa paradisiaca, Solanum nigrum* and *Asparagus racemosus* and have been used in acid peptic disorders.

Azadirachta indica (Meliaceae), popularly known as neem is known for its several medicinal values. Medicinal properties like antiseptic, healing of wounds, curing of skin diseases and anti-ulcer activity of various parts of this plant have been reported. The plant consists of the active constituents nimbin, nimbidin and nimbidiol.

Eupatorium triplinerve, commonly known as ayappana, a native of Brazil, is cultivated in various parts of India in damp places, meadows and river banks. The herb contains an essential volatile oil and neutral crystalline principle, ayapanin. The hot infusion of the drug is given in the cold stage of ague and in acute inflammatory affections. It has been reported that the fresh leaves are applied to foul ulcers, sores and to bites of venomous reptiles (Nadkarni, 1976).

Nadkarni (1976) has reported about the wound healing effects of *Eupatorium triplinerve*. Administration of *Azadirachta indica* extract has been observed to reduce gastric acid secretion (Raji *et al.*, 2004).

In the present study, an attempt has been made to evaluate the anti-ulcer potentialities of alcoholic extract and powder of *Azadirachta indica* and *Eupatorium triplinerve* leaves at various dose levels in aspirin induced gastric ulcer in adult albino rats of either sex. In addition, the study was also directed towards elucidation of the mechanism of gastroprotective actions of these herbal preparations.

2. REVIEW OF LITERATURE

2.1. ULCER INDUCTION

Okabe *et al.* (1974) proposed back diffusion of acid as the principle aetiologic factor for lesion formation in response to aspirin administration. The fact that aspirin produced a significant reduction of volume and H^+ and increment of Na^+ and K^+ in gastric juices confirmed this theory. However pepsin activity was not changed with aspirin at all.

Manekar and Waghmare (1980) found that the mean number of ulcers and ulcer index were optimum at the dose of 200mg/kg of aspirin. The results of the study showed that several factors like increase in the serotonin level, damage to the mucosal barrier, changes in mucosal permeability and mucosal blood flow and inhibition of prostaglandin synthesis could be responsible for aspirin induced gastric damage.

Sairam *et al.* (2002) studied the antiulcerogenic effect of methanolic extract of *Emblica officinalis* in different ulcer models. Ulcer was induced by administering aspirin at a dose of 200mg/kg and the stomach was taken out and cut open along the greater curvature and the ulcers were scored after four hours. The total severity of the ulcers was determined by recording the severity of each ulcer in pluses after histological confirmation.

In the studies conducted by Rao *et al.* (2004) different acute and chronic gastric ulcer models were used. Ulcer was induced by administering aspirin at a dose of 200mg/kg, ethanol at the rate of 1ml/200g, by subjecting animals to cold-restraint stress at temperature of 4-6^{0}C and by pylorus ligation. Chronic ulcers were induced by treatment with 50 per cent acetic acid.

2.2. ULCER SCORE

Gupta *et al.* (1988) devised an arbitrary scoring system for grading the severity of lesions. (i). denuded epithelium=10. (ii). Petechial and frank

haemorrhages=20. (iii). One or two ulcers=30. (iv). Multiple ulcers=40. (v). Perforated ulcers=50. The severity of ulceration was expressed in terms of ulcer index which was the mean score of all the animals in a group.

The incidence and severity of lesions were recorded with the help of an arbitrary scoring system. (i). Shedding of epithelium=10; (ii). Petechial and frank haemorrhages=20; (iii). One or two ulcers=30; (iv). More than two ulcers=40; (v). Perforated ulcers=50. The ulcer index, percentage protection index and healing index were calculated from the scorings (Dharmani *et al.*, 2004).

2.3. FAMOTIDINE

Sener *et al.* (2004) conducted studies on the protective effect of increasing doses of famotidine, omeprazole, lansoprazole, and melatonin against ethanol-induced gastric damage in rats. The treatment with higher doses of famotidine(12mg/kg), omeprazole(2.4mg/kg), or lansoprazole(10mg/kg) prevented the gastric ulcerogenesis significantly and decreased the ulcer index values. They caused a decrease in gastric acidity and also reduced the oxidative damage to the gastric mucosa as indicated by lower levels of lipid peroxide and myeloperoxidase activity and increased levels of glutathione.

2.4. PHARMACOLOGICAL EFFECTS OF PLANTS UNDER STUDY

2.4.1 *Azadirachta indica*

Pillai *et al.* (1978) undertook investigation on the anti-gastric ulcer activity of nimbidin, the main active principle of *Azadirachta indica* isolated from the oil of seeds and the trunk bark. Nimbidin at a dose of 20-40mg/kg significantly prevented ulceration and also was found to suppress gastric secretion by about 50 per cent. The anti-ulcer activity of the drug could also be attributed to its anti-peptic activity, besides the anti-secretory effect.

Studies conducted by Pillai and Santhakumari (1981) showed that Neem oil (2.5ml) and Nimbidine (200mg/kg), the active principle isolated from Neem oil exhibited significant hypoglycaemic activity by reducing blood sugar level by 24

and 26 per cent respectively at the fifth hour of feeding. The pattern of glucose tolerance curves of both were almost similar to that of tolbutamide which indicated that they may be acting through the release of endogenous insulin as in the case of sulphonylureas.

The *in vitro* antibacterial activity of Neem oil against pathogenic strains isolated from clinical sources were studied by the disc diffusion method. The results indicated that Neem oil has significant antibacterial properties against pathogenic gram negative bacilli such as *Pseudomonas, Escherichia* and gram positive cocci such as *Staphylococcus* (Rao *et al.*, 1986).

Chattopadhyay *et al.* (1992) conducted studies on the hepatoprotective activity of *Azadirachta indica* leaves on paracetamol induced hepatic damage in rats. *A.indica* (one g/kg, p.o.) showed significant reversal effects on the elevated serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, acid phosphatase and alkaline phosphatase in paracetamol hepatotoxicity.

Studies conducted by Sen *et al.* (1992) showed that *Azadirachta indica* lowered blood glucose, triglyceride and serum glutamate oxaloacetate transaminase levels and attenuated the stress induced elevations of cholesterol and urea levels. *A.indica* also enhanced the humoral antibody response to the antigen as well as leucocyte migration in immunized rats, indicating the humoral and cell mediated immune enhancement.

Bopanna *et al.* (1997) observed that treatment with neem kernel powder along with glibenclamide significantly decreased the concentration of serum lipids, blood glucose and activities of serum enzymes like alkaline phosphatase, acid phosphatase, lactate dehydrogenase, liver glucose-6-phosphatase and HMG CoA reductase activity in liver and intestine of alloxan diabetic rabbits. The results suggest a significant antidiabetic and antihyperlipaemic effect of the neem kernel powder.

Khosla *et al.* (2000) conducted studies on the hypoglycaemic effects of *Azadirachta indica* in normal and alloxan diabetic rabbits. Both the leaf extract as well as seed oil significantly reduced the blood glucose levels and the effects were

comparable to that of glibenclamide. The pretreatment with the drug prior to alloxan administration was also shown to partially prevent the rise in blood glucose levels as compared to control diabetic animals.

The *in vitro* antiviral activity of aqueous neem leaves extract assessed in C6/36 (cloned cells of larvae of *Aedes alboplicatus*) cells employing virus inhibition assay showed inhibition in dose-dependent manner. The aqueous extract of neem leaves at its maximum non-toxic concentration of 1.897mg/ml completely inhibited 100-10,000 $TCID_{50}$ of virus as indicated by absence of cytopathic effects. The *in vivo* protection studies with neem leaves extract at its maximum non-toxic concentrations 120-30mg/ml resulted in inhibition of the virus replication as confirmed by the absence of Dengue related clinical symptoms in suckling mice and absence of virus specific 511 bp amplicon in RT-PCR (Parida *et al.*, 2002).

Clinical studies were conducted by Bandyopadhyay *et al.* (2004) on the effect of *Azadirachta indica* bark extract on gastric secretion and gastroduodenal ulcer. The studies showed that the extract when administered for ten days at the dose of 30mg twice daily caused significant reduction in the gastric acid secretion as well as the pepsin activity by 63 per cent and 50 per cent respectively, in patients suffering from acid-related problems and gastroduodenal ulcers. The healing effect was confirmed by barium meal X-ray or by endoscopy.

Chattopadhyay *et al.* (2004) conducted studies on the anti-ulcer effect of *Azadirachta indica* leaf aqueous extract. It was found that the extract inhibit gastric lesions and also inhibit H^+K^+ATPase activity *in vitro* to inhibit acid secretion in different ulcer models. The oxidative membrane damage by hydroxyl radical as measured by lipid peroxidation in stress ulcer was significantly blocked by the leaf extract. The stress- induced apoptotic DNA fragmentation as well as hydroxyl radical mediated mucosal DNA damage *in vitro* was shown to be prevented by the extract.

Dorababu *et al.* (2004) found that *Bacopa monniera* extract did not show any significant effect on blood glucose level in both normal and non-insulin dependent diabetes mellitus (NIDDM) rats, while *Azadirachta indica* significantly decreased it.

Both *B.monniera* (50mg/kg) and *A.indica* (500mg/kg) extracts showed significant anti-ulcer and ulcer-healing activities in normal and NIDDM rats. The anti-ulcer and ulcer-healing activities may be due to their effects on mucosal offensive and defensive factors, and correction of blood sugar level by *A.indica* may help to have more ulcer protective effect in NIDDM rats.

The protective role of extracts of Neem seeds (*Azadirachta indica*) in diabetes caused by streptozotocin in rats was studied by Gupta *et al.* (2004). The decrease in activities of superoxide dismutase and catalase and the increase in lipid peroxidation of erythrocytes as observed in diabetes were regained following treatment with neem seed kernel and husk. It could be concluded from the results that the neem seed kernel and husk prevented oxidative stress caused by streptozotocin in heart and erythrocytes, indicating its potent antioxidant activity.

The effect of *Azadirachta indica* extract on gastric ulceration was studied in albino rats. The extract at a dose of 250mg/kg, i.p. significantly inhibited gastric ulceration induced by indomethacin, accompanied by a dose-dependent decrease in the total gastric acidity. The effect of the extract alone and in combination with histamine and cimetidine, on gastric acid secretion was studied and it was found that *A.indica* significantly inhibited the basal and histamine-induced gastric acid secretion. The results concluded that the gastric cytoprotection of the extract could probably be due to its acid reduction effect through an anti-histaminergic mechanism (Raji *et al.*, 2004).

2.4.2. *Eupatorium triplinerve*

Nadkarni (1976) reported on the medicinal properties of *Eupatorium triplinerve*. The infusion of the herb is given in derangement of stomach and bowels, dyspepsia, cough and ague. The hot infusion is prescribed for acute inflammatory affections. Fresh leaves bruised are applied to foul ulcers, sores and to bites of venomous reptiles. The drug is also given internally as an anti-dote to snake bites.

The wound healing effect of *Eupatorium odoratum* was compared with Himax in albino rats. Sardar *et al.* (2002) found that complete filling and healing of experimentally created wounds averaged 9.66-12.66 days after daily application of the aqueous leaf extract. The anti-bacterial activity *in vitro* in different isolates of bacteria was also studied and the results suggested that the plant might be having anti-bacterial activity since the bacterial load was found to be reduced following exposure to aqueous leaf extract.

2.5. OTHER INDIGENOUS AGENTS WITH ANTI-ULCER EFFECT

Gambhir *et al.* (1987) conducted studies on the flavonoid compound, Amentoflavone and found that the compound significantly reduced the mean ulcer index in pylorus ligation, restraint stress and histamine induced gastric ulcer models when administered at the dose of 50mg/kg. Amentoflavone also exhibited significant dose-dependent anti-inflammatory activity against carrageenan- induced paw oedema model.

Goel *et al.* (1988) studied the anti-inflammatory and anti-ulcer effects of a flavonoid Kaempferol, isolated from *Rhamnus procumbens.* The drug significantly reduced the increase in paw volume in different paw oedema models. Kaempferol decreased both the acid and pepsin output without affecting the volume of gastric juice. The total carbohydrate to protein ratio was also found to increase indicating strengthening of mucosal resistance.

Gupta *et al.* (1988) evaluated the anti-ulcer activity of Sodium valproate in rats and guinea pigs and was shown to reduce gastric ulceration in different ulcer models. The drug was found to exert an anti-secretory effect as indicated by the reduction in gastric volume, total and free acidity and peptic activity.

Mandal *et al.* (1993) observed that the extract of *Ocimum sanctum.* Linn reduced the ulcer index, free and total acidity and thereby exert an anti-ulcerogenic effect. The extract was also found to increase the mucus secretion.

Studies conducted by Matsumoto *et al.* (1993) suggested that the white blood cells which retained high NADPH oxidase activity thereby producing oxygen

radicals play important role in gastric mucosal lesions in mice induced by HCl or ethanol. The activated neutrophils injured microvasculature via the release of oxy radicals or proteases. The anti-ulcer polysaccharide bupleuran, isolated from the roots of *Bupleurum falcatum* was found to have potent hydroxyl radical scavenging activity which was found to protect the gastric mucosa.

The effect of Thamrabhasma, an indigenous preparation of copper, on the gastric mucosal resistance was studied and it was found to increase the glycoprotein and sialomucin fractions which play important role in protecting mucosa from noxious stimuli. The drug was found to have no effect on cellular proliferation as is evident from its lack of effect on DNA and [^{3} H]-thymidine incorporation into DNA. Goel *et al.* (1994) also reported that the drug reduced the rate of mucosal shedding as indicated by decreased gastric juice DNA and protein contents.

The anti-ulcerogenic effect of UL-409, a herbal formulation against experimentally induced gastric ulcer in rats was studied by Vanisree *et al.* (1996). The protective action may be via the increase and maintenance near normalcy in the activity of superoxide dismutase which prevent neutrophil induced damage. The UL-409 pretreatment also resulted in increase in the activity of glutathione peroxidase thus preventing oxidation of lipids. Thus, it could be concluded that its anti-ulcerogenic activity is mediated through antioxidant defence mechanism.

The studies conducted on *Pteleopsis suberosa* by Germano *et al.* (1998) showed a protective action of the decoction and methanolic extract against ethanol-induced gastric mucosal damage as demonstrated by the reduction of the ulcer index and by the increased gastric mucus secretion. The mechanisms of the mucosal protective action of *P.suberosa* decoction could partly be due to the stimulation of prostaglandin synthesis. The study also demonstrated an antimicrobial activity against *Helicobacter pylori* which is generally associated with peptic or gastroduodenal ulcers, with minimum inhibitory concentrations ranging from 31.25-250microgram/ml.

Venkataranganna *et al.* (1998) evaluated the possible mechanism of anti-ulcerogenic activity of UL-409, a herbal formulation. Oral administration of the

preparation at dose of 600mg/kg reduced the offensive factors like the volume of gastric secretion, total and free acidity in aspirin and pylorus-ligation induced ulcer models. The muco-protective property of the preparation was indicated by the significant increase in total carbohydrate to protein ratio. The major mechanism involved appears to be due to promotion of mucosal protection by augmenting gastric mucin activity.

Akah and Nwafor (1999) found that the extract of *Cissampelos mucronata* demonstrated potent anti-ulcer activity, protecting rats from ulcerogenesis induced by various agents and mechanisms. The anti-ulcer activity may be attributed to the reduction in intestinal motility and the inhibition of microbial growth.

The ethanolic extract of *Bidens pilosa* L. var. *radiata* was assessed for its anti-ulcer properties in indomethacin and ethanol induced gastric ulcer models. The extract was found to decrease the gastric juice volume, acid secretion, as well as pepsin activity indicating its anti-secretory effect. The extract was also found to stimulate mucus secretion, thereby strengthening the gastric mucus-bicarbonate barrier. Alvarez *et al.* (1999) concluded from the study that the extract exerts a cytoprotective effect in addition to its gastric anti-secretory activity that could be due partly at least to the presence of flavonoids of which quercetin was identified by HPLC.

Dhuley (1999) studied the protective effect of Rhinax, a herbal formulation in different gastroduodenal ulcer models. Rhinax was found to increase the mucus and decrease the acid volume, free and total acid content in rats. It could be concluded that the gastroprotective effect elucidated by Rhinax could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition and free radical scavenging properties.

The studies conducted by Lewis *et al.* (1999) isolated the active anti-ulcerogenic ingredient from unripe plantain banana. The dried unripe plantain banana powder, the extracted leucocyanidin and a purified synthetic leucocyanidin demonstrated a significant protective effect against aspirin-induced erosions. The

mechanisms by which these natural flavonoids exert anti-ulcerogenic activity have been shown to be due to the reduction of acid secretion from gastric parietal cells.

The anti-ulcer activity of *Ocimum sanctum* was studied in comparison with famotidine in rats. Both the powder and the extract were shown to reduce the ulcer index significantly in comparison to the aspirin treated controls, but did not produce statistically significant healing effect comparable to famotidine groups (Sanjay *et al.*, 1999).

The gastric anti-ulcer activity of the fixed oil of *Ocimum basilicum* against various ulcerogens in different animal models were studied. The oil at dose levels of 1 to 3ml/kg i.p. significantly suppressed the development of ulcers in a dose-dependent manner. Singh (1999) concluded from the study that the fixed oil of *O.sanctum* possessed significant anti-ulcer activity which may be due to lipoxygenase inhibitory, histamine antagonistic and antisecretory effects of the oil.

