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Studies on anti-inflammatory, antibacterial and immuno-modulatory activity of Noni *(Morinda citrifolia L.)* in experimental animals

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Foreword

This Technical Bulletin is the final report of the project "Studies on anti-inflammatory, antibacterial and immuno-modulatory activity of noni (*Morinda citrifolia* L.) in experimental animals" funded by World Noni Research Foundation. I had opportunities to watch closely the whole process of the research work being carried out through skilled, thoughtful and well designed investigations by Dr. R. K. Sharma and Dr. (Mrs.) Varsha Sharma from College of Veterinary Sciences and Animal Husbandry, Jabalpur, MP.

The project aimed at exploring a range of activities of the Noni fruit especially the anti-inflammatory, anti-pyretic, analgesic, immuno- modulatory and anti-bacterial properties. The studies also included the phytochemistry, acute and chronic toxicity of the fruit extracts.

I congratulate the authors for their dedicated efforts to bring out these properties of Noni fruit and also for the preparation of the WNRF Technical Bulletin - 8.

Chennai
September, 2011

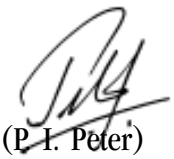
(Kirti Singh)
Chairperson

About the Project

The World Noni Research Foundation (WNRF), Chennai, is committed to undertake research and development on Noni with the ultimate objective of human wellness, nutrition, health, prosperity and empowerment. Noni is going to occupy an important place in the forthcoming safe drugs of new generation. Noni was not quite recognized as a fruit of any special importance in our life in whatever manner but I proudly put forth that the intense and focused attempts of WNRF were proved to be pioneering and vital in showing the extraordinary properties that this fruit possessed in curing a wide range of health problems. Also, it opens up an opportunity for being a new economic fruit crop in which India can take a definite upper edge in the world. This potential of Noni has made the dream of achieving the above said objectives of the Foundation as a reality.

The project was sanctioned to Dr. R. K. Sharma, the Principal Investigator, to explore the various actions of Noni using approved and dependable pharmacological and microbiological methodology. Dr. R. K. Sharma carried out anti-inflammatory, anti-bacterial and immunomodulatory studies of Noni along with its analgesic and anti pyretic activities with aqueous and alcoholic extracts. Studies were conducted at Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.).

The results of the research work are very encouraging and form a terrafirma leading to advancement of the foundation nearer to its objectives. I congratulate the investigators for their earnest attempt to bring this useful Technical bulletin.



Chennai
September, 2011

(P. I. Peter)
Chairman, Noni BioTech

Preface

The traditional medical knowledge on herbs and trees to deal with diseases led to a safe therapeutic line of treatment the best adapted to the human body. The herbs and trees, in fact, have a treasure house of wellness characteristics and qualities.

I have great pleasure in bringing out the outcome of the project entitled "Studies onanti-inflammatory, anti-bacterial and immunomodulatory activity of Noni (*Morinda citrifolia* L.) in experimental animals" sponsored by World Noni Research Foundation, Chennai.

The studies revealed the potentials of Noni fruits having anti-inflammatory effect in acute as well as chronic inflammation, analgesic and anti-pyretic properties. Its immunomodulatory and antibacterial actions open a door of hope to withstand against the most dreaded diseases causing failure of immune system on one hand and on the other, the threat of increased resistance to antimicrobial drugs.

I take this opportunity to express my gratitude and sincere thanks to World Noni Research Foundation (WNRF), Chennai for granting this project. To Prof. P. I. Peter, Chairman, Noni BioTech, I owe a lot and express my sincere gratitude.

I convey my deep sense of gratitude to Dr. Kirti Singh, Chairperson, WNRF, Chennai for his guidance. I vividly convey my heartfelt thanks to his invaluable help, constant inspiration and healthy criticism rendered during the course of this study with zest and zeal.

I am thankful to Dr. K.V.Peter, Director and Dr. T. Marimuthu, Joint Director, World Noni Research Foundation, Chennai for unfailing and abiding inspiration, benevolence and valuable guidance during the tenure of this study.

I am grateful to Dr. Govind Prasad Mishra, Vice- Chancellor, M.P. Pashu Chikitsa Vigyan Vishwavidyalaya, Jabalpur and Dr. S.N.S. Parmar, Director Research Services, M.P. Pashu Chikitsa Vigyan Vishwa vidyalaya, Jabalpur for granting permission to carry out the research project.

I express my sincere thanks and gratitude to Dr. R.P.S.Baghel, Dean Faculty and Dr. A.B. Srivastav, Dean College and Director Wild Life Health Centre for extending the necessary facilities to conduct this study and for their constructive supervision and valuable suggestions during the course of the study.

I thank Dr. Y.P. Sahni, Professor and Head, Dept of Pharmacology and Toxicology, College of Veterinary Science & A.H., Jabalpur for the support and cooperation rendered during the execution of this research work.

I thank Dr. N. Punniamurthy, Professor and Head, University Training Centre, TANUVAS, Thanjavur, and Head Pharmacology, WNRF for his critical editing.

I thank Dr. Bharti Jethani, Research Associate for her dedication in compiling the findings from the project.

Jabalpur
September, 2011 R.K. Sharma

1. Introduction

Since time immemorial, man is using herbs or plant products as medicine for developing immunity or resistance against various diseases. Natural herbal products are in demand to control various ailments. The strong historical bond between plants and human health is well substantiated by plant species diversity and related knowledge of their use as herbal medicines

Over the past a few years natural products have become increasingly popular and the field of natural herbal remedies has flourished considerably. One up coming botanical fruit of *Morinda citrifolia* L. whose Polynesian name is Noni, is currently the subject of much science, myth, and wellness. Despite comparatively less traditional use of Noni fruit in relation to other parts of the plant, it is the fruit itself in a variety of forms that is gaining popularity in today's herbal market. Either dried and crushed, juiced and bottled, or freeze-dried. Noni fruit is being used in mitigating diabetes, cardiovascular diseases, cancer, headaches, arthritis and a host of degenerative diseases.