The anti-ulcer effect of *Microgramma squamulosa* was assessed in acute gastric ulcer models like ethanol/ HCl-induced ulcers, using cimetidine and misoprostol as reference drugs. The different fractions of the extract and cimetidine were tested through subchronic induction test with acetic acid. The sub chronic toxicity test was performed using a dose of 800mg/kg of the crude extract orally administered for 30 days. The results demonstrated significant ulcer protective effects, which means that there is a compound probably acting as antagonist to H_2 receptors (Suffredini *et al.*, 1999).

Treatment with butanol extract of the water fraction of *Phyllanthus emblica* fruits at the dose of 100mg/kg was found to enhance secretion of the cytoprotective gastric mucus and hexosamine in indomethacin-induced ulceration of rats. Its cytoprotective action on the gastric mucosa was also supported by morphological observations. Bandyopadhyay *et al.* (2000) found that a strong antioxidant property was also responsible for the cytoprotective action of the drug.

The anti-ulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* was analysed and it was found that the extract decreased the ulcerative

lesion index produced by indomethacin, ethanol and reserpine in rats. However no anti-secretory activity was observed on pyloric ligation model. According to Dias *et al.* (2000), the pharmacological mechanism of anti-ulcerogenic activity of the extract could be attributed to the activity of antioxidant compounds found in the extract which are believed to increase the cytoprotective mucosal non-protein sulfhydryl group content.

The studies conducted by El-Dakhakhny *et al.* (2000) on the effects of *Nigella sativa* on the gastrointestinal tract revealed that the *N.sativa* oil protected the gastric mucosa against ethanol-induced damage by a protective ratio of 52.65 per cent as compared to the control group. The protective action could be attributed to the significant increase in the cytoprotective mucin and glutathione level and a significant decrease in the gastric mucosal histamine content.

Nwafor *et al.* (2000) conducted studies on the anti-ulcerogenic and anti-diarrhoeal effects of methanolic extract of *Asparagus pubescens* root in rats. The extract dose-dependently reduced the intestinal propulsive movement, castor oil-induced diarrhoea and intestinal fluid accumulation. The extract also reduced the ulcer indices induced by indomethacin and ethanol in a dose-related manner. The results indicated that its anti-diarrhoeal and anti-ulcerogenic effects might in part be due to its alpha-2 adrenoceptor stimulation and its active constituents like tannins, saponins and flavonoids respectively.

The antiulcerogenic activity of flavonoid and alkaloid fractions of *Synclisia scabrida* was studied by Obi *et al.* (2000) using aspirin and 0.6 N NaOH ulcer models. These fractions were found to reduce the ulcer index significantly and also the alkaline phosphatase activity, which is observed to be elevated in tissue necrosis and damage associated with gastrointestinal ulceration. In this study, the alkaline phosphatase is used as a biochemical basis of antiulcerogenecity of *S.scabrida.*

Okwari *et al.* (2000) conducted studies on the anti-ulcerogenic effects of *Dombeya buettneri* in rats. Intragastric perfusion with the aqueous extract caused significant reduction in basal and histamine stimulated gastric acid secretion. Pretreatment with the extract was also found to reduce the extent of gastric mucosal

damage induced by oral ethanol, but had no much effect on the mucus secretion. Hence it was concluded from the study that the anti-ulcerogenic effect of the extract may be related to its anti-secretory action rather than a cytoprotective mechanism.

Studies conducted by Sanjay *et al.* (2000a) observed that the alcoholic extract of *Withania somnifera* at the dose of 250-500mg/kg produced healing of gastric ulcer comparable to famotidine at the dose of 40mg/kg. Histopathological findings showed that re-epithelialization was almost complete and the junctional morphology was restored in the famotidine as well as extract treated group.

The alcoholic extract of *Musa* (AAB group, "Nendran") unripe fruit produced a dose-dependent increase in anti-ulcer activity while healing effect produced by *Musa* powder were not dose-dependent but the effects were comparable with famotidine groups administered for 10 days and 20 days respectively. All the groups produced a significant decrease in ulcer index when compared with aspirin treated controls (Sanjay *et al.*, 2000b).

Shetty *et al.* (2000) found that the extract of *Gingko biloba* at a dose of 300mg/kg significantly protected rats from ethanol-induced mucosal injury, which may be due to its antioxidant property. *G.biloba* has been found to exert its beneficial effect by neutralizing hydroxyl and peroxyl radicals.

The anti-ulcer actions of the bark methanol extract of *Voacanga africana* was studied in different experimental ulcer models in rats. Significant decreases of the ulcer index scores were obtained when the extract was administered at 500-750mg/kg. Tan *et al.* (2000) concluded from the results that the anti-ulcer properties were not related to an anti-secretory effect, rather it was due to a mechanism involving the physico-chemical re-inforcement of the gastric mucous layer or by effects similar to endogenous prostaglandins.

The petroleum ether and ethanol extracts of *Indigofera longeracemosa* was observed to afford protection against restraint stress or pylorus ligation induced gastric ulcers in rats. The mechanism of anti-ulcer effect could be due to decrease in

acid-pepsin secretion or augmentation of mucin secretion as indicated by the increased total carbohydrate to protein ratio (Thangadurai and Viswanathan, 2000).

The extract of *Musa sapientum* var. *paradisiaca* at the dose of 50mg/kg was evaluated for its antioxidant and anti-*Helicobacter pylori* activities in stress-induced gastric ulcer models. Goel *et al.* (2001) demonstrated that the extract significantly reversed the increase in ulcer index, lipid peroxidation and superoxide dismutase values induced by stress, though no much significant effect was observed in the catalase values. The extract however did not show any anti-*H.pylori* activity. The study indicated that antioxidant activity appear to be involved in the ulcer protective effect of *M.sapientum* extract.

The hydroalcoholic extract and the fractions of *Davilla rugosa* were shown to protect rats from developing gastric ulcers induced by HCl/ethanol and immersion restraint stress. The extract when tested for the possible toxic effects were not found to produce much modifications in body weight or in the external aspect of the internal organs. The active ingredients like flavonoids, saponins present in the extract may be implicated in the anti-ulcer activity of the species (Guaraldo *et al.*, 2001).

The studies conducted by Jafri *et al.* (2001) found that the crude methanolic extract of fruits of *Amomum subulatum* (large cardamom) and its fractions inhibited gastric lesions induced by ethanol significantly, but not those which were induced by pylorus ligation and aspirin. The ethyl acetate fraction increased the wall mucus in pylorus ligated rats. The results suggested a direct protective effect of ethyl acetate fraction on gastric mucosal barrier while the gastroprotective action of petroleum ether and the essential oil was due to their lowering effect on gastric motility.

The gastric anti-ulcer effect of the freeze-dried aqueous extract of *Rhizophora mangle* was investigated on the acidified-ethanol induced ulcer model in rats. A high level of gastric protection was obtained following treatment with the extract at 500mg/kg. The anti-ulcer effect could be attributed to its cytoprotective property as indicated by the increase in mucus content accompanied by a proportional increase in proteins (Perera *et al.*, 2001).

The effect of *Convolvulus pluricaulis* Chois on gastric ulceration and secretion in rats was studied and the juice of the plant showed anti-ulcerogenic effect in all the experimental gastric ulcer models and was comparable to the reference drug, sucralfate. Sairam *et al.* (2001) concluded that the anti-ulcerogenic effect of the juice was found to be due to augmentation of mucosal defensive factors like mucin secretion, life span of mucosal cells and glycoproteins rather than on the offensive factors like acid-pepsin.

Studies conducted on the antioxidant and gastroprotective activity of *Hibiscus tiliaceus* showed that single dose treatment of the methanolic extract produced significant decrease in ulcer index values. The extract was found to scavenge the superoxide and nitric oxide radicals *in vitro* and also showed potent *in vivo* antioxidant properties. The histopathological findings also confirmed the cytoprotective effect of the extract. The antioxidant and gastroprotective effect of *H.tiliaceus* is believed to be due to the presence of flavonoids and polyphenols (Ajaikumar *et al.*, 2002).

The extracts of the leaves and fruits of *Sapindus saponaria* were studied for its anti-ulcer activity and the gastric secretion parameters were evaluated after pylorus ligation. The administration was observed to bring about a decrease in gastric secretion volume and hydrochloric acid secretion, with a slight pH increase, when compared to the control group. Albiero *et al.* (2002) also found that the inhibitory effects of the extract on lesions induced by stress were comparable to that of cimetidine.

The gastroprotective effects of Fenugreek seeds compared to omeprazole was studied on ethanol-induced gastric ulcers. The cytoprotective effects of the seed were indicated by the near normal levels of the mucosal glycoproteins. The significantly lower levels of pepsin activity, protein content and volume of gastric juice confirmed the anti-secretory activity which is important in protecting gastric mucosa. Pandian *et al.* (2002) also found that fenugreek seeds prevented the rise in lipid peroxidation presumably by enhancing the antioxidant potential of the gastric mucosa thereby lowering mucosal injury.

The ulcer protective potential of methanolic extract of *Emblica officinalis* was assessed in different acute gastric ulcer models, and the healing effect was assessed in chronic gastric ulcers induced by acetic acid in rats. The extract at doses of 20-50mg/kg was found to significantly reduce the offensive factors like the volume and concentration of acid as well as pepsin activity. Sairam *et al.* (2002) observed that the extract increased the defensive factors like mucin secretion, cellular mucus and life span of the mucosal cells. It was also found to have a significant antioxidant effect which contributes to the cytoprotective effect.

The methanolic extract of *Punica granatum* at the dose of 500mg/kg showed significant reduction in gastric mucosal injury induced by aspirin and ethanol. Ajaikumar *et al.* (2003) showed that in the treated group of animals, a significant increase in free radical scavenging enzymes were obtained which revealed the potent antioxidant activity of the extract. The histopathological studies also confirmed the cytoprotective activity. The *in vivo* antioxidant and anti-ulcer activity of *P.granatum* may be due to the presence of flavonoids and terpenes.

Biochemical studies on the anti-ulcerogenic potential of the aqueous ethanolic extracts of the roots of *Hemidesmus indicus* R. Br. var. *indicus* were conducted by Anoop and Jegadeesan (2003) in different gastric ulcer models. The extract was found to bring about reduction in the aggressive factors like gastric volume, free and total acidity as well as the pepsin activity. An increased carbohydrate to protein ratio was observed which is a reliable index for an effective mucosal barrier.

The aqueous extract of the roots of *Tephrosia purpurea* was assessed for its anti-ulcer activity and the ulcer index in the treated animals was found to be significantly less in all the ulcer models when compared to the vehicle control group. Deshpande *et al.* (2003) concluded from the results that the aqueous extract of *T.purpurea* possess significant anti-ulcer property which could be either due to cytoprotective action of the drug or by strengthening of gastric and duodenal mucosa and thus enhancing the mucosal defence.

The *Nigella* oil and its constituent thymoquinone, was found to have a marked protective action against ischaemia/reperfusion induced gastric mucosal lesions, as indicated by the suppression in the levels of lipid peroxides and lactate dehydrogenase, and an increase in those of glutathione and superoxide dismutase. Thus the anti-ulcerogenic effect of *Nigella* oil could be attributed to the improvement of the antioxidant status or the presence of free radical scavenging substances such as thymoquinone (El-Abhar *et al.*, 2003).

The effect of ethanol extract of *Piper betle* leaf on healing of indomethacin-induced experimental ulcer was studied by Majumdar *et al.* (2003). On treatment with the extract, the antioxidant enzyme activity as well as the gastric mucosal barrier, primarily the hexosamine and mucus content gradually increased indicating significant protective as well as healing action of the drug. In contrast, the oxidized lipids and oxidatively modified proteins were reduced to near normalcy. The results suggested that the antioxidant or free radical scavenging activity of the plant extract may be responsible for its healing action.

The anti-inflammatory effect of the methanolic extract of *Bambusa arundinacea* against carrageenan-induced as well as immunologically-induced paw oedema as well as the anti-ulcer activity were found to be significant when compared to the standard drugs. These effects were found to be probably due to inhibition of mediators of inflammation like the platelet activation factor (PAF) receptors. Muniappan and Sundararaj (2003) also found that the combination of the extract and phenylbutazone gave potent anti-inflammatory action with least ulcerogenic activity.

Nwafor and Akah (2003) observed that the methanolic extract of *Cissampelos mucronata* exhibited significant protection against indomethacin-induced ulcers. The anti-ulcer effects could be attributed to the influence of flavonoids and alkaloids, present in the extract, or arachidonic acid metabolism, their vasoprotective action and their ability to interfere with formation of histamine in the gastric mucosa.

The chloroform extract of *Tanacetum larvatum* was observed to produce a dose-dependent anti-inflammatory effect in the carrageenan-induced rat paw oedema model. The extract at a dose of 200mg/kg when concomitantly given with indomethacin, the anti-inflammatory effect was enhanced, but the gastric lesions were significantly reduced. The anti-inflammatory and anti-ulcer activity may be mainly due to the inhibition of DNA binding of the transcription factor NF-kB by components of the extract (Petrovic *et al.*, 2003).

The ulcer protective effect of the methanolic extract of *Pongamia pinnata* roots were studied against various experimental gastric ulcer models and was found to show significant protection. The ulcer protective effect was due to the augmentation of mucosal defensive factors like mucin secretion, life span of mucosal cells, mucosal cell glycoproteins and cell proliferation as well as prevention of lipid peroxidation. The extract was observed to have little effect on the offensive acid-pepsin secretion (Prabha *et al.*, 2003).

The methanolic extract of *Phyllanthus amarus* significantly inhibited gastric lesions, induced by intragastric administration of absolute ethanol. Mortality, increased stomach weight, ulcer index, and intraluminal bleeding were reduced by *P.amarus*. The reduced glutathione produced by ethanol administration was significantly elevated by treatment with the extract. The extract also produced an inhibition of rat paw oedema upto 42 per cent compared to the control. The antioxidant activity of the extract as well as the presence of tannins in the extract may be responsible for these observed activities (Raphael and Kuttan, 2003).

The ulcer protective effect of methanolic extract of fresh roots of *Asparagus racemosus* was studied on different gastroduodenal ulcer models. The gastric juice and mucosal studies showed that the extract significantly increased the mucosal defensive factors like mucus secretion, cellular mucus as well as mucosal glycoproteins. The extract was also found to have significant antioxidant activity (Sairam *et al.*, 2003).

The anti-ulcerogenic activity of ethanol extract of *Solanum variabile* at different doses was investigated by Antonio *et al.* (2004). The compounds in the

extract were found to interfere with the ulcerogenic mechanism of ethanol indicating a cytoprotective effect. Similarly the extract was also found to decrease the lesions produced by indomethacin and interfered with the stress induced gastric secretion. Thus the extract was found to act both by inhibiting gastric secretion and increasing prostaglandin release, with subsequent mucus production and active re-epithelialization indicating a cytoprotective role.

Bafna and Balaraman (2004a) studied the anti-ulcer activity of Normacid, a herbomineral formulation. The reduction in ulcer index in all the ulcer models along with reduction in total acidity and an increase in pH of the gastric fluid in pylorus-ligated rats proved the anti-ulcer activity of Normacid. The increase in the levels of free radical scavenging enzymes and membrane bound enzymes, as well as decrease in lipid peroxidation showed the antioxidant activity of the formulation. It could be concluded from the results that the anti-ulcer effects of Normacid could be mainly due to the modulation of defensive factors by improvement in gastric cytoprotection and partly due to the antioxidant activity.

Bafna and Balaraman (2004b) tested DHC-1, a herbal formulation for its anti-ulcer and antioxidant activity in pylorus ligation and ethanol induced gastric mucosal damage in rats. The reduction in ulcer index, along with the reduction in volume and total acidity proved the anti-ulcer activity of DHC-1. The increase in levels of superoxide dismutase, catalase, reduced glutathione and the membrane bound enzymes indicate the antioxidant activity of the formulation.

Dharmani *et al.* (2004) conducted studies to investigate the anti-ulcer effect of *Ocimum sanctum* in different ulcer models like cold-restraint stress, aspirin, alcohol, pylorus ligation-induced gastric ulcer and histamine-induced duodenal ulcer and the ulcer healing effect in chronic acetic acid- induced ulcer model in comparison to the standard anti-ulcer drug omeprazole. The extract at a dose of 100mg/kg was found to reduce the free and total acidity and increase the mucin secretion in comparison to control. It was concluded that a cytoprotective effect substantiated by the increase in mucin content and the free radical scavenging effect

rather than anti-secretory effect was responsible for the anti-ulcer efficacy of *O.sanctum*.

Urtica dioica, a Mediterranean herb was found to exhibit powerful antioxidant, anti-ulcer, antimicrobial and analgesic effects by Gulcin *et al.* (2004). The anti-ulcer effect of the water extract of *U.dioica* was studied using the ethanol-induced ulcer model and the mucosal injury was inhibited at the dose of 200mg/kg. The extract had effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities which may contribute to the anti-ulcer effect.

The studies conducted by Kumar and Nirmala (2004) demonstrated that the pretreatment of rats with the extract of *Caesalpinia pulcherrima* prevented the formation of gastric lesions in HCl/ ethanol-induced ulcer model, revealing a probable cytoprotective effect of the extract. In the aspirin and pylorus ligation model, the extract was able to significantly reduce ulcer formation and increase the mucus content, but had no effect on the gastric volume or acid secretion. Thus the results indicated that the anti-ulcerogenic action of the extract could be attributed to augmentation of gastric defensive mechanisms.

Phytic acid was observed to exhibit anti-inflammatory activity at a dose of 150mg/kg in carrageenan-induced rat paw oedema model and showed significant protection from ulcers induced by different ulcerogenic agents. The gastroprotective effect was found to be mediated by the antioxidant activity and cytoprotection of gastric mucosa as indicated by the decrease in malondialdehyde levels and scavenging of the reactive oxygen metabolites in gastrointestinal tract (Kumar *et al.*, 2004)

The polyherbal drug, "Ambrex", was shown to afford protection against ethanol-induced gastric mucosal damage. There was significant reduction in ulcer index, an increase in mucosal DNA content which is a reliable index of cell proliferation. The increased alkaline phosphatase activity associated with various models of gastrointestinal ulceration was brought to near normal levels following Ambrex pretreatment. Histopathologically upon Ambrex pretreatment, the mucosal

epithelium had normal architecture and it had less haemorrhage as against the ethanol-induced damages in the mucosal epithelium (Narayan *et al.*, 2004).

Rao *et al.* (2004) studied the anti-ulcer activity of *Utleria salicifolia* rhizome extract in different acute and chronic gastric ulcer models in rats. The extract reduced the ulcer index with significant decrease in plasma corticosterone, lipid peroxidation, superoxide dismutase and increase in catalase activity indicating the antioxidant activity which protects the gastric wall mucosa from free radical injury. The gastric wall mucus was also significantly enhanced with concomitant increase in prostaglandins which contribute to its cytoprotective property.

Vineesh *et al.* (2004) examined the anti-ulcerogenic activity of methanolic extract of *Tribulus terrestris* which is commonly used in folklore medicine for various diseases. The gastric ulcers induced by oral administration of 80 per cent ethanol to rats were reduced dose-dependently when the extract at a dose of 125-250mg/kg was administered orally. The extract prevented the alcohol-induced reduction of glutathione level within the gastric mucosa. The results were comparable to that of ranitidine, a standard anti-ulcer drug.

Sannomiya *et al.* (2005) conducted studies on the anti-ulcerogenic activity of the hydromethanolic, methanolic and chloroformic extracts of *Brysonima crassa* leaves. It could be concluded that polar extracts provided better anti-ulcer activity than the apolar extract. Phytochemical evaluation indicated the presence of flavonoids like amentoflavone, catechins and quercetin derivatives which are believed to afford protection against gastric ulcers, owing to their potent free radical scavenging effect.

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

The study was conducted in adult albino rats weighing 150-200g of either sex purchased from the Small Animal Breeding Station, Kerala Agricultural University, Mannuthy. The animals were reared under standardized environmental conditions (22 to 28^0 C, 60-70 per cent relative humidity, 12hr dark/light cycle) and fed with standard rat feed and water *ad libitum*.

3.2. PREPARATION AND ADMINISTRATION OF DRUGS

3.2.1. Preparation of alcoholic extract

The leaves of *Azadirachta indica* and *Eupatorium triplinerve* were collected fresh and were dried in the shade at room temperature. The dried leaves were then powdered well in a pulveriser. The powdered leaves were subjected to extraction using 70 per cent ethanol in a Soxhlet apparatus for 16 hours. The liquid extract so obtained was collected in a wide mouthed vessel and the solvent was allowed to evaporate by keeping them in a water bath at low temperature so as to obtain a semi-solid/solid residue. The yield of the extract was 18 percent and 18.6 per cent for *A.indica* and *E.triplinerve* respectively. The crude extract thus prepared was kept in the refrigerator at 4^0C for further use.

3.2.2. Preparation of powder

The leaves of *Azadirachta indica* and *Eupatorium triplinerve* were collected fresh and were dried in shade at room temperature. The dried leaves were then powdered well in a pulveriser. The powder was dispersed in five percent gum acacia using Teflon homogenizer at a speed of 2500rpm. Administration was effected in a volume of 5ml/kg, once daily for 20 days. To ensure accurate dosing, suspension was prepared fresh and administered by slow syringing through the orogastric tube.

Fig. 1. *Azadirachta indica* (Neem)

Fig. 2. *Eupatorium triplinerve* (Ayappana)

3.2.3. Preparation of aspirin for ulcer induction

Aspirin (Marco Pharmaceuticals) was purchased locally and 200mg was suspended in two ml of five percent gum acacia. The suspension contained 100mg/ml of aspirin and was administered orally at the dose of 200mg/kg bodyweight for each rat.

3.2.4. Preparation of Famotidine for oral administration

Famotidine (Torrent Pharmaceuticals Ltd) 40mg tablets were purchased locally and suspended in two ml of five percent gum acacia. The suspension contained 20mg of famotidine/ml and was given orally at the dose of 40mg/kg bodyweight for each rat.

3.3. EXPERIMENTAL DESIGN

Ninety six rats of either sex were used for the study. They were divided into twelve groups with each group consisting of eight rats.

3.3.1. Treatment schedule

Group 1 : Vehicle alone i.e. gum acacia was administered at the dose of 5ml/kg body weight for 7 days. On eighth day, rats were sacrificed and normal gastric mucosa was studied.

Group 2 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day, rats were sacrificed and ulcer index assessed (Manekar and Waghmare,1980)

Group 3 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, feed and water were given *ad lib*. On 28^{th} day, rats were sacrificed and assessed the natural healing.

Group 4 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth

day onwards, Famotidine at the dose of 40mg/kg was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 5 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, alcoholic extract of *Azadirachta indica* leaves at a dose of 250mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 6 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, alcoholic extract of *Azadirachta indica* leaves at a dose of 500mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 7 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, powdered leaves of *Azadirachta indica* at a dose of 500mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 8 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, powdered leaves of *Azadirachta indica* at a dose of 1000mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 9 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, alcoholic extract of *Eupatorium triplinerve* leaves at a dose of 250mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 10 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, alcoholic extract of *Eupatorium triplinerve* leaves at a dose of 500mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 11 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, powdered leaves of *Eupatorium triplinerve* at a dose of 500mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

Group 12 : Aspirin was administered at the dose of 200mg/kg body weight for seven days. Feed was restricted and water given *ad lib*. On eighth day onwards, powdered leaves of *Eupatorium triplinerve* at a dose of 1000mg/kg body weight was given orally for 20 days. On 28th day, rats were sacrificed and healing index was assessed.

The animals were sacrificed and stomach dissected out. The pyloric end was ligated and ten percent formal saline was injected through the oesophageal end and then that end was also ligated. The stomachs were dissected along the greater curvature and examined under a magnifying lens to determine the incidence and severity of ulceration.

The ulcer index and healing index was calculated after sacrificing the rats using the method employed by Sahni *et al.* (1990).

$$\text{Ulcer index} = \text{Number of ulcers} + \text{Severity of Ulceration} + \frac{\% \text{ incidence}}{\text{no.of rats}}$$

$$\text{Healing index} = \frac{\text{Ulcer index (control)}^* - \text{Ulcer index (drug)}}{\text{Ulcer index (control)}^*} \times 100$$

(control)*- rats given aspirin for seven days and sacrificed on eighth day.

Severity of ulceration was assessed by a method employed by Gupta *et al.* (1988) (arbitrary scoring system).

(i).denuded epithelium=10; (ii).petechial and frank haemorrhage=20; (iii).One or two ulcers=30; (iv).multiple ulcers=40; (v).perforated ulcers=50.

The data obtained were compared with those of standard anti-ulcer drug, famotidine.

3.4. PHYTOCHEMICAL SCREENING

The alcoholic extracts of *Azadirachta indica* and *Eupatorium triplinerve* were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

3.4.1. Test for Detection of Steroids

3.4.1.1. Salkowski Test

About five mg of extract was dissolved in three ml of chloroform and then shaken with three ml of concentrated sulphuric acid. Development of a red colour indicates the presence of steroids.

3.4.1.2.Lieberman Burchardt Test

About five mg of the extract was dissolved in three ml of chloroform. Then five drops of acetic anhydride and one ml of concentrated sulphuric acid was added to it through the sides. A reddish ring at the junction of two layers indicates the presence of steroids.

3.4.2. Test for Detection of Alkaloids

About 0.5 g of the extract was mixed with five ml ammonia and then extracted with equal volume of chloroform. To this 0.1 N hydrochloric acid was added. The acid layer obtained was used for chemical test for the alkaloids.

3.4.2.1. Mayer's Test (potassium mercuric iodide)

To one ml of the acid layer obtained few drops of Mayer's reagent were added. If a creamy white precipitate was formed, it indicates the presence of alkaloids.

3.4.2.2. Hager's Test (saturated solution of picric acid)

To one ml of acid layer, few drops of Hager's reagent were mixed. A yellow precipitate is formed when alkaloids are present.

3.4.2.3. Dragendroff's Test (Solution of potassium bismuth iodide)

Two drops of Dragendroff's reagent was mixed with one ml of acid layer. Presence of alkaloids is indicated if a reddish brown precipitate is seen.

3.4.3. Tests for Detection of Tannins

3.4.3.1. Ferric Chloride Test

Two milligram of extract was mixed with three ml of one per cent ferric chloride solution .If blue green or brownish green colour is obtained it indicates the presence of tannins.

3.4.4. Tests for Detection of Flavonoids

3.4.4.1. Ferric chloride Test

To two ml of alcoholic solution of extract (0.5g extract in 10ml methanol) few drops of neutral ferric chloride solution was mixed. Presence of flavonoids is indicated by green colour.

3.4.4.2. Lead Acetate Test

To two ml of alcoholic solution of extract, (0.5g extract in 10 ml methanol), few drops of 10 per cent lead acetate was mixed. Yellow precipitate indicates the presence of flavonoids.

3.4.5. Tests for Presence of Glycosides

3.4.5.1. Benedict's Test

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

3.4.5.2. Sodium Hydroxide Test

Dissolved a small amount of the extract (about five mg) in one ml water and added five to six drops of sodium hydroxide solution. Yellow colour indicates the presence of glycosides.

3.4.6. Tests for Presence of Phenolic Compounds

About five mg of the extract was dissolved in one ml of water and five drops of ten percent ferric chloride solution was added to it. Development of dark brown colour occurs if phenolic compounds are present.

3.4.7. Tests for Detection of Diterpenes

About five mg of the extract was mixed with three ml of copper acetate solution. Presence of diterpenes is indicated by the development of green colour.

3.4.8. Tests for Presence of Triterpenes

3.4.8.1. Salkowski Test

About three mg of extract was dissolved in three ml chloroform and then shaken with concentrated sulphuric acid. Lower layer turning yellow on standing indicates presence of triterpenes.

3.4.8.2. Liebermann Burchardt Test

Few drops of acetic acid and one ml concentrated sulphuric acid were added to 30ml chloroform solution of the extract (about three mg of extract in three ml chloroform). Deep red ring at the junction of the two layers indicates presence of triterpenes.

3.4.9. Test for Presence of Saponins

3.4.9.1. Foam Test

A small amount of extract (about five mg) was shaken with three ml of water. If the foam produced persists for 10 minutes, presence of saponins is confirmed.

3.5. COLLECTION OF BIOLOGICAL SAMPLES

3.5.1. Blood

Blood was collected from retroorbital plexus in the inner canthus of the eye under light ether anaesthesia using sodium heparinised capillary tubes (microhaematocrit capillaries). Blood was collected in fresh vials containing disodium salt of Ethylene Diamine Tetra Acetic acid (EDTA, 1mg/ml) as anticoagulant.

3.5.2. Serum

Blood was collected in fresh vials without any anticoagulant and kept at room temperature for one hour. Then it was centrifuged at 2000 rpm for 20 minutes. The serum was aspirated into another vial and used for alkaline phosphatase estimation.

3.5.3. Gastric mucosa

The animals were euthanized and dissected upon and the stomach was collected. After the measurement of gastric lesions, the mucosa of the glandular stomach was removed by scrapping with a blunt scalpel blade and subjected to biochemical analysis.

3.6. ESTIMATION OF BIOCHEMICAL PARAMETERS

3.6.1. Measurement of lipid peroxide level (Okhawa *et al.*, 1979).

Reagents

Potassium chloride 150mM

Sodium dodecyl sulphate 8.1 per cent (SDS)

Acetic acid 20 per cent; pH adjusted to 3.5

Aqueous solution of Thiobarbituric acid 0.8 per cent (TBA)

n-butanol :pyridine mixture (15:1)

Procedure

1. One gram of tissue was mixed with 9 ml of 150 mM potassium chloride and homogenized in a tissue homogenizer
2. Tissue homogenates (0.2ml) were taken in test tubes, added 0.2 ml of 8.1 percent SDS, 1.5 ml of 20 per cent acetic acid and 1.5 ml of TBA. Blank contained 0.2ml of potassium chloride instead of tissue homogenate.
3. Made up the volume to 4 ml with distilled water and heated on a water bath at 95^0 C for 60 minutes.
4. Added 1ml of distilled water and 5ml of n-butanol: pyridine mixture. It was shaken well and centrifuged at 15xg for 10 minutes.
5. The absorbance of the colour of the organic layer was measured at 532nm.
6. The lipid peroxide level was calculated by using extinction coefficient of $1.56x10^5$ and the values were expressed in nmol of malondialdehyde (MDA)/ g of wet tissue.

3.6.2. Measurement of superoxide dismutase level (Mimami and Yoshikawa., 1979).

Reagents

Sodium chloride

Tris cacodylic acid buffer (50mM, pH8.2)

Tris cacodylic acid 50mM

Diethylene triamine penta acetic acid

Nitroblue tetrazolium 0.1 M

Triton X 100 (0.001 percent).

All reagents were mixed in equal quantities and the pH was adjusted to 8.2 using 0.1N sodium hydroxide.

Pyrogallol 0.2mM

Procedure

1. Freshly excised liver was homogenized with 10 volumes of 0.9 per cent sodium chloride followed by centrifugation at 15xg for 10 minutes at 4^0C to harvest the supernatant.
2. The assay mixture in a total volume of 3ml consists of 1.4 ml of 50mM tris cacodylic acid buffer, 1.4ml of 0.2mM pyrogallol and 0.2ml of enzyme preparation.
3. Blank contained distilled water instead of enzyme preparation.
4. The absorbance due to autooxidation of pyrogallol was read at 420nm using spectrophotometer.
5. One unit of SOD activity was the amount of enzyme that inhibited pyrogallol autooxidation by 50 per cent under experimental conditions .
6. The values were expressed in units/mg of protein after quantifying the protein content of supernatant by method of Lowry *et al.* (1951).

3.6.3. Esitmation of catalase (Cohen *et al.*, 1970).

Reagents

Phosphate buffer- hydrogen peroxide solution(10mM)

Phosphate buffer (0.05M pH 7.0)

0.2M sodiumdihydrogen phosphate 39ml

0.2M disodium hydrogen phosphate 61ml

Distilled water 300ml

Immediately before use 0.2ml of hydrogen peroxide was added to 100ml buffer.

Procedure

1. Three ml of the phosphate buffer – hydrogen peroxide solutions were taken in test tubes.
2. Blank contained distilled water instead of hydrogen peroxide solution.
3. Samples prepared in sodium chloride (as described in case of superoxide dismutase) were added to both and the absorbance was read at 240nm at the 40^{th} second of addition of the sample.
4. The time required for the initial absorbance to decrease by 0.05 units was noted.
5. The catalase activity in units / assay mixture was calculated by using the formula logE1/E2X2300/6.93X1/Δt.

E1 – Initial absorbance

E2 – Absorbance after decrease by 0.05 units

Δt – Time taken for the decrease in absorbance by 0.05 units (seconds)

3.7. ESTIMATION OF ALKALINE PHOSPHATASE

3.7.1. Principle

Estimation of alkaline phosphatase in serum was carried out according to the recommendations of Bergmeyer (1972). The rate of increase in 4 –nitrophenolate is determined photo metrically and is directly proportional to the activity of ALP in the sample. The reagent contains 4-nitro phenyl phosphate which is converted into phosphate and 4-nitro phenolate by ALP.

3.7.2. Procedure

1.20µl serum dispensed into serum tubes .

2.1000µl of reagent solution dispensed into serum tubes contain serum.

3. Mixed well and kept it for 3 minutes.

4. After 3 min. reading was taken at 405 nanometer.

5. The enzyme activity is calculated by the following formula.

$$\text{Enzyme activity(U/L)} = (\Delta A/\text{min}) \times 2754$$

U- Units ; L-Litre ; ΔA/min- Absorbance/minute

3.8. BODY WEIGHT

The body weight of rats of both the treated and control groups were taken and recorded before the commencement of the experiment, at weekly intervals, during the experiment and at the end of the experiment and data was analyzed.

3.9. HAEMATOLOGICAL PARAMETERS

3.9.1. Total leukocyte count

The leukocytes were counted by standard dilution technique using Thomas fluid diluent. Counting of cells was done in the zone for leukocytes in the haemocytometer placed under low power of the microscope (Benjamin, 1985).

3.9.2. Differential leukocyte count

Blood smears were prepared from freshly drawn blood without anticoagulant by using slide technique. After staining with Wright's stain, counting was done under oil immersion (Benjamin, 1985).

3.9.3. Haemoglobin concentration

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

3.10. GROSS AND HISTOPATHOLOGICAL EXAMINATION OF GASTRIC MUCOSA

3.10.1. Gross lesions

The gross lesions in the gastric mucosa of treated groups were compared with the control group. Representative samples were collected for histopathological examination.