Noni (*Morinda citrifolia* L.) is a tropical fruit from tree also known as Indian mulberry, great morinda, beach mulberry, Tahitian Noni and cheese fruit. It belongs to family Rubiaceae. Noni is used since ancient times and is the ancient health miracle for modern times. Its barks, root, leaves and fruits are all utilized as dye, medication and food supplement. Noni is reported to have a broad range of health benefits for cancer, infectious arthritis, diabetes, asthma, hypertension and pain. Noni tree is local to South East Asia but is also cultivated in Pacific Islands, India, Australia, South Africa and New Zealand. Noni grows in shady forests as well as in open rocky or sandy shores. It reaches maturity in about 18 months and then yields between 4-8 kg of fruit every month throughout the year. The fruits of Noni are ovoid, grenade-like, and yellow in colour with foul taste and odour. It can grow up to 12 cm and has a lumpy surface covered by polygonal shaped sections. Its fruit has been used as a food in tropical regions throughout the world (McClatchey, 2002).

A number of major components were identified in the Noni plant such as octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones

(such as nordamnacanthal, morindone, rubiadin, and rubiadin-1-methyl ether, anthraquinone glycoside), flavonoids,carotene, vitamin A, Iridoids trisaccharide fatty acid esters, "Noniosides" resulting from combination of an alcohol and an acid in Noni fruit(Wang, *et al* 1999), linoleic acid, Alizarin, amino acids, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative proxeronine (Levand and Larson, 1979), flavonol glycosides (Sang *et al* 2001), an iridoid glycoside from the Noni leaves, rutin lignans (Lin, *et al.* 2007), an asperulosidic acid from the fruit., free fatty acids (caprylic acid and hexanoic acid) responsible for unique pungent (cheese-like) aroma of ripe Noni fruit, Scopoletin (it binds to serotonin, keeping serotonin levels elevated), Beta-sitosterol (a plant sterol with potential for anti-cholesterol activity), Damnacanthal (a potentially toxic anthraquinone, putatively an inhibitor of HIV viral proteins), Terpene (Helps with cell rejuvenation, thus increasing nutrient-toxin exchange), Phytonutrients & Selenium and Xeronine.

Chronic inflammatory diseases remain one of the world's major health problems (Li *et al*., 2003). Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair. (Vane and Bolting, 1995). Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

Man has ransacked the entire globe to obtain drugs with which he might correct inflammatory disorders. Non steroidal anti-inflammatory drugs are the heterogeneous group of compounds which are not chemically similar. Different agents namely corticosteroids, acetyl salicylic acid, phenylbutazone, indomethacin and ibuprofen gave temporary relief by exerting an analgesic or anti-inflammatory effect but do not cure the disease permanently. At the same time their side effects such as disturbance of gastro-intestinal function culminating in peptic ulcer, disorders of central nervous system and haemopoietic system, ocular, renal and dermatological disorders are well known. The nature of inflammation and disorders produced, indicated the search for anti-inflammatory drugs. Therefore, new anti-inflammatory and analgesic drugs lacking these side effects are being researched as alternatives to NSAID and opiates (Kumar, 2001).

Numerous herbal agents are claimed to possess anti-inflammatory, analgesic and antipyretic properties. Most of the plants and plant products are easily available and thus may suit to a common man who can not afford to have costly drugs. Noni is unique in view of the large number of medical indications which characterize claims for its efficacy, the little that is known about its pharmacologic potential compared with other popularly used botanicals (Dixon *et al.,* 1999).

Morinda is reputed to have antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects. Originally, the leaves were applied directly to the skin to treat ulcerations and minor infections.

Even though 10 thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is amazingly rapid. In order to keep pace with this ever increasing need for new antimicrobials, it is imperative that new compounds be discovered (Gerson and Palu, 2006).

In an era of nutraceutical research, "Noni" fruit is considered as a panacea because of the profound claim on its medicinal uses in treating many diseases inclusive of infections of microbial origin. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. The control of bacteria infection has been remarkably effective since the discovery of antibacterial drugs. However some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants are shown to be a potential source for new anti-microbial agents. The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literatures. (Rajrajan *et al.,* 2009).

When penicillin was first discovered in the early 1900's, its popularity as an antibacterial agent grew. This led to frequent usage of antibiotics, causing people to become increasingly dependent on them to cure their illnesses. Further development and advanced technology allowed for the production of more powerful antibiotics. Through time, antibiotics were prescribed and used to an extent so great that they were misused unnecessarily as well. Their use has also been employed to treat cases of nonbacterial related illnesses. Thus, certain types of bacteria have undergone mutations and developed resistance towards antibiotics and can no longer be eliminated or controlled by them. For centuries, medicinal herbs are used to alleviate symptoms of various illnesses, as well as to cure them. *Morinda citrifolia* is one such plant that has been investigated in the research. Commonly known as Noni, the plant is found in Tahiti, Samoa, Hawaii, India, Fiji and Australia. It is used for centuries to cure a variety of sicknesses, namely, arthritis, diabetes and diarrhoea, inflammation, skin and stomach ulcers (Plate 1.)

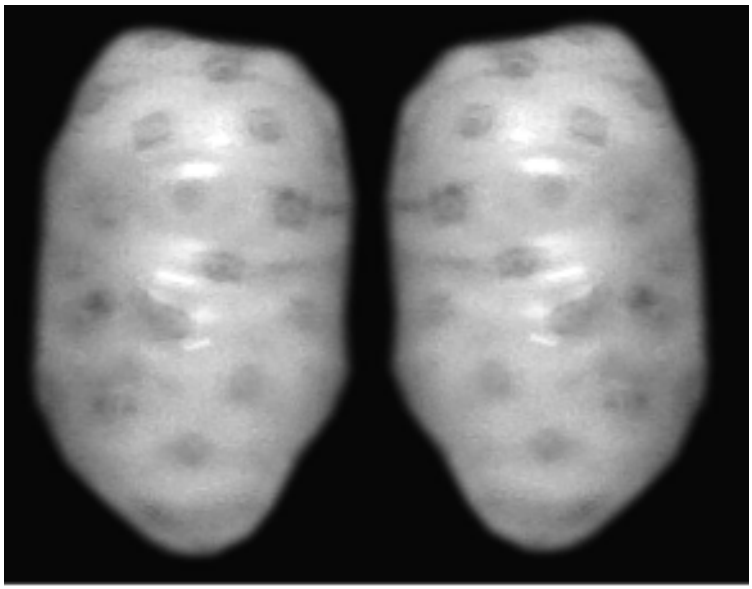


Plate 1 : Noni Fruit

2. State of knowledge

Extraction

Osol (1975) gave the procedure for preparation of aqueous extract, viz a weighed amount of powdered substance was macerated in distilled water at room temperature and agitated occasionally for one day . Filtered through a fine filter paper and marc was pressed to avoid loss. The extract obtained was in liquid form at room temperature. The extract was stored in a sterilized PVC bottle at 4 °C

Rosario et al. (2005) studied the procedure for preparation of the aqueous extract in which 100 g of the dried powdered leaves, were boiled in 500 ml of distilled water for 25 min. The aqueous extract was filtered, the filtrates obtained were lyophilized.

Phytochemical analysis

Kapoor *et al.* (1969) determined the presence of flavonoids by using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium and potassium hydroxide solution.

Culer (1982) gave the test for alkaloids ,that when 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath and few drops of Dragendorff's reagent was used to treat 1 ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids.

Goodwin and Mercer (1983) observed that plant steroids are mainly restricted to the plant membrane and may function there as cholesterol does in animal membranes.

Said *et al.* (1990) gave the test for triterpenes that when 300 mg of extract was mixed with 5 ml chloroform and warmed at 80°C for 30 min. Few drops of concentrated sulfuric acid was added to it and mixed well. The appearance of red color indicates the presence of triterpenes.

Sharma and Srivastava (1991) reported the presence of alkaloids, glycosides, reducing sugars, tannins and resins on phytochemical analysis of aqueous and alcoholic extracts of Teeburb.

Middleton and Kandaswami (1993) reported that the flavonoids have long been recognized to possess anti allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities.

Sofowora (1993) gave the test for saponin when 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins.

Trease and Evans (2002) gave a method for tannins when 2.5 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins

Okwu (2004) reported that alkaloids have analgesic, anti-spasmodic and bactericidal effects and this is the basis for their use as basic medicinal agents.The infections may also be controlled by the presence of saponins. Saponins have antibiotic properties and so help the body to fight infections and microbial invasion.

Morinda citrifolia L. (Noni)

Okwu and Okwu (2004) reported that tannins have astringent properties and hasten the healing of wounds and inflamed mucous membranes.

Ahmad and Bano (1980) reported that plant sterols can assist in inhibiting inflammatory response, which causes swelling and pain. ?-sitosterol in Noni has been shown in laboratory tests to possess anti-inflammatory properties that rival the steroid cortisone, anti-fever actions comparable to aspirin and worked to reduce swelling - all occurring with no side effects of any kind.

Heinicke (1985) states that the Noni fruit contains a natural precursor for Xeronine that he named Proxeronine. Proxeronine is converted to the alkaloid, Xeronine, in the body by an enzyme he calls Proxeroninase. Xeronine is able to modify the molecular structure of proteins.

Scortichini and Pia Rossi (1991) evaluated that triterpenoids and flavanoids in Noni are known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization.

Sim (1993) isolate and characterize scopoletin from the fruit of *Morinda citrifolia* (Noni).

Farine *et al.* (1996) isolated some volatile components from ripe fruits of Morinda citrifolia like acids, Alcohols, Esters, Ketones, Lactones, Hexanamide, Limonene, Ethylthiomethyl benzene, Scopoletin, Vomifoliol, Aucubin, Asperuloside. All of the above volatile components are readily vaporizable at a relatively low temperature.

Song *et al.* (1998) reported eight frequently occurring isoflavones (genistin, genistein, daidzin, daidzein, glycitin, glycitein, formonoetin, biochaninA) in Tahitian Noni juice.

Wang *et al.* (1999) isolated two known glycosides and a novel trisaccharide fatty acid ester from *Morinda citrifolia* (Noni) fruit. The novel trisaccharide fatty acid ester was 2,6-di-O- (beta-D-glucopyranosyl)-1-O-octanoyl-beta-Dglucopyranose.The known compounds were rutin and asperulosidic acid.

Sang *et al.* (2001) reported a new unusual iridoid with inhibition of activator protein-1 (AP-1) in the leaves of *Morinda citrifolia*.

Chopra *et al.* (2002) reported that unriped berries of Noni fruit are chard & mixed with salt & apply successfully to spongy gums.

McClatchey (2002) reported that Noni fruit has been used as a food in tropical regions throughout the world and it appears to have many health benefits.

Wang *et al.* (2002) reported that Noni has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelminth, analgesic, hypotensive, anti-inflammatory and immune enhancing effects.

Teglund and Myers (2002) reported oligo and polysaccharides long rchain sugar molecules in Noni that serve a prebiotic function as dietary fiber fermentable by colonic bacteria, yielding short-chain fatty acids with numerous potential health properties.

Stalman *et al.* (2003) reported that cell cultures of *Morinda citrifolia* L. are capable of accumulating substantial amounts of anthraquinones. Chorismate formed by the shikimate pathway is an important precursor of these secondary metabolites. Isochorismate synthase the enzyme that channels chorismate into the direction of theanthraquinones, is involved in the regulation of anthraquinone biosynthesis.

Singh *et al.* (2004) reported that *Morinda citrifolia* (Noni), have anthraquinones in their fruit, roots and leaves These chemicals have potential to be modified as therapeutic agents, yet possess toxicity in animals.

Wei *et al.* (2004) reported that in an extensive GC-MS analysis of the volatile components in ripe Noni fruit, a range of free fatty acids, methyl and ethyl esters of fatty acids ,aliphatic and aromatic alcohols, ketones, lactones and traces of monoterpenes were identified.

Kamiya *et. al.* (2005) identified anthraquinones in Noni fruit. From the fruits one new anthraquinone, 5,15-O-dimethylmorindol, together with five known anthraquinones and one new iridoid, morindacin, together with two known iridoids, were isolated. Their structures were elucidated by analysis of spectroscopic data.

Nandhasri *et al.* (2005) studied that in addition to vitamins, minerals and phytochemicals, Noni contains amino acids, fatty acids and sugars. There is a wide variety of nutrient levels in various Noni products.

Biu *et al.* (2006) reported that the polysaccharide fraction of the fruit juice of *Morinda citrifolia* (Noni) consist primarily of the pectic homogalacturonan, rhamnogalaturonan I, arabinan and type I and II arabinogalactans.

Kumar *et al.* (2006) performed a modified version of high hydrostatic pressure extraction for extraction of antioxidants from M. citrifolia fruit at 5, 15, 25 bar and temperature30° to 70°C for time duration 1, 2, 4 and 6 hours.