3.10.2. Histopathological examination

Representative samples of gastric mucosa obtained from the dissected animals were fixed in 10 percent neutral buffered formalin. They were then processed and paraffin embedded as described by Sheehan and Hrapchak, 1980. The sections were stained with Haematoxylin and Eosin as per the technique followed by Bancroft and Cook, 1984. The sections were examined in detail under light microscope and lesions were classified.

4. RESULTS

Results obtained were tabulated and presented in Tables and the statistical analysis was done using One way ANOVA as described by Snedecor and Cochran (1985). Results are expressed as mean ± standard error.

All the plant preparations under study significantly reduced the ulcer index of aspirin treated control. The ulcer index and healing index of all the plant preparations, famotidine, natural healing and control groups were determined, tabulated and presented in Tables 1-11 and graphically represented in Figures 3 to 6b.

4.1. PHYTOCHEMICAL SCREENING

4.1.1 Steroids

As per Salkowski test, red colour was obtained and Lieberman Burchadt test gave a reddish ring at the junction for *Eupatorium triplinerve* but not for *Azadirachta indica al*coholic extract. Thus it could be concluded that steroids are present in alcoholic extract of *Eupatorium*, but no detectable level of steroids could be obtained in *Azadirachta* extract.

4.1.2. Alkaloids

A creamy white precipitate as per Mayer's test and a yellow coloured precipitate as per Hager's test was obtained for *Azadirachta* and *Eupatorium* extracts. Dragendroff's test yielded a reddish brown precipitate for the two extracts. Thus the tests revealed detectable levels of alkaloids in the alcoholic extracts of *Azadirachta* and *Eupatorium.*

4.1.3. Tannins

Brownish green colour was obtained in Ferric chloride test for *Azadirachta* extract but not for *Eupatorium*. The results indicated the presence of tannins in *Azadirachta* alcoholic extract.

4.1.4. Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in lead acetate test indicated presence of flavonoids in *Azadirachta* and *Eupatorium* extracts.

4.1.5. Glycosides

As per Benedict's test, red colour was obtained indicating the presence of glycosides in both the extracts. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent which also indicated the presence of glycosides.

4.1.6. Phenolic compounds

The extract mixed with ten percent ferric chloride produced dark brown colour indicating the presence of phenolic compounds for *Azadirachta* and *Eupatorium* extracts.

4.1.7. Diterpenes

Diterpenes were detected in *Azadirachta* and *Eupatorium* extracts as indicated by the green colour when mixed with copper acetate solution.

4.1.8. Triterpenes

For *Azadirachta* and *Eupatorium* extracts, lower layer turned to yellow on standing as per Salkowski test, and by Lieberman Burchadt test, a deep ring appeared at the junction of the two layers. Results indicated the presence of triterpenes in *Azadirachta* and *Eupatorium* extracts.

4.1.9. Saponins

In the foam test, foam persisted for ten minutes in the case of *Azadirachta* extract and not in the case of *Eupatorium* extract, indicating the presence of saponins in *Azadirachta* only.

4.2. ANTI-ULCER AND ULCER HEALING EFFECTS

The alcoholic extract and powder of *Azadirachta indica* and *Eupatorium triplinerve* showed tendency to reduce the ulcer index against aspirin-induced gastric ulcer in rats. Aspirin administration to rats produced gastric damage with an ulcer index of 57.25 ± 0.34 (Table 1).

The mean ulcer index score was reduced from 57.25 in the aspirin control group to 19.88 ($P<0.01$, 65.5% reduction) and 17.68 ($P<0.01$, 69% reduction) following treatment with 250mg/kg and 500mg/kg of *A.indica* alcoholic extract respectively (Tables 4 and 5). Similarly the *A.indica* powder at the doses of 500mg/kg and 1000mg/kg produced ulcer indices of 17.00 and 16.88 which indicated 70.3 and 70.5 percent reduction respectively ($P<0.01$) (Tables 6 and 7) (Fig.3).

The *E.triplinerve* alcoholic extract at the doses of 250mg/kg and 500mg/kg produced a relatively higher significant response, the ulcer index being reduced to 18.00 ($P<0.01$, 68.5% reduction) and 15.06 ($P<0.01$, 73.7% reduction) respectively (Tables 8 and 9). The *E.triplinerve* powder at the doses of 500mg/kg and 1000mg/kg showed 68.5% and 73.7% reduction ($P<0.01$) with mean ulcer indices of 18.00 and 15.06 (Tables 10 and11) (Fig.4).

The results indicated a dose-dependent decrease in ulcer index by both *A.indica* and *E.triplinerve*. Both the alcoholic extract and powder significantly decreased ($P<0.01$) the haemorrhagic lesions and provided dose-response characterization. All the groups under study produced effects comparable to that of famotidine at the dose of 40mg/kg which produced significant reduction (77.8%, $P<0.01$) with an ulcer index of 12.68 (Table 3). All experimental groups under study with the different plant preparations significantly lowered the ulcer indices

Effect of *A.indica* and *E.triplinerve* on Ulcer and Healing indices
(Tables 1-11, ** P<0.01; significantly different from Aspirin control)
Table 1. Aspirin 200mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index
1	4	40	100	56.50
2	4	40	100	56.50
3	6	40	100	58.50
4	4	40	100	56.50
5	4	40	100	56.50
6	4	40	100	58.50
7	6	40	100	58.50
8	4	40	100	56.50
			Mean	**57.25****
			S.E	**0.34**

Table 2. Natural healing

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	3	40	87.5	53.93	5.79
2	3	40	87.5	53.93	5.79
3	3	40	87.5	53.93	5.79
4	0	10	87.5	20.93	63.42
5	3	40	87.5	53.93	5.79
6	2	30	87.5	42.93	25.00
7	3	40	87.5	53.93	5.79
8	3	40	87.5	53.93	5.79
			Mean	**48.43**	**15.40**
			S.E	**4.16**	**7.26**

Table 3. Famotidine 40 mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	0	0	25	3.12	94.54
2	0	0	25	3.12	94.54
3	2	30	25	35.12	38.64
4	0	0	25	3.12	94.54
5	0	0	25	3.12	94.54
6	1	20	25	24.12	57.86
7	0	0	25	3.12	94.54
8	0	0	25	3.12	94.54
			Mean	**12.68****	**77.85****
			S.E	**1.47**	**2.58**

Table 4. *A.indica* extract 250mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	2	20	50	28.25	50.65
2	0	10	50	17.25	69.86
3	1	10	50	17.25	69.86
4	2	20	50	28.25	50.65
5	0	10	50	16.25	71.61
6	0	10	50	16.25	71.61
7	3	20	50	19.25	66.37
8	0	10	50	16.25	71.61
			Mean	**19.88****	**65.28****
			S.E	**0.62**	**1.07**

Table 5. *A.indica* extract 500mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	0	10	37.50	14.68	74.34
2	1	10	37.50	15.68	72.59
3	0	10	37.50	14.68	74.34
4	1	20	37.50	25.68	55.13
5	0	10	37.50	14.68	74.34
6	0	10	37.50	14.68	74.34
7	0	10	37.50	14.68	74.34
8	2	20	37.50	26.68	53.38
			Mean	**17.68****	**69.10****
			S.E	**0.62**	**1.08**

Table 6. *A.indica* powder 500mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	1	10	50	17.25	69.86
2	1	10	50	17.25	69.86
3	1	10	50	16.25	71.61
4	1	10	50	16.25	71.61
5	1	10	50	16.25	71.61
6	2	20	50	18.25	68.12
7	1	10	50	16.25	71.61
8	2	20	50	18.25	68.12
			Mean	**17.00****	**70.30****
			S.E	**0.10**	**0.18**

Table 7. *A.indica* powder 1000mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	1	10	50	16.25	71.61
2	1	10	50	17.25	69.86
3	1	10	50	17.25	69.86
4	1	10	50	16.25	71.61
5	2	20	50	18.25	68.12
6	1	10	50	16.25	71.61
7	1	10	50	16.25	71.61
8	1	10	50	17.25	69.86
			Mean	**16.88****	**70.52****
			S.E	**0.26**	**0.15**

Table 8. *E.triplinerve* extract 250mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	1	10	50	17.25	69.86
2	1	10	50	17.25	69.86
3	0	10	50	16.25	71.61
4	0	10	50	16.25	71.61
5	1	10	50	17.25	69.86
6	0	10	50	16.25	71.61
7	0	10	50	16.25	71.61
8	1	20	50	27.25	52.4
			Mean	**18.00****	**68.55****
			S.E	**0.44**	**0.77**

Table 9. *E.triplinerve* extract 500mg/kg

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	1	10	37.50	15.68	72.59
2	0	10	37.50	14.68	74.34
3	0	10	37.50	14.68	74.34
4	1	10	37.50	15.68	72.59
5	0	10	37.50	14.68	74.34
6	0	10	37.50	15.68	72.59
7	0	10	37.50	14.68	74.34
8	0	10	37.50	14.68	74.34
			Mean	**15.06****	**73.68****
			S.E	**0.06**	**0.11**

Table 10. ***E.triplinerve*** **powder 500mg/kg**

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	1	10	50	17.25	69.86
2	1	10	50	27.25	52.4
3	1	10	50	16.25	71.61
4	1	10	50	16.25	71.61
5	1	10	50	17.25	69.86
6	1	10	50	17.25	69.86
7	1	10	50	16.25	71.61
8	1	10	50	16.25	71.61
			Mean	**18.00****	**68.55****
			S.E	**0.44**	**0.77**

Table 11. ***E.triplinerve*** **powder 1000mg/kg**

Sl.No.	No. of ulcers	Ulcer score	% of incidence	Ulcer index	Healing index
1	1	10	37.50	15.68	72.59
2	1	10	37.50	15.68	72.59
3	1	10	37.50	14.68	74.34
4	1	10	37.50	14.68	74.34
5	1	10	37.50	14.68	74.34
6	1	10	37.50	14.68	74.34
7	1	10	37.50	14.68	74.34
8	1	10	37.50	15.68	72.59
			Mean	**15.06****	**73.68****
			S.E	**0.06**	**0.11**

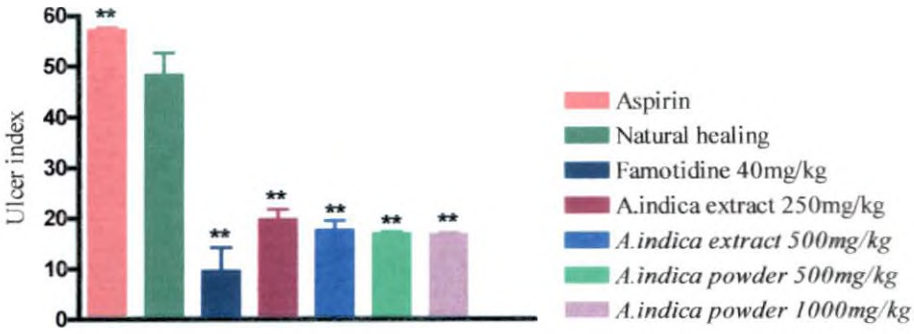


Fig.3.Comparative ulcer index of *A.indica* with Famotidine and control

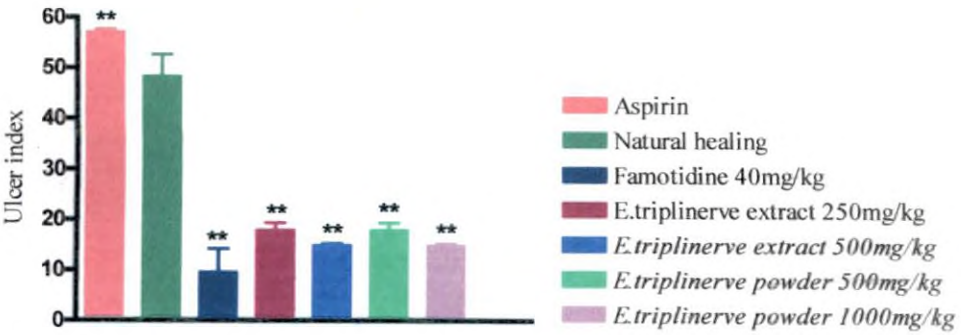


Fig.4.Comparative ulcer index of *E.triplinerve* with Famotidine and control

**P<0.01

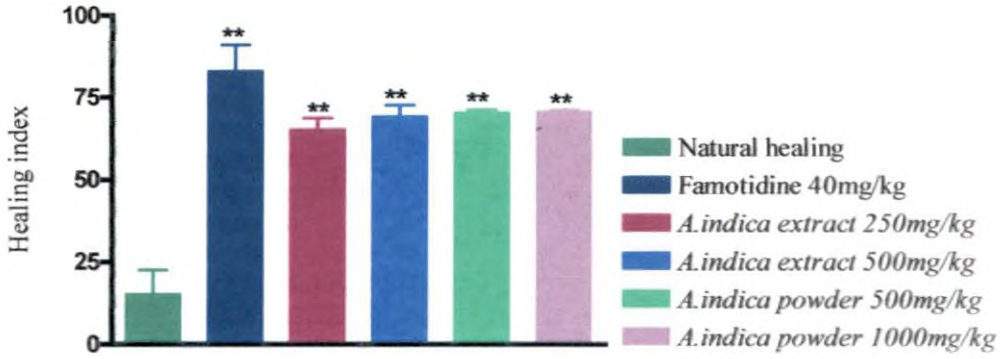


Fig.5a Comparative healing index of *A.indica* with natural healing

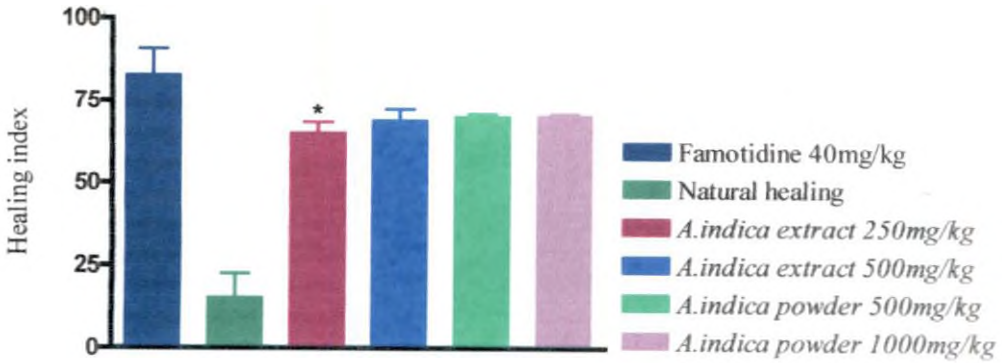


Fig.5b. Comparative healing index of *A.indica* with Famotidine

*P<0.05
**P<0.01

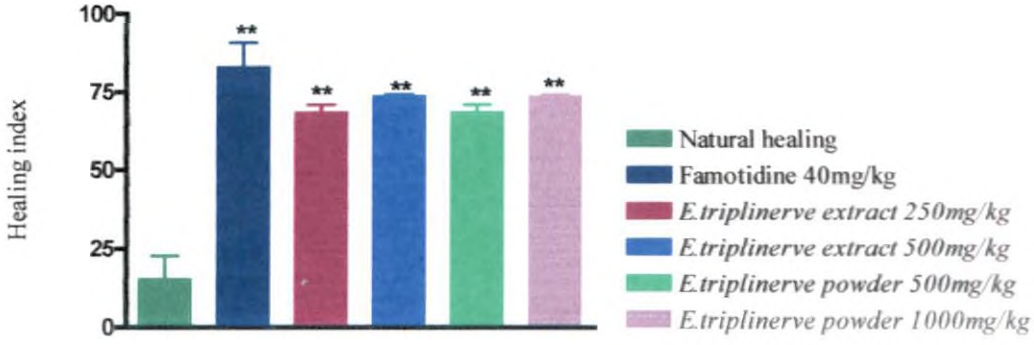


Fig.6a.Comparative healing index of *E.triplinerve* with natural healing

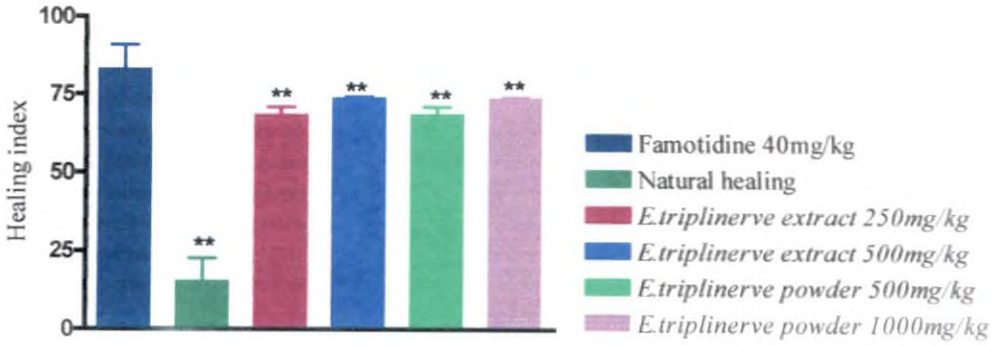


Fig.6b. Comparative healing index of *E.triplinerve* with famotidine

**P<0.01

when compared with the aspirin control group as well as the group subjected to natural healing (48.43±4.16) (Table 2).