Siddiqui *et al.* (2007) reported that the fruits of Morinda citrifolia, afforded a new constituent, morinaphthalenone (1), and three known constituents, scopoletin (2), 1, 3-dimethoxy-anthraquinone (3) and 1, 2-dihydroxy-anthraquinone (4).

Mohd Zin *et al.* (2007) studied the isolation and identification of antioxidative compound from fruit of mengkudu *Morinda citrifolia*.

Lin *et al.* (2007) isolated lignans and anthraquinones from the fruits of *Morinda citrifolia*.

Chemical mediators of inflammation

Biochemical mediators released during inflammation intensify and propagate the inflammatory response .These mediators are soluble, diffusible molecules that can act locally and systemically. Chemical mediators such as histamine, 5-hydroxytryptamine, substance-A, various chemotactic factors bradykinin, leukotriens and prostaglandins are liberated locally.

Histamine

Histamine causes arteriolar dilation, increased capillary permeability, contraction of nonvascular smooth muscle, eosinophils chemotaxis and can stimulate nociceptors responsible for the pain response. Its release is stimulated by the complement components C3a and C5a and by lysosomal proteins released from neutrophils. Histamine activity is mediated through the activation of 1 of 3 specific histamine receptors, designated H1, H2, or H3, in target cells. Most histamine-induced vascular effects are mediated by H1 receptors. H2 receptors mediate some vascular effects, but are more important for their role in histamine-induced gastric secretion. Less is understood regarding the role of H3 receptors, which may be localized to the CNS.

Linardi *et al.* (2002) reported that histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability. Sick *et al.* (2009) reported that mast cells play a vital role in allergic and inflammatory processes via distinct activation pathways.

5-Hydroxy tryptamine (serotonin)

Serotonin (5-hydroxytryptamine) is a vasoactive mediator similar to histamine found in mast cells and platelets in the GI tract and CNS. Serotonin increases vascular permeability, dilates capillaries and causes contraction of nonvascular smooth muscle.

Sharma et al. (1991) reported that 5-HT was responsible for anti- inflammatory activity of Teeburb on chronic inflammatory process against proliferative phase of inflammation and the activity of Teeburb may be due to blockade of 5-HT receptors. Cyproheptadine (10 mg/kg I/P) a 5-HT receptor antagonist increase the anti-inflammatory activity of aqueous and alcoholic extracts of Teeburb by 29.67% and 40.99% respectively. Sufka *et al.* (1992) suggest the pro inflammatory and nociceptive action of peripheral serotonin (5-HT).

Grundy (2008) reported that 5-Hydroxytryptamine (5-HT) is a major transmitter molecule within the gastrointestinal tract.

Qu *et al.* (2008) suggest that 5-HT1A, 5-HT2 and 5-HT3, but not 5-HT4 receptors, are involved in mediating 5-HT induced antinociception in the VLO.

Bradykinin

The kinins are also important inflammatory mediators. The most important kinin is bradykinin, which increases vascular permeability, cause vasodilatation, increase blood flow to the brain, heart, viscera, skeletal muscle and glands and importantly activates phospholipase A2 (PLA2) to liberate arachidonic acid (AA). Bradykinin is also a major mediator involved in the pain response. The polypeptides-Kallidin and bradykinin, are collectively known as plasmakinin. These peptides stimulate the endothelium to release the eicosanoid.

Campos & Calixto (1995) reported that injection of bradykinin into the rat paw, produced edema, which was potentiated by co-administration of low doses of prostaglandin, hydroxy tryptamine and substance P, however there was no potentiation of inflammation with co-administration of histamine.

Leukotrines

Leukotriene B4 (LTB4) and 5-hydroxyeicosatetranoate (5-HETE) are strong chemo attractants stimulating polymorph nuclear leukocyte movement. LTB4 also stimulates the production of cytokines in neutrophils, monocytes, and eosinophils and enhances expression of C3b receptors. Other leukotrienes facilitate the release of histamine and other autacoids from mast cells and stimulate bronchiolar constriction and mucous secretion. In some species, leukotrienes C4 and D4 are more potent than histamine in contracting bronchial smooth muscle.

Amorim *et al.* (1994) demonstrated the involvement of leukotrienes in the anaphylactic paw edema in sensitized boosted or unboosted mice. No difference was noted in the intensity of the antigen-induced paw edema between boosted and unboosted animals. Busse (1998) reported that leukotrienes are potent pro-inflammatory mediators that appear to contribute to pathophysiologic features of asthma.

Prostaglandins

Various prostaglandins are associated with the development of pain that accompanies injury or inflammation. Prostaglandins have ability to sensitize pain receptors to mechanical or chemical stimuli. The prostaglandins of E series (PGE) are accepted as nociceptive agents which act by stimulating pain receptors either directly or indirectly by sensitizing them to other algogenic agents like bradykinins, acetylcholine or 5-HT. Bacterial endotoxins or other pyrogens act by stimulating the biosynthesis and release of endogenous pyrogens by neutrophils and other cells. Endogenous pyrogens reach central nervous system where they act upon discrete sites within the brain, especially the pre-optic hypothalamic area, which results in elevation of body temperature, mediated by release of prostaglandins.

Scher and Pillinger (2009) reported that prostaglandin D2 (PGD2) and its dehydration end product 15-deoxy-Delta-prostaglandin J2 (15d-PGJ2) seem to play important role in regulating inflammation, via both receptor-dependent (DP1 and DP2 receptors) and receptor-independent mechanisms.

Cytokines

Two of the more important cytokines, interleukin-1 (IL-1) and TNF-?, mobilize and activate leukocytes, enhance proliferation of B and T cells, cause natural killer cell cytotoxicity and are involved in the biologic response to endotoxins (Cannon, 2000).

Antagonist of mediators

Mahajan *et al.* (1984) reported that cimetidine (20 mg/kg, I/P) inhibited the peptic ulcer index in histamine treated adult albino rats.

Sharma and Srivastava (1991) studied the interaction of different antagonist of mediators of inflammation with aqueous and alcoholic extracts of Teeburb on carrageenin induced rat paw edema and reported that maximum increase in anti- inflammatory was produced by cyproheptadine which persisted for four hours followed with cimetidine, promethazine and paracetamol.

Nguyen *et al.* (2001) reported that the H1 antagonists, doxepin, cinnarizine and promethazine exhibit high affinity for binding to the H4 receptor.

Leurs *et al.* (2002) reported that the H1-antihistamines are actually inverse agonists at the histamine H1-receptor, rather than antagonists.

Fumagalli *et al.* (2004) reported that antihistamines can modulate part of immunological mechanisms involved in the pathogenesis of allergic inflammation reducing mediator release expression of adhesion molecules, regulating the release of cytokines, chemokines and consequently inflammatory cells recruitment.

Schooley *et al.* (2007) determine that the oral administration of cyproheptadine or cetirizine blocks the action of serotonin and histamine.

Chimona *et al.* (2008) reported the histopathologic effects of H1 histamine receptor antagonists in an experimental histamine-induced middle ear inflammation model.

Non steroidal anti-inflammatory drugs

Jain (1995) observed a significant antipyretic effect of aspirin and diclofenac sodium against yeast induced pyrexia in rats. Diclofenac was found to be effective at the dose rates of 10 mg/kg body weight I/P whereas aspirin was found to be effective at the dose rates of 80 mg/kg body weight I/P.

Colville-Nash and Gilroy (2001) suggested that COX-2 over expression may lead to increased angiogenesis and inflammatory reaction. Therefore the inhibition of COX-2 might have a general cancer preventive effect via anti-inflammatory activity and decrease angiogenesis. The selectivity of COX-2 inhibition of TNJ versus COX-1 in vitro was investigated. The inhibitions of TNJ on COX-2 and COX-1 activities were compared with that of the traditional NSAIDs such as aspirin, indomethacin and a known selective COX-2 inhibitor, celebrex.

Antman *et al.* (2005) studied that primary property of NSAID is the inhibition of COX enzyme. There are 2 major COX isoenzymes: COX-1 which is expressed constitutively (constantly) in most tissues, whereas COX-2 which is induced in inflammation.

Aronoff *et al.* (2006) reported that aspirin inhibits the production of prostaglandins and also inhibit the cyclooxygenase (COX) family of enzymes. Thus acts via two pathways.

Indigenous drugs

Subhash *et al.* (2000) evaluated the anti-inflammatory activity of Ficus racemosa extract on carrageenin, serotonin, histamine and dextran-induced rat hind paw edema models. The extract at doses of 200 and 400 mg/kg has been found to possess significant anti-inflammatory activity on the tested experimental models. The effect produced by the extract was comparable to that of phenylbutazone, a prototype non-steroidal anti-inflammatory agent.

Kim *et al.* (2004) reported that plant flavonoids show anti-inflammatory activity in vitro and in vivo. One of the important mechanisms is the inhibition of eicosanoid generating enzymes including phospholipase A2,

cyclooxygenases and lipoxygenases, thereby reducing the concentrations of prostanoids and leukotrienes. Recent studies have also shown that certain flavonoids, especially flavones derivatives, express their anti-inflammatory activity at least in part by modulation of proinflammatory gene expression such as cyclooxygenase-2, inducible nitric oxide synthetase and several pivotal cytokines. Due to these unique action mechanisms and significant in vivo activity, flavonoids are considered to be reasonable candidates for new anti-inflammatory drugs.

Gupta *et al.* (2005) investigated the anti-inflammatory, analgesic and antipyretic effects of 50, 100 and 200 mg/kg body weight of methanol extract obtained from Bauhinia racemosa stem bark, the so-called MEBR. The effects of MEBR on the acute and chronic phases of inflammation were studied in carrageenin, dextran and mediators (histamine and serotonin) induced paw edema and cotton pellet-induced granuloma, respectively. Analgesic effect of MEBR was evaluated in acetic acid-induced writhing and hot plate tests. Antipyretic activity of MEBR was evaluated by yeast-induced hyperpyrexia in rats. The anti-edema effect of MEBR was compared with 10 mg/kg of indomethacin orally.

Number of herbal agents has been found useful in different inflammatory conditions. *Morinda citrifolia* (Noni), an indigenous medicinal plant has been reported to possess anti-inflammatory, analgesic and anti-ulcerogenic properties.

Younos *et al.* (1990) reported that the analgesic efficacy of the Noni extract is 75 % as strong as morphine, yet non-addictive and side effect free. The lyophilized aqueous extract of roots of *M. citrifolia* was evaluated for analgesic and behavioral effects in mice. The extract did not exhibit any toxic effects but did show a significant, dose-related, central analgesic activity in the writhing and hotplate tests; this effect was confirmed by the antagonistic action of naloxone. Furthermore, administration of *M. citrifolia* extract at high dosages decreased all behavioral parameters in the two compartment test, the light/dark choice situation test and the staircase test together with the induced sleeping time. These results are suggestive of sedative properties.

Su et al. (2001) reported that Tahitian Noni juice cause selective COX-2 enzyme inhibition.

Mc. koy *et al.* (2002) evaluated the anti-inflammatory potency of both orally and intraperitonealy injected extract of Noni fruit juice.

Wang *et al.* (2002) reported that Noni has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, anti tumor, anti helminthes, analgesic, hypotensive, anti-inflammatory and immune enhancing effects.

Punjanon and Nandhasri (2003) evaluated the alcoholic extract from the fruits of *Morinda citrifolia* (Noni) for analgesic effect in mice using the acetic acid-induced writhing test. The inhibitory effect of the 4 g kg-1 dose of extract was similar to that produced by morphine in a dose of 1.5 mg kg-1. The antinoniceptive effect in writhing test was statistically significant ($p<0.001$) for 15 min until 5 hr of administration.

Palu *et al.* (2005) reported that Noni inhibits both COX-2 & LOX (lipoxygenase) enzymes & produce anti-inflammatory & anti cancerous activity.

Deng *et al.* (2006) isolate several phytochemicals from Noni fruit which selectively inhibit COX-2 in vitro, indicating potential anti-inflammatory activity.

Srivastava (2006) reported Noni to be effective in allaying pain and controlling painful inflammation and swellings. Studies in mice have further demonstrated that the aqueous root extract of Noni did show dose related central analgesic activity in writhing and hot plate tests .Two types of COX enzymes, the COX-1 and COX-2 have been discovered indicating COX-2 enzyme largely responsible for causing pain and inflammation, whereas, COX-1 enzyme is responsible for protecting the stomach lining and kidneys. Noni juice selectively inhibits COX-2 enzyme while allowing the COX-1 enzyme to continue functioning. Scopoletin, an important ingredient of Noni has been found to exert strong anti-inflammatory activity. Scopoletin has also been reported to be an analgesic as it significantly increased the reaction time in mice on hot plate analgesiometer.