The healing effect produced by alcoholic extract of *A.indica* at the dose of 500mg/kg (69.10 ±1.08) (Table 5); *A.indica* powder at the dose of 500mg/kg (70.30 ±0.18) (Table 6) and 1000mg/kg (70.52±0.15) (Table 7) were found to be comparable to famotidine, the standard anti-ulcer drug at the dose of 40mg/kg (77.85 ± 2.58) (Table 3) (Fig.5b). The alcoholic extract at the dose of 250mg/kg (65.28 ±1.07) (Table 4) produced significant healing effect comparable to the famotidine administered group (Fig.5b).

The *E.triplinerve* alcoholic extract produced healing effects at the dose of 500mg/kg (73.68 ± 0.11) (Table 9) as well as the powder at a dose of 1000mg/kg (73.68 ±0.11) (Table 11) which was comparable to the standard anti-ulcer drug, famotidine. The healing obtained with *E.triplinerve* alcoholic extract at the dose of 250mg/kg (68.55 ±0.77) (Table 8) and the powder at the dose of 500mg/kg (68.55 ± 0.77) (Table 10) were also comparable to the effect produced by famotidine, though comparatively lesser than the higher doses (Fig.6b).

Both *A.indica* and *E.triplinerve* preparations as well as famotidine produced significant healing effects when compared to the natural healing group (15.40 ± 7.26) (Table 2) (Fig.5a and 6a).

4.3. BIOCHEMICAL PARAMETERS

4.3.1. Anti-oxidant effect

4.3.1.1. Lipid peroxide level

Aspirin caused ulceration with significant increase in lipid peroxide (LPO) levels (495.30 ±22.40 nmol MDA/g, $P<0.01$) (Table 12). Aspirin induced ulceration caused about two fold increase in LPO levels when compared with that of control group (222.30±30.89). Free radical and lipid peroxidation play an important role in ulcerogenesis.

Table 12. Effect of *A.indica* on lipid peroxide levels (nmol/g)

SL.No.	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*A.indica* extract		*A.indica* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	202.80	499.20	349.00	298.00	312.00	187.20	187.20	171.60
2	187.20	436.80	312.00	204.00	296.40	156.00	171.60	320.00
3	171.60	499.20	298.20	186.00	265.20	249.60	265.20	156.00
4	327.60	546.00	348.00	271.00	187.20	202.00	187.20	208.40
Mean	**222.30**	**495.30****	**326.80**	**239.75****	**265.20****	**198.70****	**202.80****	**214.00****
S.E	**30.89**	**22.40**	**11.12**	**26.67**	**27.76**	**19.49**	**21.12**	**37.00**

** P<0.01; significantly different from Aspirin control

Table 13. Effect of *E.triplinerve* on lipid peroxide levels (nmol/g)

SL.No.	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*E.triplinerve* extract		*E.triplinerve* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	202.80	499.20	349.00	298.00	187.20	171.60	187.20	171.60
2	187.20	436.80	312.00	204.00	327.60	156.00	202.00	181.00
3	171.60	499.20	298.20	186.00	171.60	187.20	212.00	249.00
4	327.60	546.00	348.00	271.00	187.60	327.60	219.60	240.00
Mean	**222.30**	**495.30****	**326.80**	**239.75****	**218.50****	**210.60****	**205.20****	**210.40****
S.E	**30.89**	**22.40**	**11.12**	**26.67**	**36.56**	**39.52**	**7.00**	**19.87**

** P<0.01; significantly different from Aspirin control

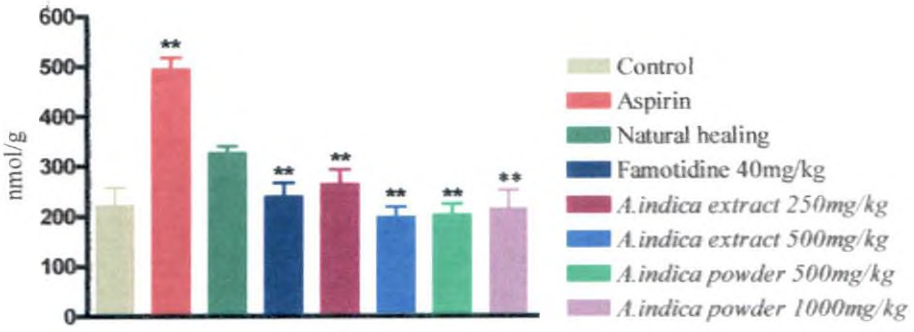


Fig.7.Effect of *A.indica* on lipid peroxide levels

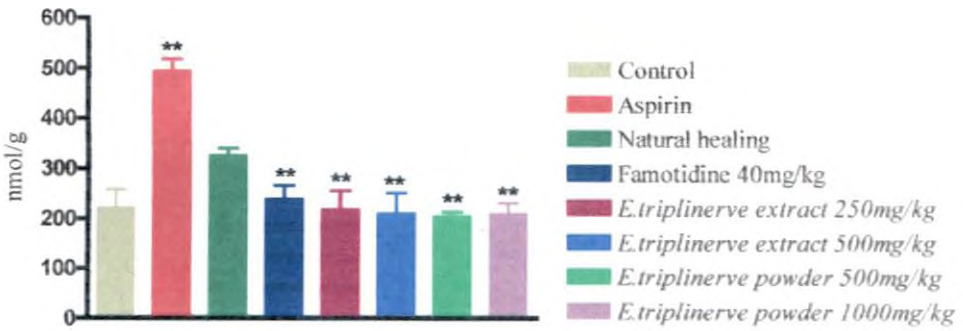


Fig.8.Effect of *E.triplinerve* on lipid peroxide levels

** P<0.01

The treatment groups showed significant reduction in lipid peroxide levels when compared with the animals administered aspirin. The LPO levels were significantly reduced following treatment with 250mg/kg and 500mg/kg of alcoholic extract of *A.indica* with the values close to normal, 265.20±27.76 (P<0.01) and 198.70 ±19.49 (P<0.01) respectively, while the powder at 500mg/kg and 1000mg/kg produced values of 202.80±21.12 (P<0.01) and 214.00 ±37.00 (P<0.01) respectively (Table 12) (Fig.7). Similarly the alcoholic extract and powder of *E.triplinerve* at the two dose levels mentioned produced significant reduction in LPO levels, 218.5± 36.56 (P<0.01), 210.60 ±39.52 (P<0.01), 205.20 ± 7.00 (P<0.01) and 210.40±19.87 (P<0.01) respectively (Table 13) (Fig.8). The reference anti-ulcer drug, famotidine, also exhibited significant anti-oxidant properties as seen from the reduced LPO levels (239.75±26.67) (P<0.01), whereas in the natural healing group the LPO levels were elevated (326.80 ±11.12) as in the aspirin control group.

The results indicate that the treatment groups showed potent inhibition in the tissue lipid peroxidation activities, which indicate their potent anti-oxidant properties.

4.3.1.2. Superoxide dismutase

The values of superoxide dismutase (SOD) obtained are presented in Tables 14, 15 and Figures 9, 10. A significant increase in the SOD level was noted in all the treatment groups compared to the aspirin control (49.74±2.58) (P<0.01). Following treatment with *A.indica* extract at 250mg/kg and 500mg/kg and the powder at 500mg/kg and 1000mg/kg, the SOD levels were found to be 62.94±3.99 (P<0.01), 75.37 ±3.33 (P<0.01), 58.16±3.10 (P<0.01) and 59.88±6.63 (P<0.01) respectively (Table 14) (Fig.9). Elevated levels of SOD were obtained with treatment of *E.triplinerve* extract and powder at the two dose levels, 78.75±4.97 (P<0.01), 79.56±3.22 (P<0.01), 78.06±3.09 (P<0.01) and 78.91±5.70 (P<0.01) respectively (Table 15) (Fig.10). Similarly treatment with famotidine brought about significant increase in SOD levels (71.80±3.71) (P<0.01), while in the natural healing group the SOD levels (49.20 ±1.23) were considerably reduced.

Table 14. Effect of *A.indica* on superoxide dismutase levels (U/mg of protein)

Sl.No.	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*A.indica* extract		*A.indica* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	105.00	50.27	45.81	82.25	73.84	84.54	52.22	50.26
2	90.96	56.03	48.00	68.21	61.81	75.00	66.86	48.00
3	82.45	43.46	52.10	71.50	61.42	73.20	57.27	76.25
4	75.87	49.20	50.90	65.23	54.67	68.72	56.27	65.00
Mean	**88.57**	**49.74****	**49.20**	**71.80****	**62.94****	**75.37****	**58.16****	**59.88****
S.E	**5.45**	**2.58**	**1.23**	**3.71**	**3.99**	**3.33**	**3.10**	**6.63**

** P<0.01; significantly different from Aspirin control

Table 15. Effect of *E.triplinerve* on superoxide dismutase levels (U/mg of protein)

Sl.No.	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*E.triplinerve* extract		*E.triplinerve* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	105.00	50.27	45.81	82.25	69.28	88.92	81.85	84.05
2	90.96	56.03	48.00	68.21	76.20	78.00	80.46	92.22
3	82.45	43.46	52.10	71.50	92.75	74.25	81.08	66.88
4	75.87	49.20	50.90	65.23	76.76	77.07	68.83	72.50
Mean	**88.57**	**49.74****	**49.20**	**71.80****	**78.75****	**79.56****	**78.06****	**78.91****
S.E	**5.45**	**2.58**	**1.23**	**3.71**	**4.97**	**3.22**	**3.09**	**5.70**

** P<0.01; significantly different from Aspirin control

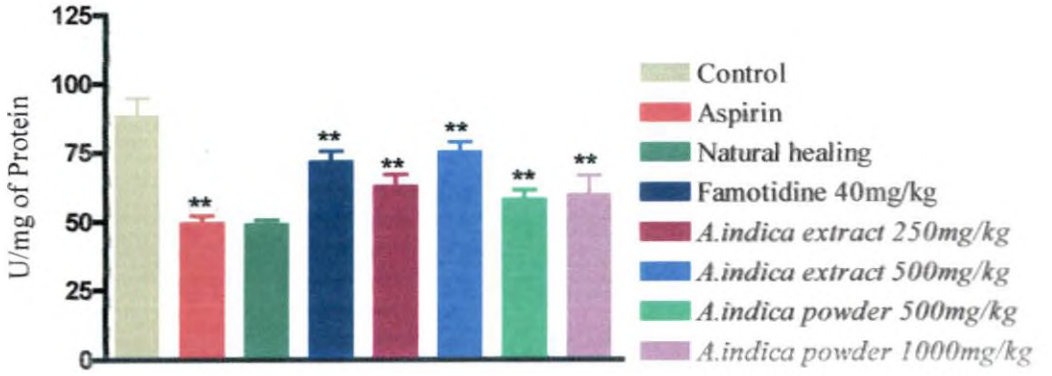


Fig.9.Effect of *A.indica* on superoxide dismutase levels

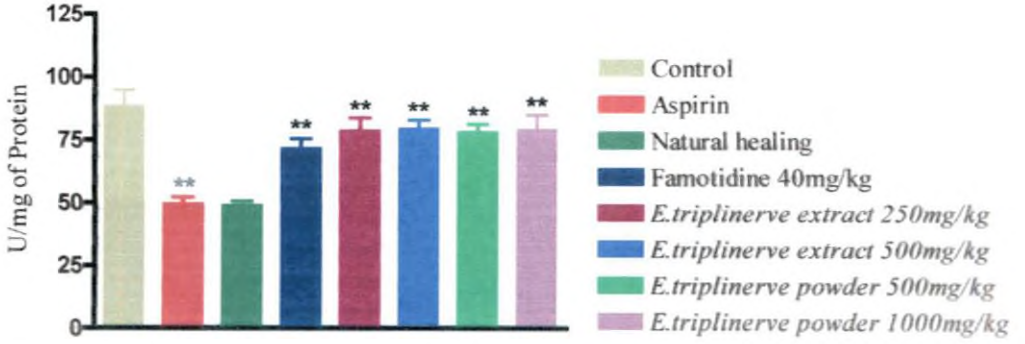


Fig.10.Effect of *E.triplinerve* on superoxide dismutase levels

** P<0.01

The plant preparations significantly protected the animals against aspirin induced free radical damage as seen from the increase in SOD levels which were found to be closer to the normal level.

4.3.1.3. Catalase

The values of catalase are given in Tables 16, 17 and Figures 11, 12. There was significant increase in the mean catalase (CAT) level of treatment groups when compared with the aspirin control (15.49±0.98) ($P<0.01$). The observed CAT values of the *A.indica* extract at the dose levels of 250mg/kg, 500mg/kg and powder at the dose levels of 500mg/kg and 1000mg/kg were 33.10±1.03 ($P<0.01$), 34.23±1.14 ($P<0.01$), 33.69±1.51 ($P<0.01$) and 35.08±1.61 ($P<0.01$) respectively (Table 16) (Fig.11). The CAT values of similar doses of *E.triplinerve* extract and powder were 31.90 ± 0.64 ($P<0.01$), 33.73±0.84 ($P<0.01$), 33.44±1.66 ($P<0.01$) and 34.75±1.42 ($P<0.01$) respectively (Table 17) (Fig.12). Famotidine administration also brought about pronounced elevation in CAT levels (32.73±2.18) ($P<0.01$) whereas natural healing reduced the CAT levels (19.70±0.48) considerably.

The significant increase in CAT levels indicates the potent anti-oxidant properties of the plant preparations.

The results indicate that aspirin administration was found to increase lipid peroxidation and decrease SOD and CAT, thus leading to oxidative stress. Administration of the different plant preparations at the various dose levels mentioned, brought about a significant reduction in lipid peroxidation and an increase in the activities of anti-oxidant enzymes namely, SOD and catalase.

4.3.2. Serum Alkaline Phosphatase estimation

The results obtained are presented in Tables (18a) and (18b). The mean serum alkaline phosphatase activity was increased in the aspirin treated group (154.63±7.11), whereas the control group showed alkaline phosphatase activity of 90.38±2.44. The treatment groups showed significant reduction in the alkaline phosphatase activity when compared to the aspirin control group ($P<0.01$).

Table 16. Effect of *A.indica* on catalase levels (Units)

SL.No.	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*A.indica* extract		*A.indica* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	33.10	18.20	18.60	30.60	32.10	37.30	31.74	33.10
2	39.40	15.61	20.20	27.80	36.00	34.50	36.41	32.50
3	45.50	14.22	21.00	39.20	33.00	32.10	30.50	35.10
4	43.61	13.91	19.01	31.30	31.30	33.00	36.10	39.60
Mean	**40.40**	**15.49****	**19.70**	**32.23****	**33.10****	**34.23****	**33.69****	**35.08****
S.E	**2.38**	**0.98**	**0.48**	**2.44**	**1.03**	**1.14**	**1.51**	**1.61**

** P<0.01; significantly different from Aspirin control

Table 17. Effect of *E.triplinerve* on catalase levels (Units)

SL.No.	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*E.triplinerve* extract		*E.triplinerve* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	33.10	18.20	18.60	30.60	30.94	33.30	30.74	38.00
2	39.40	15.61	20.20	29.80	30.64	35.50	30.61	33.50
3	45.50	14.22	21.00	39.20	33.10	31.60	37.30	31.50
4	43.61	13.91	19.01	31.30	32.90	34.50	35.10	36.00
Mean	**40.40**	**15.49****	**19.70**	**32.73****	**31.90****	**33.73****	**33.44****	**34.75****
S.E	**2.38**	**0.98**	**0.48**	**2.18**	**0.64**	**0.84**	**1.66**	**1.42**

** P<0.01; significantly different from Aspirin control

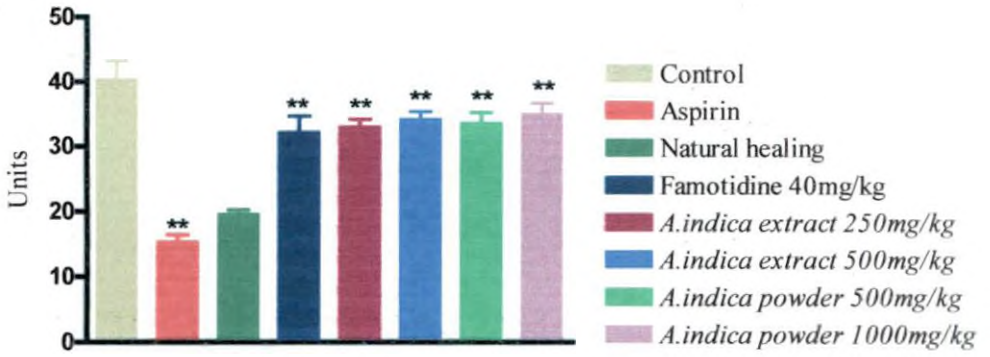


Fig.11.Effect of *A.indica* on catalase levels

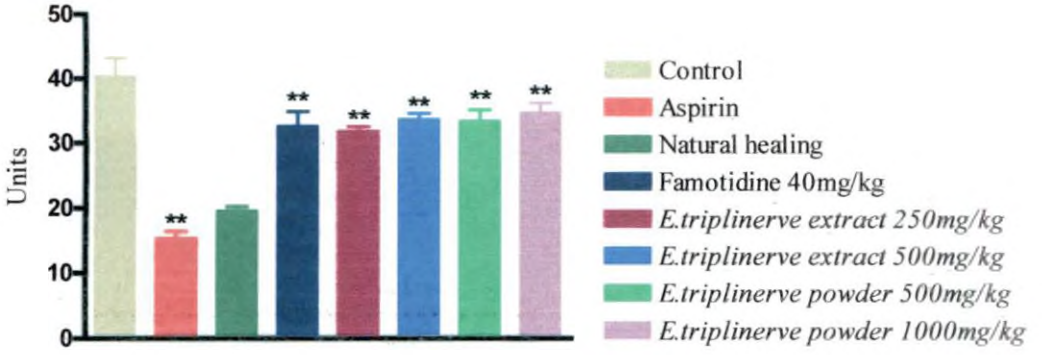


Fig.12.Effect of *E.triplinerve* on catalase levels

**P<0.01

Table 18a. Effect of *A.indica* on serum alkaline phosphatase levels (U/L)

SL.No	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*A.indica* extract		*A.indica* powder	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	98.00	196.00	88.00	87.00	114.00	84.00	121.00	78.00
2	86.00	152.00	135.00	94.00	101.00	85.00	88.00	113.00
3	95.00	130.00	113.00	89.00	97.00	105.00	101.00	87.00
4	88.00	140.00	103.00	100.00	111.00	102.00	112.00	91.00
5	78.00	160.00	110.00	99.00	120.00	90.00	98.00	101.00
6	90.00	140.00	111.00	88.00	88.00	93.00	112.00	113.00
7	101.00	160.00	128.00	95.00	86.00	87.00	103.00	89.00
8	87.00	159.00	138.00	101.00	125.00	99.00	95.00	90.00
Mean	**90.38**	**154.63****	**115.75**	**94.13****	**105.25****	**93.13****	**103.75****	**95.25****
S.E	**2.44**	**7.11**	**5.61**	**1.99**	**5.13**	**2.84**	**3.78**	**4.46**

** P<0.01; significantly different from Aspirin control

Table 18b. Effect of *E.triplinerve* on serum alkaline phosphatase levels (U/L)

SL.No	Control	Aspirin control	Natural healing	Famotidine 40mg/kg	*E.triplinerve extract*		*E.triplinerve powder*	
					250mg/kg	500mg/kg	500mg/kg	1000mg/kg
1	98.00	196.00	88.00	87.00	99.00	84.00	100.00	96.00
2	86.00	152.00	135.00	94.00	112.00	77.00	90.00	107.00
3	95.00	130.00	113.00	89.00	107.00	111.00	114.00	77.00
4	88.00	140.00	103.00	100.00	109.00	98.00	99.00	97.00
5	78.00	160.00	110.00	99.00	100.00	99.00	87.00	92.00
6	90.00	140.00	111.00	88.00	84.00	106.00	120.00	88.00
7	101.00	160.00	128.00	95.00	93.00	93.00	104.00	85.00
8	87.00	159.00	138.00	101.00	98.00	99.00	91.00	100.00
Mean	**90.38**	**154.63****	**115.75**	**94.13****	**100.25****	**95.88****	**100.63****	**92.75****
S.E	**2.44**	**7.11**	**5.61**	**1.99**	**3.23**	**3.92**	**4.14**	**3.32**

** P<0.01; significantly different from Aspirin control

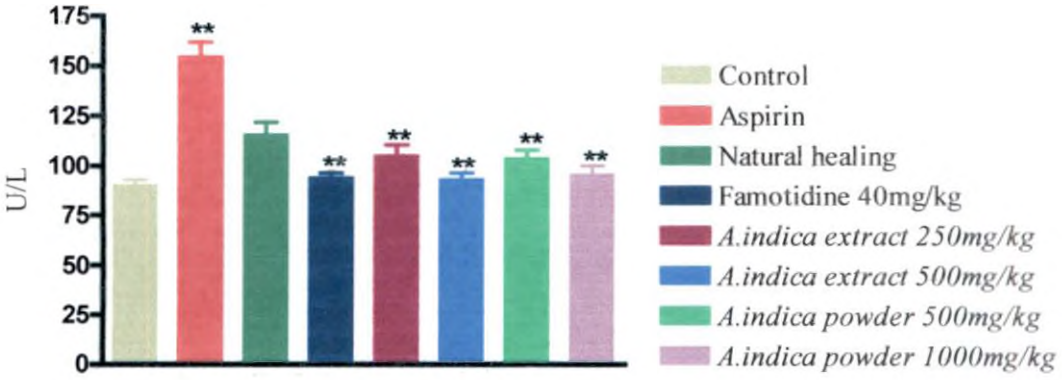


Fig.13.Effect of *A.indica* on alkaline phosphatase levels

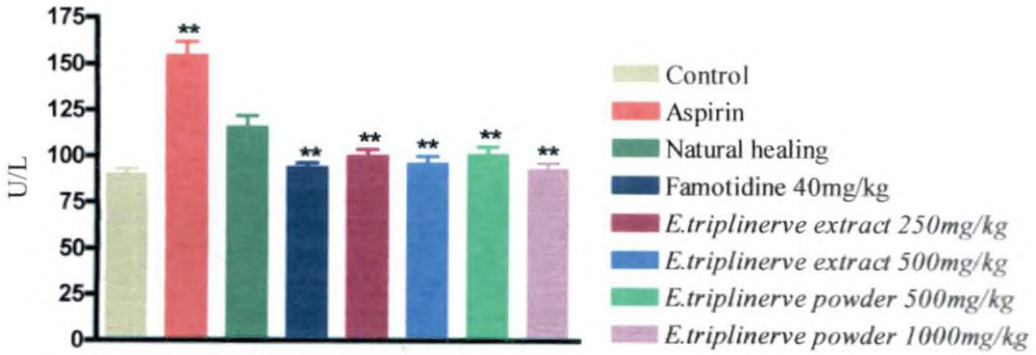


Fig.14.Effect of *E.triplinerve* on alkaline phosphatase levels

** P<0.01

A.indica treatment groups significantly reduced the enzyme levels to near normal, the values being 105.25± 5.13, 93.13±2.84, 103.75±3.78, 95.25±4.46 (Fig.13). The values of *E.triplinerve* groups were 100.25±3.23, 95.88± 3.92, 100.63±4.14 and 92.75±3.32 (Fig.14).

4.4. BODY WEIGHT

The results obtained are presented in Table 19 and Figure 15. There was no significant difference between the treatment and control groups in body weight.

4.5. HEAMOTOLOGICAL PARAMETERS

The study of haematological parameters of all the groups revealed no significant changes at $P<0.01$ and all the values fall within the normal range of blood values for the species under study. The details of the observations are given in (Tables 20 to 22).

4.6. HISTOPATHOLOGICAL STUDY

Grossly, in the aspirin administered group the lesions observed were petechial haemorrhages, shallow erosions and deep ulcers. The lesions were mostly petechial haemorrhages and shallow erosions. The haemorrhagic ulcers were linear, mostly occuring in the glandular corpus (Fig. 16).

Histopathological studies showed severe erosion of gastric mucosa, with necrotic patches, submucosal oedema and neutrophil infiltration. The mucosal ulcers revealed areas of coagulative necrosis characterized by capillary haemorrhages in the lamina propria, desquamation of the epithelial layer and development of a superficial area of ulceration. The lesions whether shallow erosion or deep ulcers were covered by an exudate consisting of mucus, fibrin and necrotic debris (Fig. 19).

Table 19. Effects of *A. indica* and *E. triplinerve* on body weight in Albino rats

Group			0 day	1st week	2 nd week	3rd week	4th week
1	Control	Mean	171.25	178.75	-	-	-
		S.E	5.43	5.71	-	-	-
2	Aspirin Control	Mean	172.50	180.00	-	-	-
		S.E	3.42	3.06	-	-	-
3	Natural Healing	Mean	175.00	178.75	186.25	193.75	201.25
		S.E	4.68	4.12	4.31	4.66	3.72
4	Famoditine 40mg/kg	Mean	175.00	177.50	185.00	195.00	200.00
		S.E	4.68	5.23	4.68	4.68	5.00
5	*A.indica* Extract(250mg/kg)	Mean	182.50	187.50	196.25	203.75	213.75
		S.E	4.24	4.24	3.51	3.03	3.03
6	*A.indica* Extract(500mg/kg)	Mean	180.00	186.25	193.75	200.00	207.50
		S.E	5.00	4.66	3.93	5.00	4.59
7	*A.indica* Powder (500mg/kg)	Mean	177.50	181.25	190.00	198.75	206.25
		S.E	5.52	5.98	5.30	5.13	5.28
8	*A.indica* Powder (1000mg/kg)	Mean	177.50	183.75	193.75	201.25	207.50
		S.E	4.24	4.98	4.98	4.12	4.92
9	*E.triplinerve* Extract(250mg/kg)	Mean	172.50	180.00	187.50	195.00	203.75
		S.E	4.92	5.30	4.24	5.00	4.98
10	*E.triplinerve* Extract(500mg/kg)	Mean	177.50	183.75	193.75	200.00	208.75
		S.E	5.52	5.57	5.57	4.68	4.12
11	*E.triplinerve* Powder(500mg/kg)	Mean	177.50	185.00	191.25	200.00	207.50
		S.E	5.52	5.00	4.49	4.68	3.42
12	*E.triplinerve* Powder(1000mg/kg)	Mean	175.00	183.75	190.00	198.75	205.00
		S.E	4.68	5.28	5.00	4.82	5.00

Mean;S.E;n=8

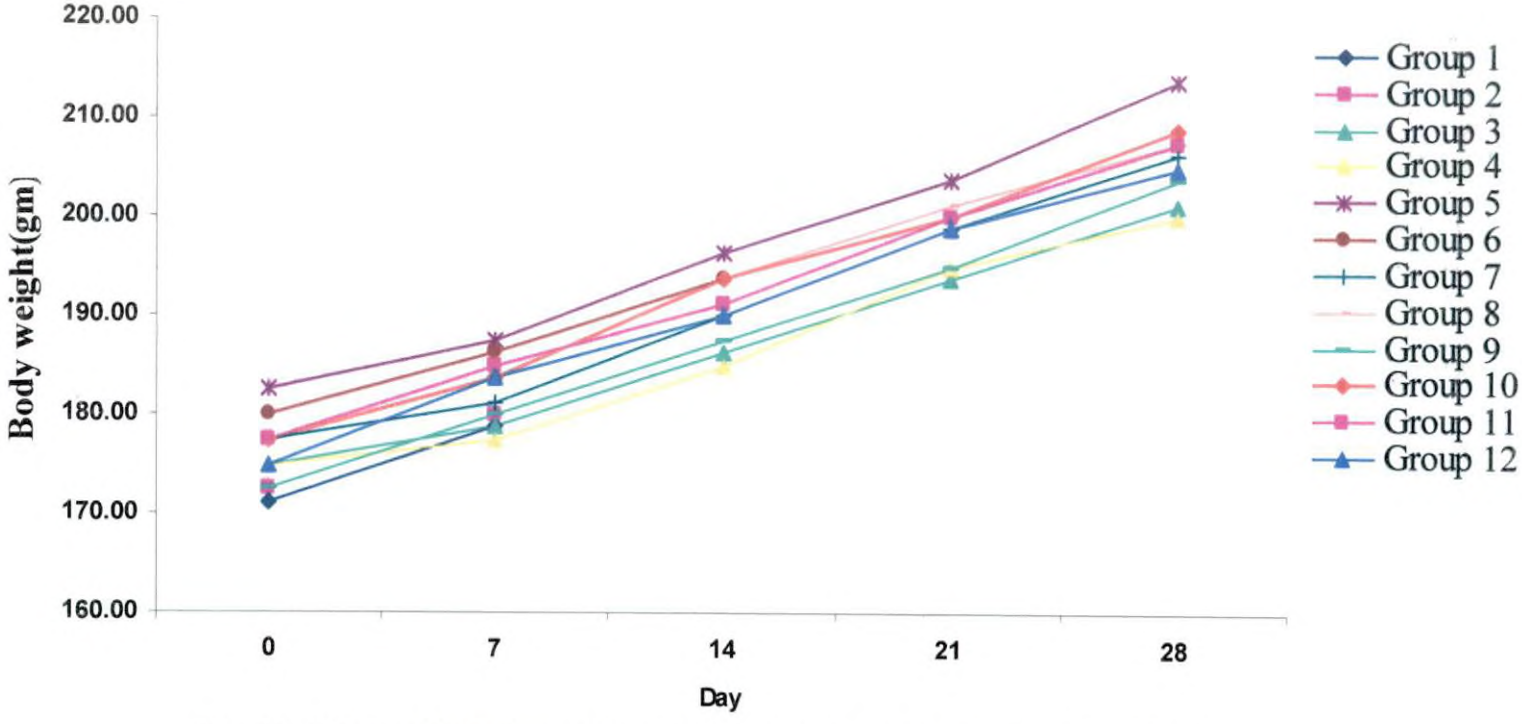


Fig.15. Effect of *A.indica* and *E.triplinerve* treatment on body weight

Table 20. Effect of *A.indica* and *E.triplinerve* on haematological parameters on day 1

Group		Leucocyte count 10^3/ cmm	Differential count (%)					Haemoglobin (gm%)
			Neutrophil	Lymphocyte	Monocyte	Basophil	Eosinophil	
1	Mean	11.07	18.50	79.88	0.75	0.13	0.75	13.76
	S.E	0.29	0.47	0.37	0.23	0.12	0.23	0.17
2	Mean	11.47	18.50	80.13	0.63	0.25	0.50	13.65
	S.E	0.38	0.31	0.21	0.25	0.15	0.18	0.19
3	Mean	11.25	18.38	80.25	0.50	0.25	0.63	13.68
	S.E	0.29	0.35	0.23	0.25	0.15	0.25	0.24
4	Mean	11.69	18.38	79.88	1.00	0.13	0.63	13.30
	S.E	0.32	0.30	0.33	0.25	0.12	0.25	0.17
5	Mean	10.88	18.63	79.63	0.88	0.25	0.63	13.18
	S.E	0.25	0.43	0.17	0.28	0.15	0.25	0.19
6	Mean	11.45	18.75	79.88	0.75	0.13	0.50	13.33
	S.E	0.31	0.46	0.21	0.29	0.12	0.25	0.21
7	Mean	11.03	19.25	79.75	0.75	0.13	0.13	13.40
	S.E	0.27	0.46	0.23	0.29	0.12	0.12	0.19
8	Mean	10.61	18.75	79.50	1.00	0.25	0.50	13.38
	S.E	0.19	0.49	0.18	0.31	0.15	0.25	0.18
9	Mean	11.40	18.88	79.25	1.13	0.25	0.50	13.45
	S.E	0.29	0.37	0.34	0.28	0.15	0.25	0.19
10	Mean	11.07	19.25	79.50	0.63	0.13	0.50	13.30
	S.E	0.30	0.39	0.25	0.17	0.12	0.25	0.20
11	Mean	11.08	19.38	79.38	0.63	0.25	0.38	13.35
	S.E	0.31	0.50	0.17	0.30	0.15	0.17	0.19
12	Mean	11.04	18.63	79.63	0.88	0.25	0.63	13.23
	S.E	0.26	0.43	0.17	0.28	0.15	0.25	0.22
Normal Range	Mean	9-10.0	15-40	50-80	0-3	0-1.5	0-5	10.8-17.5

Mean; S.E.; n=8

Table 21. Effect of *A.indica* and *E.triplinerve* on haematological parameters on day 7

Group		Leucocyte count 10^3/ cmm	Differential count (%)					Haemoglobin (gm%)
			Neutrophil	Lymphocyte	Monocyte	Basophil	Eosinophil	
1	Mean	11.34	18.63	80.13	0.63	0.13	0.50	13.80
	S.E	0.31	0.35	0.33	0.25	0.12	0.18	0.17
2	Mean	11.79	18.88	79.75	0.50	0.38	0.50	13.35
	S.E	0.37	0.45	0.29	0.25	0.17	0.25	0.21
3	Mean	12.65	18.63	79.63	0.88	0.25	0.63	13.45
	S.E	0.44	0.43	0.17	0.28	0.15	0.25	0.24
4	Mean	11.32	18.75	79.75	1.25	0.13	0.13	13.15
	S.E	0.40	0.34	0.15	0.29	0.12	0.12	0.19
5	Mean	11.14	19.38	79.75	0.63	0.13	0.13	13.40
	S.E	0.33	0.35	0.23	0.25	0.12	0.12	0.20
6	Mean	11.12	19.50	78.63	0.75	0.38	0.75	13.25
	S.E	0.21	0.31	0.30	0.23	0.17	0.29	0.18
7	Mean	11.38	19.38	79.63	0.63	0.13	0.25	13.40
	S.E	0.35	0.43	0.17	0.25	0.12	0.15	0.23
8	Mean	11.20	18.75	79.50	1.00	0.25	0.50	13.25
	S.E	0.30	0.49	0.18	0.31	0.15	0.25	0.21
9	Mean	10.85	18.88	79.75	0.63	0.25	0.50	13.20
	S.E	0.22	0.37	0.29	0.25	0.15	0.25	0.18
10	Mean	11.20	19.00	79.25	0.88	0.25	0.63	13.15
	S.E	0.35	0.40	0.39	0.21	0.15	0.25	0.15
11	Mean	11.07	19.00	79.38	0.75	0.13	0.75	13.13
	S.E	0.24	0.47	0.30	0.29	0.12	0.29	0.20
12	Mean	10.89	19.38	79.75	0.63	0.13	0.13	13.28
	S.E	0.25	0.35	0.23	0.25	0.12	0.12	0.25
Normal Range	Mean	9-10.0	15-40	50-80	0-3	0-1.5	0-5	10.8-17.5

Mean; S.E.; n=8

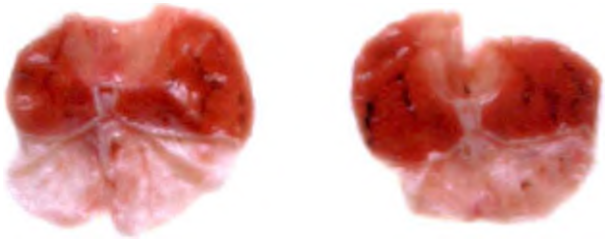
Table 22. Effect of *A.indica* and *E.triplinerve* on haematological parameters on day 28

Group		Leucocyte count 10^3/ cmm	Differential count (%)					Haemoglobin (gm%)
			Neutrophil	Lymphocyte	Monocyte	Basophil	Eosinophil	
3	Mean	12.00	19.25	79.25	0.63	0.13	0.75	13.23
	S.E	0.52	0.49	0.29	0.30	0.12	0.23	0.15
4	Mean	11.41	19.38	79.75	0.50	0.13	0.25	13.40
	S.E	0.36	0.39	0.29	0.25	0.12	0.15	0.17
5	Mean	11.05	19.25	79.25	0.88	0.25	0.38	13.23
	S.E	0.32	0.49	0.15	0.28	0.15	0.17	0.22
6	Mean	11.40	18.63	79.75	1.00	0.13	0.50	13.25
	S.E	0.40	0.39	0.29	0.31	0.12	0.25	0.19
7	Mean	10.86	19.25	79.50	0.63	0.13	0.50	13.25
	S.E	0.27	0.49	0.18	0.30	0.12	0.25	0.21
8	Mean	11.06	18.75	79.75	0.75	0.13	0.63	13.15
	S.E	0.27	0.49	0.29	0.29	0.12	0.30	0.15
9	Mean	11.20	19.38	79.50	0.63	0.00	0.50	13.10
	S.E	0.30	0.35	0.18	0.25	0.00	0.25	0.15
10	Mean	10.87	19.88	78.63	0.50	0.38	0.63	13.25
	S.E	0.21	0.33	0.30	0.25	0.17	0.25	0.16
11	Mean	10.84	19.50	78.75	0.63	0.25	0.88	13.40
	S.E	0.30	0.25	0.34	0.25	0.15	0.28	0.19
12	Mean	11.12	19.25	79.25	0.88	0.25	0.38	13.23
	S.E	0.38	0.49	0.15	0.28	0.15	0.17	0.22
Normal Range	Mean	9-10.0	15-40	50-80	0-3	0-1.5	0-5	10.8-17.5

Mean; S.E.; n=8

The ulcers which were largely limited to the glandular corpus were resolved by granulation tissue formation and subsequently by re-epithelialization in the treatment groups.