Xu *et al.* (2006) evaluated the anti-inflammatory action of Noni juice. The test foals were fed orally 60 ml of Tahitian Noni Equine Essentials, twice daily for 60 days. Blood samples were taken at days 10 and 60, from which

the peripheral monocytes were isolated and separated into test and control groups. The test group was then stimulated with lipopolysaccharide (LPS) for 2 hrs. The treated foals showed a dramatic fold reduction in COX-2, TNF-? (tumor necrosis factor), ILs-1? and mRNA (messenger RNA), compared to the untreated controls. A similar, but less dramatic reduction was observed at 60 days. These results indicate that foals receiving the Tahitian Noni Equine Essentials may experience promising anti-inflammatory therapy.

Akihisa *et al.* (2007) isolate a new anthraquinone, 1,5,15-tri-O-methylmorindo from the methanolic extract of the fruits of Morinda citrifolia (Noni) having anti-inflammatory and potential cancer chemo preventive activity.

Deng *et al.* (2007) isolated the lipoxygenase inhibitory constituents of the fruits of Noni (*Morinda citrifolia*). A phytochemical study of the fruits of Noni (*Morinda citrifolia*) collected in Tahiti led to the isolation of two new lignans, (+)-3,4,3',4'-tetrahydroxy-9,7'?-epoxylignano-7?,9'-lactone (1) and (+)-3,3'-bisdemethyltanegool (2), as well as seven known compounds, (-)-pinoresinol (3), (-)-3,3'-bisdemethylpinoresinol (4), quercetin (5), kaempferol (6), scopoletin (7), isoscopoletin (8), and vanillin. Compounds 1-8 were shown to inhibit 5- and/or 15-lipoxygenase, with IC50 values ranging from 0.43 to 16.5 ? M. Compound 5 exhibited weak inhibitory activity toward cyclooxygenase.

Pak Dek *et al.* (2008) reported that Morinda citrifolia has an inhibitory effect on lipoprotein lipase activity. Efficacy of *Morinda citrifolia* L. leaf (MLE) and fruit extracts (MFE) in inhibiting lipoprotein lipase (LPL) was determined in vitro and showed that the highest inhibition on the LPL activity was exhibited by MLE (66%± 2.1%), which is significantly higher than that demonstrated by MFE (54.5%± 2.5%), green tea extract (GTE) (54.5%± 2.6%), and catechin (43.6%± 6.1%).

Antibacterial activity of *Morinda citrifolia :*

Bushnell *et al.* (1950) reported on the antibacterial properties of some plants found in Hawaii, including Noni. He further reported that Noni was

traditionally used to treat broken bones, deep cuts, bruises, sores and wounds. Extracts from the ripe noni fruit exhibited moderate antibacterial properties against Ps aeruginosa, M pyrogenes and E coli, and were also shown to have moderate antibacterial properties against Salmonella typhosa, Salmonella montevideo, Salmonella schottmuelleri, Shigella paradys, BH and Shigella paradys, III-Z.

Atkinson *et al.* (1956) Acubin, L-asperuloside, and alizarin in the Noni fruit, as well as some other anthraquinone compounds in Noni roots, are all proven antibacterial agents. These compounds have been shown to fight against infectious bacteria strains such as Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Baciillis subtilis, Escherichia coli, Salmonella, and Shigela. These antibacterial elements within Noni are responsible for the treatment of skin infections, colds, fevers, and other bacterial-caused health problems.

Leach *et al.* (1988) demonstrated that acetone extracts obtained from Cycas circinalis, *Morinda citrifolia, Bridelia penangiana, Tridax Procumbens, Hibiscus tiliaceus, and Hypericum papuanun* showed antibacterial activity. The widespread medicinal use of these plants would suggest that they do contain pharmacologically active substances and alternative methods of extraction and screening should be utilized to find the major bioactive component in the plants for the purpose of new drug development.

Locher *et al.* (1995) reported that selected plants including *Morinda citrifolia* have a history of use in Polynesian traditional medicine for the treatments of infectious disease. These plants were investigated for anti-viral, anti-fungal, and anti-bacterial activity in vitro. Their study using biological assays in vitro confirmed that some of the ethnobotanical reports of Hawaiian medicinal plants have curative properties against infectious diseases.

Duncan *et al.* (1998) demonstrated that scopoletin, a health promotor in Noni, inhibits the activity of E coli, commonly associated with recent outbreaks resulting in hundreds of serious infections and even death. Noni also helps stomach ulcer through inhibition of the bacteria H pylori.

Saludes and colleagues (2002) A crude ethanol extract and hexane fraction from *Morinda citrifolia* show antitubercular activity. The major constituents

of the hexane fraction are E-phytol, cycloartenol, stigmasterol, sitosterol, campesta-5,7,22,trien-3-ol and the ketosteroides stigmasta -4-en-3-one and stigmasta -4-22-dien-3-one.E-phytol, a mixture of the two ketosteroids, and the epidioxysterol derived from campesta-5,7,22-trien-3-ol all show pronounced antitubercular activity.

Wang *et al.* (2002) stated that *Morinda citrifolia* L (Noni) has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelmin, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects.

Makato *et al.* (2003) studied antibacterial activity of extracts of Noni, cat's claw, Maca, Pashuchaka, in a methanol+chloroform mixture and in water and reported that all extracts (including Noni) showed antibacterial activity against Staphylococcus aureus and the potency was in order of Maca > Pashuchaca = Non i> cat's claw. None of water extracts showed any activity against St. aureus. Some of extracts (Maca and Pashuchaca) exhibited antimicrobial activity against E. coli while cat's claw and Noni extracts did not. Extracts of Noni in a chloroform + methanol mixture and in water were examined for their antioxidative and antimicrobial activities. The extracts (chloroform+ methanol and water) were found to contain low molecular weight substances that turned brown upon denaturation, and ?- tocopherol. It seems likely that interaction among these low molecular weight substances are responsible for the antioxidative and antimicrobial activities of these extracts, and that the difference in the potency of their activities.

Sekander (2004) investigated and explores its bioactive (antibiotic) properties, as well as synergistic potential as a result of combining its extracts with known antibiotics in certain ratios. It focuses on the extraction processes carried out to prepare the extracts for bioactivity /synergy testing and includes results obtained upon completion of the tests. The report states that bioactive properties were exhibited by *Morinda citrifolia*. However, it does not confirm that synergism was observed. This is due to the lack of a solid definition of synergism that is described in the report. Nonetheless, it reports on the observation of slightly enhanced bioactivity when combinations of the plant extracts and Penicillin were tested.