The granulation tissue consisted of proliferating capillaries along with the proliferation of fibroblasts (Fig.20). Besides, there was infiltration by a mixed inflammatory cell population and the healing lesion was overlaid by a usually thin layer of necrotic debris. The phase of re-epithelialization was characterized by mucus secreting goblet cell metaplasia and glandular hyperplasia (Fig.21). The re-epithelializtion was almost complete and the functional morphology was almost restored in the famotidine treated group (Fig. 22). The above healing processes were observed to a lesser degree in the *Azadirachta indica* and *Eupatorium triplinerve* treated groups (Fig. 17 and 18). The treated groups showed evidences of restoration of mucosal epithelium, continued clearance of inflammatory exudates and better secretory activity of normally arranged glands.



Aspirin 200 mg/kg

Fig. 16. Gastric mucosa showing linear haemorrhages, shallow erosions and deep ulcers

Fig. 17. Gastric mucosa following treatment with *Azadirachta indica*

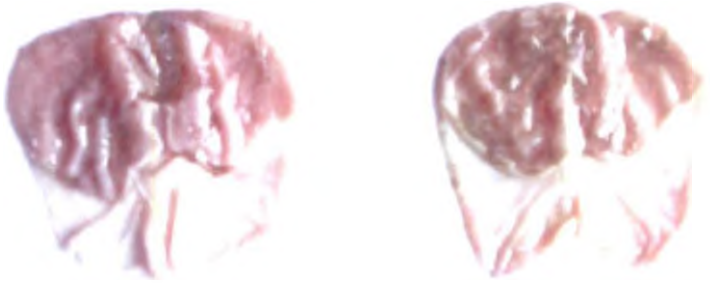


Fig. 18. Gastric mucosa following treatment with *Eupatorium triplinerve*

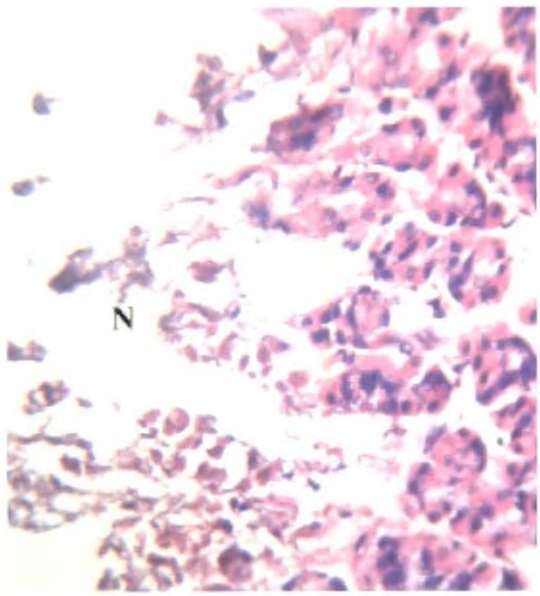


Fig. 19.

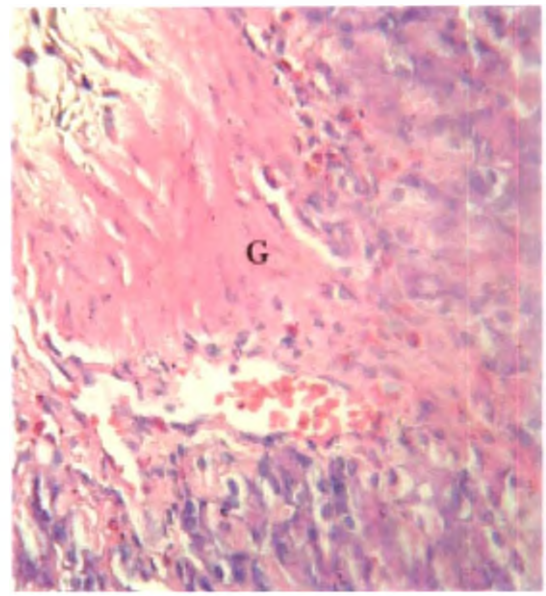


Fig. 20.

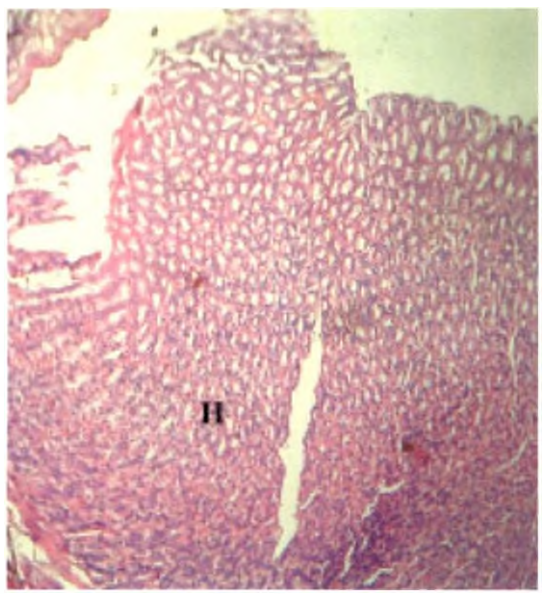


Fig. 21.

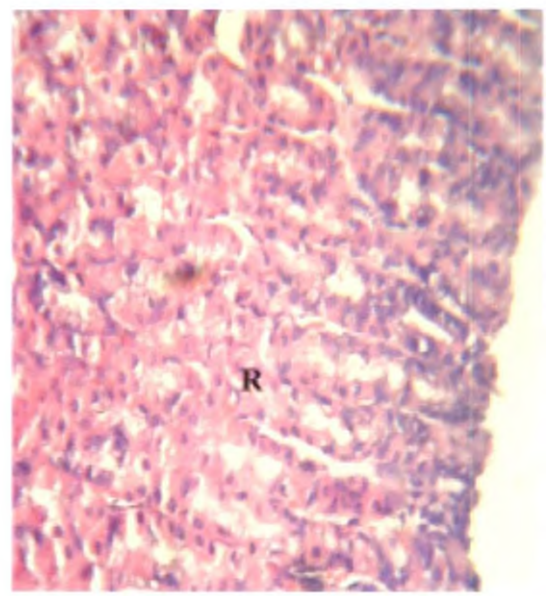


Fig. 22.

Fig. 19. Gastric mucosa- cross section of ulcer covered by an exudate consisting of mucus, fibrin and necrotic debris (N) (H&Ex125)

Fig. 20. Gastric mucosa- cross section of ulcer showing healing by granulation tissue proliferation (G) (H&Ex 400)

Fig. 21. Gastric mucosa- cross section showing re-epithelialization marked by goblet cell metaplasia and glandular hyperplasia (H) (H&Ex125)

Fig. 22. Gastric mucosa- cross section showing almost complete re-epithelialization of the gastric mucosa (R) (H&Ex125)

Discussion

5. DISCUSSION

Peptic ulcer is one of the major gastrointestinal disorders which occur due to an imbalance between offensive and defensive factors. Major offensive factors are acid, pepsin, *Helicobacter pylori* and bile salts. Defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins. Consequently reduction of gastric acid production as well as re-inforcement of gastric mucosal protection has been the major approaches for the therapy of peptic ulcer disease.

The use of various herbal medicines for various disorders is now widely accepted. The presumptions based on nature of traditional use and evaluation of specific beneficial activity of indigenous drugs has been found to be a successful approach in medicinal plant research. The present study was undertaken to evaluate the anti-ulcerogenic effect of *Azadirachta indica* and *Eupatorium triplinerve,* that are mentioned in Indian system of medicine (Ayurveda) for their remedial properties.

5.1. PHYTOCHEMICAL SCREENING

Phytochemical analysis of the extracts revealed the presence of tannins, saponins, flavonoids, triterpenes, alkaloids, phenolic compounds which are known to affect the integrity of mucous membranes. The HPTLC evaluation of the aqueous extract of *Azadirachta indica* demonstrated the presence of approximately 0.4% of total phytosterols, 1.25% total flavonoids, 0.41% rutin and 0.08% quercetin (Dorababu *et al.*, 2004).

Tannins being an astringent may have precipitated microproteins on the site of the ulcer thereby forming an impervious pellicle over the lining and resist the attack of proteolytic enzymes (Nwafor *et al.*, 2000). Saponins, especially those of the triterpene type like glycyrrhetic acid and carbenoxolone, have also been implicated as anti-ulcer agents whose action is mediated by the formation of protective mucus on the gastric mucosa (Guaraldo *et al.*, 2001). Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. It was reported that phenolic compounds were associated with antioxidant

activity and play an important role in stabilizing lipid peroxidation (Gulcin *et al.*, 2004).

Flavonoids present in many plant species are reported to be associated with a broad scale of biological activities, mainly anti-inflammatory and anti-ulcer. Flavonoids, by virtue of their high chemical reactivity with membrane phospholipids, have been reported to affect enzymes altering endogenous phospholipids metabolism leading to either stimulation or inhibition of the products of arachidonic metabolism which protect the gastric mucosa against damage (Dorababu *et al.*, 2004). Flavonoids improve microcirculation which renders the cells less injurious to precipitating factors. Alkaloids are substances known to affect the integrity of the mucous membrane and many alkaloids have been used to suppress acid secretion. The ulcer healing activity of the plants under this study may be attributed to the phytochemical constituents like flavonoids, alkaloids, saponins and tannins present in them.

5.2. ANTI-ULCER AND ULCER HEALING EFFECTS

The present study demonstrated significant ulcer healing property of *Azadirachta indica* and *Eupatorium triplinerve* in aspirin-induced ulcer model and the effect was comparable to the reference drug, famotidine. All the groups under study produced a significant decrease in ulcer index when compared with aspirin treated control group. The alcoholic extract at the two dose levels 250mg/kg and 500mg/kg as well as powder, at the two dose levels 500mg/kg and 1000mg/kg of both *A.indica* and *E.triplinerve* were found to produce a dose-dependent healing effect which is comparable to that of famotidine. However, the present findings indicate that *Eupatorium triplinerve* possesses slightly higher healing effect than *Azadirachta indica* as shown by the healing indices.

Raji *et al.* (2004) reported that *Azadirachta indica* significantly inhibited basal and histamine induced gastric acid secretion by 9.1 and 11 percent respectively. *A.indica* also produced a dose-dependent reduction in total gastric acidity. Administration of the drug at a dose of 800mg/kg orally produced 100 per cent inhibition of gastric ulceration. The mechanism by which *A.indica* produced

anti-ulcer effect might be through inhibition of gastric acid secretion by the parietal cell. The findings indicate that the extract probably inhibits H_2 receptor, causing blockade of histamine release, a potent acid secretagogue. It is postulated that the *A.indica* extract might contain a histamine antagonist acting via H_2 receptor and is capable of producing anti-ulcer activity.

Nimbidin, the active principle isolated from the oil of seeds and the trunk bark of *Azadirachta indica*, administered at the dose of 20mg/kg orally exhibited significant reduction in both free and total acid output and peptic activity of the gastric fluid. It also accorded significant protection in histamine induced gastric lesions in guinea pigs. The anti-ulcer activity could be attributed to the anti-peptic activity as well as the anti-secretory effect (Pillai *et al.*, 1978).

Clinical studies conducted by Bandyopadhyay *et al.* (2004) found that the lyophilized powder of the extract of *A.indica* when administered at the dose of 30mg twice daily inhibited the volume of gastric secretion and pepsin activity by 63 and 50 percent respectively.

Chattopadhyay *et al.* (2004) reported that the Neem leaf extract inhibit H^+K^+ATPase activity *in vitro* in concentration dependent manner to inhibit acid secretion, indicating its anti-secretory effect. In stress ulcer model, it is more effective than ranitidine but less effective than omeprazole.

The non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin are known to induce gastric ulceration. Aspirin is an irreversible inhibitor of cyclooxygenase pathway of arachidonic acid metabolism which brings about inhibition of biosynthesis of "cytoprotective prostaglandins", PGEs and PGI_2. This in turn results in over production of leukotrienes and other products of 5-lipoxygenase pathway which exhibits damaging effect (Singh, 1999). On the mucosal epithelial factors, it decreases mucin, surface-active phospholipids, bicarbonate secretion, mucosal proliferation and on the microvasculature produced damage by formation of free radicals. These changes permit back diffusion of acid through the breached surfaces of the mucosal barrier to destroy cells, capillaries and vein causing haemorrhagic ulcer. Aspirin could interfere with gastric mucus either

by reducing the rate of secretion of mucus or by inhibiting the *in vivo* biosynthesis of the mucoproteins (Rainsford *et al.*, 1968). Aspirin increases the acid secretion and back diffusion of H^+ ions; at the same time decreases mucosal ATPsynthesis and cell turn over process.

In the present study, the fact that *A.indica* and *E.triplinerve* significantly reduced ulcer index in a dose-dependent manner supports their cytoprotective effect, which may be mediated by prostaglandins. The prostaglandins are believed to enhance mucosal resistance, perhaps by enhancing the secretion of mucus and bicarbonates, strengthening the mucosal barrier, maintaining gastric microcirculation, increasing the release of endogenous mediators scavenging the free radicals, decreasing the release of endogenous amines and stimulation of cellular growth and repair (Anoop and Jegadeesan, 2003).

Since ulcers are essentially due to imbalance between offensive and defensive factors, an effective anti-ulcer agent should have both anti-secretory as well as cytoprotective effect. Earlier studies have proved the anti-secretory effect of *A.indica*. However, data are not available to prove the anti-secretory effect of *E.triplinerve*.

Recent studies have demonstrated that anti-ulcer drugs like omeprazole, lansoprazole and famotidine are protective against aspirin-induced damage through their anti-oxidant property, as well as through their anti-secretory effect (Sener *et al.*, 2004).

5.3. BIOCHEMICAL PARAMETERS

5.3.1. Anti-oxidant effect

In the present study, the alcoholic extract as well as powder of *Azadirachta indica* and *Eupatorium triplinerve* exhibited significant healing effect on NSAID induced gastric ulcer, as evident from various biochemical parameters. Aspirin administration was found to increase lipid peroxidation and decrease superoxide dismutase (SOD) and catalase (CAT), thus leading to oxidative stress. Administration of the herbal formulation at the various dose levels brought about a

significant reduction in lipid peroxidation and an increase in the activities of anti-oxidant enzymes namely, SOD and CAT.

5.3.1.1. Lipid peroxide

In the present study, there was significant increase in tissue malondialdehyde levels in aspirin treated rats. This is consistent with earlier studies that associate lipid peroxidation and the subsequent generation of oxygen derived free radicals with the pathogenesis of gastric ulcer. NSAIDs are known to induce peptic ulcer not only by denaturing mucous glycoproteins but also by free radical formation. Detection and measurement of lipid peroxidation is the evidence cited to support the involvement of free radicals. Thiobarbituric assay is the most popular method to estimate malondialdehyde level, which is an indication of lipid peroxidation and free radical activity. Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and hence these membranes are susceptible to peroxidative attack (Kumar *et al.*, 2004).

The tissue peroxidised lipid, malondialdehyde levels in tissue were lowered following treatment with *A.indica* and *E.triplinerve*, which was correlated with its anti-ulcer effect. The fact that the treatment significantly reduced lipid peroxide (LPO) levels suggests decreased lipid peroxidation and free radical induced damage.

Involvement of reactive oxygen species (ROS) in pathogenesis of gastric ulceration was first evident from the studies on ischaemia-reoxygenation-induced gastric mucosal injury (Yoshikawa *et al.*, 1989). Clinical evidences suggest that gastric mucosal damage by ethanol, non-steroidal anti-inflammatory drugs is mediated through reactive oxygen species. The NSAIDs inhibit gastric peroxidase and increase mucosal H_2O_2 and OH level to cause oxidative mucosal damage. This OH causes lipid peroxidation and increases gastric lesions induced by aspirin (Pihan *et al.*, 1987).

Studies conducted by Chattopadhyay *et al.* (2004) found that the oxidative membrane damage by hydroxyl radical (OH-) as measured by lipid peroxidation in stress ulcer is significantly blocked by the *Azadirachta indica* extract. The extract also prevents OH^- mediated mucosal damage *in vitro* by scavenging the OH^-, indicating its anti-ulcer activity by preventing oxidative damage and apoptosis.

5.3.1.2. Superoxide dismutase

The aspirin induced ulcer group showed significant reduction in the SOD levels, and this results in increased levels of superoxide and peroxyl radicals which are not effectively scavenged, again causing increased lipid peroxidation. The SOD activity showed significant increase during the process of ulcer healing, the activity of the treatment groups being comparable to that of the control group.

Increase in lipid peroxide levels (LPO) indicates increase of reactive oxygen species (ROS), the major radicals being superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). Preventive anti-oxidants, such as superoxide dismutase (SOD) and catalase (CAT) enzymes are the first line of defence against reactive oxygen species. The superoxide dismutase scavenges the superoxide radical and this reaction leads to increase in generation of peroxyl radical ($H_2O_2^-$) which is also capable of producing more oxidative damage. The catalases and other peroxidases further reduce $H_2O_2^-$ (Sairam *et al.*, 2003).

Studies conducted by Ajaikumar *et al.* (2003) found that the *in vivo* anti-oxidant levels such as SOD, catalase, glutathione and glutathione peroxidase levels were increased in *Punica granatum* treated group of animals as compared with the aspirin control group and these levels were found to be closer to the normal levels. The tissue lipid peroxidation level was found to be lowered in treated group of animals as compared to the control group.

In the present study, the decreased SOD activity shown in the aspirin group was restored by treatment with *A.indica* and *E.triplinerve* which gives an indication of the anti-oxidant potential of these plant preparations.

5.3.1.3. Catalase

The present study showed significant reduction in the catalase levels in the aspirin induced ulcer group. The treatment with *A.indica* and *E.triplinerve* reversed the aspirin induced reduction in CAT levels bringing about significant increase in this anti-oxidant enzyme level.

Accumulation of H_2O_2 occurs in the mitochondria and cytosol, if not scavenged by catalase and thus leads to increased generation of OH^- radical. In the ulcerated condition, as the CAT levels are decreased H_2O_2 is not effectively scavenged, resulting in increased lipid peroxidation and tissue damage. The effect is further aggravated by decreased activity of gastric peroxidases (Goel *et al.*, 2001). Treatment with *A.indica* and *E.triplinerve* reversed these oxidative changes induced by NSAIDs as evident from the significant increase in anti-oxidant enzyme levels, which suggests its efficacy in preventing free radical induced damage.

The anti-oxidant activity of the constituents of *Azadirachta indica* and *Eupatorium triplinerve* may be one of the important defensive factors in addition to the other effect like the increase in prostaglandin synthesis as reported earlier. These results clearly show the cytoprotective ability of the plants under study which may be due to its free radical scavenging effect. Hence from the present study, the gastric ulcer healing effect of *A.indica* and *E.triplinerve* may be due to its predominant effect on the mucosal defensive factors rather than offensive factors.

5.3.2. Alkaline phosphatase activity

An increased activity of serum alkaline phosphatase was found in the aspirin treated group. When aspirin is in the lipid-soluble undissociated form it can damage the gastric mucosa. Treatment with the alcoholic extract and powder of *A.indica* and *E.triplinerve* has been found to significantly reduce the alkaline phosphatase activity when compared with the aspirin treated control. The reduction of alkaline phosphatase by both the plant formulations give an indication that there is a correlation between this enzyme and ulcer index, thereby implicating alkaline

phosphatase as a biochemical basis of anti-ulcerogenicity of these herbal preparations.

Alkaline phosphatase is an enzyme capable of catalyzing the hydrolysis of various phosphate esters at alkaline pH. This enzyme is produced in many cells of the body which include osteoblast, liver, intestinal mucosa, placenta, kidney and blood cells. The alkaline phosphatase activity has been reported to be increased in bone diseases, diseases of the liver and gastrointestinal lumen. The release of this enzyme has been suggested to play a role in tissue necrosis associated with various models of gastro-intestinal ulceration. Increased activity of this enzyme may be found in damaged tissues (Obi *et al.*, 2000).

5.4. BODY WEIGHT

There was no significant difference in the mean body weight gain between any of the groups.

In earlier studies, Raji *et al.* (2004) observed that administration of *Azadirachta indica* extract at varying doses of 100-800mg/kg in indomethacin-induced ulcer model produced no significant change in daily body weight or organ weight. Sen *et al.* (1992) observed that treatment with *A.indica* at a dose of 100mg/kg did not produce any significant change in general behaviour, food intake or body weight in stress-induced animal models.

5.5. HAEMATOLOGICAL FINDINGS

None of the treatment groups produced any significant changes in the haematological parameters.

The anti-ulcer activity of *Ocimum sanctum* (Thulasi), *Musa* (AAB Group, "Nendran") and *Withania somnifera* (Amukkiram) in rats were studied by Sanjay (1998). It was found that study of haematological parameters of all the groups revealed no significant changes and the values were within the normal range of blood values for the species under study.

5.6. HISTOPATHOLOGICAL FINDINGS

Grossly, in the aspirin administered group the lesions observed were petechial haemorrhages, shallow erosions and deep ulcers. The lesions were mostly petechial haemorrhages and shallow erosions. The haemorrhagic ulcers were linear, mostly occuring in the glandular corpus.

Histopathological studies showed severe erosion of gastric mucosa, with necrotic patches, submucosal oedema and neutrophil infiltration. The mucosal ulcers revealed areas of coagulative necrosis characterized by capillary haemorrhages in the lamina propria, desquamation of the epithelial layer and development of a superficial area of ulceration. The lesions whether shallow erosion or deep ulcers were covered by an exudate consisting of mucus, fibrin and necrotic debris.

The ulcers which were largely limited to the glandular corpus were resolved by granulation tissue formation and subsequently by re-epithelialization in the treatment groups.

The granulation tissue consisted of proliferating capillaries along with the proliferation of fibroblasts. Besides, there was infiltration by a mixed inflammatory cell population and the healing lesion was overlaid by a usually thin layer of necrotic debris. The phase of re-epithelialization was characterized by mucus secreting goblet cell metaplasia and glandular hyperplasia. The re-epithelializtion was almost complete and the functional morphology was almost restored in the famotidine treated group. The above healing processes were observed to a lesser degree in the *Azadirachta indica* and *Eupatorium triplinerve* treated groups. The treated groups showed evidences of restoration of mucosal epithelium, continued clearance of inflammatory exudates and better secretory activity of normally arranged glands.

Studies conducted by Sanjay *et al.* (2000a) found that aspirin administration produced mucosal ulcers that revealed a rough wedge shaped zone of coagulative necrosis characterized further by capillary haemorrhages in the lamina propria, desquamation of the epithelial layer and development of a superficial area of

ulceration. Following treatment with *Withania somnifera*, the ulcers were resolved by granulation tissue formation and subsequently by re-epithelialization.

The histopathological studies of ethanol and aspirin induced ulceration models show severe erosion of gastric mucosa, with necrotic patches, sub-mucosal oedema and neutrophil infiltration in control animals. The control group also showed presence of necrotic debri in the lamina propria of mucosa, infiltrated with polymorphonuclear leucocytes. All of these symptoms were found to be normal in animals treated with *Punica granatum* methanolic extract (Ajaikumar *et al.*, 2003).

The histopathological findings in the present study confirmed the cytoprotective effect of *A.indica* and *E.triplinerve* as it inhibited the aspirin-induced necrosis, haemorrhage and congestion in gastric mucosa.

Summary

6. SUMMARY

The present study was undertaken in albino rats to evaluate the anti-ulcer activity of alcoholic extract and powder of leaves of *Azadirachta indica* (Neem) and *Eupatorium triplinerve* (Ayappana) in comparison with famotidine, a standard anti-ulcer drug.

Ninety six rats weighing 150-200gm body weight of either sex divided into twelve groups were used for the study with eight rats in each group. Group 1 was administered the vehicle, five per cent gum acacia for seven days where as group 2 was administered aspirin at the dose of 200mg/kg for seven days. Group 3 was administered aspirin for seven days and from 8^{th} day onwards, they were maintained by normal feeding and watering for 20 days to assess the natural healing. Famotidine, a standard anti-ulcer drug was given at the dose of 40mg/kg for 20 days following aspirin administration to the group 4 animals. The alcoholic extract and powder of *A.indica* and *E.triplinerve* were administered to the treatment groups (Groups 5-12) at the two dose levels of 250mg/kg, 500mg/kg and 500mg/kg, 1000mg/kg respectively for 20 days following aspirin administration.

The vehicle group as well as the aspirin group were sacrificed on the 8^{th} day, whereas all the other groups were sacrificed on 28^{th} day. The number of ulcers and severity (ulcer score) were determined with the help of magnifying lens. The ulcer index and healing index were calculated based on the formulae:

$$\text{Ulcer index} = \text{Number of ulcers} + \text{Severity of ulceration} + \frac{\%\ \text{incidence}}{\text{no.of rats}}$$

$$\text{Healing index} = \frac{\text{Ulcer index(control)*} - \text{Ulcer index(drug)}}{\text{Ulcer index (control)*}} \times 100$$

(control)*- rats given aspirin for seven days and sacrificed on eighth day.

The reduction in ulcer index observed as a result of treatment were compared with that of the aspirin-induced ulcer group whereas the anti-ulcer activity of the plants under study was compared with famotidine.

The body weight gain were studied at weekly intervals. Haematological study of the treatment as well as control groups were done initially and after sacrificing the rats to assess any change in the total leucocyte count, differential count and haemoglobin count. Biochemical parameters like lipid peroxide, superoxide dismutase and catalase levels in gastric mucosa and the serum alkaline phosphatase levels were studied on 28th day of the experiment. Histopathological study was also conducted to evaluate the severity of ulceration and healing process.

The alcoholic extract and powder of *Azadirachta indica* and *Eupatorium triplinerve* showed tendency to reduce the ulcer index against aspirin-induced gastric ulcer in rats. The result indicated a significant dose-dependent decrease in ulcer index by both *A.indica* and *E.triplinerve*. All the treatment groups under the study with the plant preparations, have significantly higher healing effects than the aspirin control group as well as the group subjected to natural healing. The alcoholic extract at the two dose levels 250mg/kg and 500mg/kg as well as powder at the two dose levels 500mg/kg and 1000mg/kg of both *A.indica* and *E.triplinerve* were found to produce a dose-dependent healing effect which is comparable to that of famotidine.

Aspirin significantly caused ulceration with significant increase in lipid peroxide (LPO) levels and decrease in superoxide dismutase (SOD) and catalase (CAT) levels. Aspirin-induced ulceration caused about 2-fold increase in LPO levels while the SOD and CAT levels were reduced to half of its normal value.

Administration of the herbal formulation at the various dose levels brought about a significant reduction in lipid peroxidation and an increase in the activities of anti-oxidant enzymes namely, superoxide dismutase and catalase, which suggest its efficacy in preventing free radical induced damage. The treatment reversed the increased activity of serum alkaline phosphatase observed in the aspirin treated group.

There was no significant difference in the mean body weight gain between any of the groups. The study of haematological parameters of the entire groups revealed no significant changes and all the values fall within the normal range of blood values for the species under study. The results are substantiated by the histopathological studies, which confirmed that treatment with *A.indica* and *E.triplinerve* inhibited aspirin-induced necrosis, haemorrhage and congestion in gastric mucosa. All the treated groups showed evidences of restoration of mucosal epithelium, continued clearance of inflammatory exudates and better secretory activity of normally arranged glands.

Phytochemical analysis of the extracts revealed the presence of tannins, saponins, flavonoids, triterpenes, alkaloids, phenolic compounds which are known to affect the integrity of mucous membranes. Saponins, especially those of the triterpene type like glycyrrhetic acid and carbenoxolone, have also been implicated as anti-ulcer agents whose action is mediated by the formation of protective mucus on the gastric mucosa. It was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. Flavonoids improve microcirculation which renders the cells less injurious to precipitating factors. Alkaloids are substances known to affect the integrity of the mucous membrane and many alkaloids have been used to suppress acid secretion. The ulcer healing activity of the plants under this study may be attributed to the phytochemical constituents like flavonoids, alkaloids, saponins and tannins present in them.

In the present study, the fact that *A.indica* and *E.triplinerve* significantly reduced ulcer index of the aspirin treated animals in a dose-dependent manner supports their cytoprotective effect, which may be mediated by prostaglandins, as aspirin is known to irreversibly inhibit the prostaglandin synthesis. The anti-oxidant activity of the constituents of *Azadirachta indica* and *Eupatorium triplinerve* may also be one of the important defensive factors in addition to the other effect like the increase in prostaglandin synthesis. The results clearly show the cytoprotective

ability of the plants under study which may be due to its free radical scavenging effect. Hence from the present study, the gastric ulcer healing effect of *A.indica* and *E.triplinerve* may be due to its predominant effect on the mucosal defensive factors rather than offensive factors.

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ANTI-ULCER EFFECT OF *Azadirachta indica* (NEEM) AND *Eupatorium triplinerve* (AYAPPANA) IN RATS

SANGEETHA SATHEESAN

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

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

ABSTRACT

The present study was conducted in adult albino rats to assess the anti-ulcer effect of alcoholic extract and powder of leaves of *Azadirachta indica* and *Eupatorium triplinerve* in comparison with famotidine, a standard anti-ulcer drug.

Ninety six rats weighing 150-200gm body weight of either sex divided into twelve groups were used for the study with eight rats in each group. Group 1 was administered the vehicle, five per cent gum acacia for seven days where as group 2 was administered aspirin at the dose of 200mg/kg for seven days. Group 3 was administered aspirin for seven days and from 8th day onwards, they were maintained by normal feeding and watering for 20 days to assess the natural healing. Famotidine, a standard anti-ulcer drug was given at the dose of 40mg/kg for 20 days following aspirin administration to the group 4 animals. The alcoholic extract and powder of *A.indica* and *E.triplinerve* were administered to the treatment groups (Groups 5-12) at the two dose levels of 250mg/kg, 500mg/kg and 500mg/kg, 1000mg/kg respectively for 20 days following aspirin administration.

The control group as well as the aspirin group were sacrificed on the 8th day, whereas all the other groups were sacrificed on 28th day. The number of ulcers and severity (ulcer score) were determined with the help of magnifying lens and the ulcer index and healing index were calculated.

Various biochemical parameters were studied to confirm the anti-ulcer activity of the plant preparations under study. The degree of lipid peroxidation, as well as the anti-oxidant enzyme status, namely, superoxide dismutase and catalase levels were assessed in gastric mucosa. The serum alkaline phosphatase activity was estimated on 28th day of the experiment. Body weight was taken at weekly intervals.

Heamatological parameters such as total leucocyte count, differential count and haemoglobin count were determined to assess any changes in the haemogram. Histopathological study was also conducted to evaluate the severity of ulceration and healing process.

The results indicated that all the treatment groups under study produced a significant decrease in ulcer index when compared to aspirin treated control group. The alcoholic extract at the two dose levels 250mg/kg and 500mg/kg as well as powder at the two dose levels 500mg/kg and 1000mg/kg of both *A.indica* and *E.triplinerve* were found to produce a dose-dependent healing effect which is comparable to that of famotidine.

Administration of the herbal formulation at the various dose levels brought about a significant reduction in lipid peroxidation and an increase in the activities of anti-oxidant enzymes namely, superoxide dismutase and catalase, which suggest its efficacy in preventing free radical induced damage. The treatment reversed the increased activity of serum alkaline phosphatase observed in the aspirin treated group.

There was no significant difference in the mean body weight gain between any of the groups. Haematological study revealed no significant change and all values fall within the normal range of blood value for the species under study. The results are substantiated by the histopathological studies, which confirmed that treatment with *A.indica* and *E.triplinerve* inhibited aspirin-induced necrosis, haemorrhage and congestion in gastric mucosa.

Phytochemical analysis of the extracts revealed the presence of tannins, saponins, flavonoids, triterpenes, alkaloids, phenolic compounds which are known to affect the integrity of mucous membranes.

In the present study, the fact that *A.indica* and *E.triplinerve* significantly reduced ulcer index in a dose-dependent manner supports their cytoprotective effect, which may be mediated by prostaglandins and the ulcer healing effect could be attributed to its predominant effect on the mucosal defensive factors rather than offensive factors.