Rios and Recio (2005) reported Antimicrobial activity of Morinda citrifolia leaf extract is compared with the antibiotics` of the respective organism. It was found that the extract in some cases exhibited the zone of inhibition which was equal or greater than the zone of inhibition of antibiotic. leaf extract can surely inhibit the growth of these microorganisms there by preventing various disease such as skin infections, diabetes, cancer etc. *Morinda citrifolia* leaf extract thus provides safe, easy, effective and practical solutions to every day ailments leaving behind no toxins and creating a clean, pleasant atmosphere. The overall results indicate promising baseline information for the potential uses of solvent extracts of M. citrifolia leaf in the treatment of infectious disease.

Zaidan *et al.* (2005) reported that Noni inhibits growth of infectious bacteria like Pseudomonas aeruginosa, protius morgii, Staphylococcus aureus, E.coli Salmonella and Shigellla. Antibacterial activity is due to L-asperuloside and alizarine compounds. Another compound scopoletin of Noni inhibits E. coli. Noni inhibits the growth of Helicobacter pylori which is responsible gastric ulcer. The antimicrobial activities of Noni plant extracts against the five bacteria strain examined were assessed by the presence or absence of inhibition zones and MIC values. In this preliminary investigation, the leaves were used and the crude extracts were subjected to screening against five strains of bacteria species, Methicillin Resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli, using standard protocol of Disc Diffusion Method (DDM). The antibacterial activities were assessed by the presence or absence of inhibition zones and MIC values.

Jayaraman *et al.* (2008) reported the yield of M.citrifolia fruit extracts using methanol, ethyl acetate and hexane as solvent were 22.73%, 1.9%, and .11 % respectively, on dry weight basis. The in vitro antibacterial activity of methanol, ethyl acetate and hexane extracts of *Morinda citrifolia* fruits among the three solvent extracts tested, methanol extract showed maximum inhibition potential against all tested microorganisms followed by ethyl acetate and hexane extracts respectively. Methanol extract was active against both gram positive and gram negative organisms with varied extent of antibacterial activity.

Rajarajan *et al.* (2009) suggested the usefulness of Seitz filtered aqueous extracts of ripe fruit and unripe fruits of *Morinda citrifolia* for Gastroenteritic and Pyogenic bacterial infections. The Mininum Inhibitory Concentration and Minimum Bactericidal Concentration of the Seitz filtered aqueous extracts of both ripe fruit and unripe fruits have been determined for the first time. Both ripe fruit and unripe fruit have equal level of Bactericidal activity. The study was done for eight gastroenteritic bacteria and six pyogenic bacteria. The estimation of antibacterial activity of the ripe fruit & unripe fruit extract were carried out by serial two fold tube dilution techniques. Minimum Bactericidal Concentration of the ripe as well as unripe fruit extracts for the susceptible bacteria were estimated by Recovery plate method. Both the Unripe and ripe fruit extracts exhibited nearly equal and effective activity.

Selvam *et al.* (2009), studied antibacterial activity of various extracts of *Morinda citrifolia* and suggested that it exhibit moderate antibacterial activity (Zone of inhibition: 13-21 mm) against E. coli, Staphylococcus aureus and *Proteus vulgaris* compared to standard levofloxacin (Zone of inhibition: 35 mm) under similar conditions. Antibacterial activity of various extracts of the fruit powder of *Morinda citrifolia* was studied against *E coli, Staphylococcus aureus* and *Proteus vulgaris* by cup plate method in nutrient agar medium. The activity was compared with standard levofloxacin under similar conditions. All the extracts of Morinda citrifolia were found to exhibit moderate antibacterial activity against *S. aureus* and *Proteus vulgaris* compared to standard levofloxacin.

Usha *et al.* (2010) reported that it *Morinda citrifolia* L. has antifungal, antibacterial, antiinflammatory and antiviral activities.

Immunomodulatory activity of *Morinda citrifolia*

Hokama *et al.* (1993) separated ripe noni fruit juice into 50 % aqueous alcohol and precipitated fractions that stimulated the BALB/c thymus cells in the [3H]thymidine analysis. It is suggested that inhibition of Lewis lung tumors in mice, in part, may have been due to the stimulation of the T-cell immune response.

Asahina *et al.* (1994) found that an alcohol extract of Noni fruit at various concentrations inhibited the production of tumor necrosis factor-alpha (TNF-

a), which is an endogenous tumor promotor. Therefore the alcohol extract may inhibit the tumor promoting effect of TNF-a.

Hirazumi *et al.* (1996) found that nonippt contains a polysaccharide-rich substance that inhibited tumor growth. It did not exert significant cytotoxic effects in adapted cultures of lung cancer cells, but could activate peritoneal exudate cells to impart profound toxicity when co-cultured with the tumor cells. This suggested the possibility that noni-ppt may suppress tumor growth through activation of the host immune system. Noni-ppt was also capable of stimulating the release of several mediators from murine effector cells, including TNF-a, interleukin-1beta (IL-1b), IL-10, IL-12, interferon-gamma (IFN-g) and nitric oxide (NO).

Hirazumi and Furusawa (1999) reported that the fruit juice of *Morinda citrifolia* (noni) contains a polysaccharide-rich substance (noni-ppt) with antitumour activity in the Lewis lung (LLC) peritoneal carcinomatosis model.

Wang *et al.* (2002) in University of Illinois College of Medicine observed that the thymus in animals treated with TNJ (Tahitian Noni juice) was enlarged. Wong (2004) reported In the State of Hawaii, there are abundant claims of benefit from cancer patients' use of the fruit juice of *Morinda citrifolia* (Noni).

Manuele *et al.* (2006) studied Immunomodulatery effect were reported for a traditional hawaian herb, *Morinda citrifolia* (Noni), rich in scopoletin, which was found to enhance the host immune system involving macrophage and lymphocytes.

Palu *et al.* (2008) studied to investigate the mechanisms involved in the immunomodulatory effects of Morinda citrifolia L. (noni) in vitro and in vivo in mice. In vitro, Tahitian Noni Juice (TNJ) and Noni fruit juice concentrates (NFJC) (1, 5 mg/mL) potently activate cannabinoid 2 (CB2), but inhibit cannabinoid 1 (CB1) receptors in a concentration-dependant manner. In vivo, oral administration of TNJ ad libitum for 16 days decreased the production of IL-4, but increased the production of IFN-?.

Selvam *et al.* (2007) *Morinda citrifolia* have been studied for antiviral activity against replication of HIV-1 (IIIB) in MT-4 cells. Morinda citrifolia exhibited a maximum protection of 18 percent against HIV-1 (IIIB) strains and displayed marked cytostatic activity in MT-4 cells (C-type Adult T cell leukemia cells) with CC50=0.1930 ?.g/ml.

Nayak and Mengi (2009) investigated the immunostimulant effects of the extracts and the bioactive fractions, namely, polysaccharides, anthraquinones, and alkaloids, of the fruits of *Morinda citrifolia* Linn. (Rubiaceae). The extracts and the fractions were evaluated for their effect on in vitro phagocytosis of Candida albicans spores by neutrophils obtained from pooled human blood. The maximum activity was demonstrated by the hydroalcoholic extract (79.25% at 1.0 mg mL-1, $p < 0.05$) and the polysaccharide fraction (60.0% at 0.2 mg mL-1, $p < 0.05$) obtained from the fruits of the plant. The hydroalcoholic extract and the polysaccharide fraction were evaluated for their effect on serum interleukin-6 (IL-6) levels in rats sensitized by intraperitoneal injection of Bacillus Calmette Guerin (BCG) vaccine I.P. The studies indicated the potential of *Morinda citrifolia* as an immunostimulant herbal drug.

Anti bacterial activity of Ciprofloxacin (Ci)

Bauernfeind (1998) studied the in-vitro activities of the quinolones, ciprofloxacin, trovafloxacin, clinafloxacin, levofloxacin and gatifloxacin were compared. Pseudomonas spp. was most susceptible to clinafloxacin and ciprofloxacin. Gram-positive cocci were most susceptible to clinafloxacin and trovafloxacin. Against gram-positive cocci, the only agents that were more active than ciprofloxacin were those carrying an azabicyclo (trovafloxacin), 3-amino-pyrrolidinyl (clinafloxacin) or 3- methyl-piperazinyl (gatifloxacin) moiety at position C7.

Nakamura et al. (2009) compared the antimicrobial activities of oral quinolones, ciprofloxacin , gatifloxacin, garenoxacin, moxifloxacin, norfloxacin, prulifloxacin and tosufloxacin using Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus agalactiae, Streptococcus pyogenes, extended spectrum beta-lactamase (ESBL) producing Klebsiella pneumoniae,

and methicillin-susceptible Staphylococcus aureus (MSSA) isolated from clinical materials. Based on the pharmacokinetics-pharmacodynamics theory, the target attainment rate at the area under the curve MIC of 120 or more for Gram-negative and 30 or more for Gram-positive bacteria was calculated using Monte Carlo simulation (MCS), and was assessed as the efficacy. The antimicrobial activity of Ciprofloxacin against H. influenzae was most potent.

Immunomodulatory activity of ImmuPlus

Kumar *et al.* (2003) found that ImmuPlus (standard drug) when fed to calves (@ 1g/calf/day for 90 days), vaccinate with FMD vaccine (Hoechst), lead to increase in the antibody titre against the FMD vaccine TLC, absolute lymphocyte count (ALC), absolute neutrophil count (ANC), T and B lymphocyte blastogenesis, BoCD4+ and BoCD8+ cell count and interleukin 1 and 2.

Panda *et al.* (2004) found that administration of ImmuPlus, a polyherbal preparation (standard drug) @ 75-100mg/litre of drinking water to broilers gave encouraging results not only for better growth but also as anti stress factor in summer days, enhancing the antibody titre of the Ranikhet disease vaccination.

Singh *et al.* (2004a) reported that ImmuPlus (standard drug) significantly augmented the cell mediated immunity in treated group of birds. The CMI responses were evaluated by lymphocytes stimulation test (LST) and delayed type hypersensitivity (DTH) reaction was observed using dinitrochlorobenzene (DNCB) as allergen.

Singh *et al.* (2004b) found that Immu Plus (standard drug) when given @ 25 mg/kg body weight in drinking water of birds revealed high immunoglobulin (IgG, IgM and IgA).

3. Objectives

a. Preparation of aqueous & alcoholic extract of Noni fruit
b. Chemical analysis of Noni fruit to determine the presence of bioactive principles
c. Acute toxicity study to determine the lethal dose LD50
d. Chronic toxicity study of Noni fruit
e. To study the anti-inflammatory activity of aqueous & alcoholic extracts of Noni on acute and chronic inflammatory models.
f. To study the role of inflammatory mediators on anti-inflammatory activity of Noni.
g. To study the analgesic activity of aqueous & alcoholic extracts of Noni.
h. To study the antipyretic activity of aqueous and alcoholic extracts of Noni on experimentally induced pyrexia in rats.
i. To study *in vitro* and *in vivo* antibacterial activity of aqueous and alcoholic extract of Noni in experimental albino rats.
j. To study the immuno-modulatory activity of aqueous and alcoholic extract of Noni in experimental albino rats.

4. Experimental details

The experiment was conducted using approx. 48 adult albino rats (120-150g weight) and 30 mice (20-30gm) of either sex, which were randomly divided into 13 groups, each consisting of six rats and six mice (five group of mice for acute toxicity, three groups of rats for chronic toxicity, two groups of rats for motor in coordination and three groups of rats for sleeping time. The rats were maintained in polypropylene colony cages and housed in the laboratory animal section of the department under identical and good manage mental conditions. Rats and mice were fed standard ration adlibitum and free access to clean water. Deworming was done before the start of experiment using Praziquantel @ 1 tablet per 10 kg body weight as single dose.

Materials

- Noni fruits
- Pentobarbitone
- Soxhlet apparatus
- Water bath
- Dragendorff's reagent
- Fehlings reagent A & B
- Benedict's reagent
- Chloroform
- Sulphuric acid
- Ferric chloride
- Sodium hydroxide
- Copper sulphate
- Ninhydrin reagent
- Olive oil
- Ammonia solution

Methods

Preparation of extract

The Noni fruits were dried in hot air oven and then minced.

a. Alcoholic extract

Morinda citrifolia was extracted with 95% alcohol in a soxhlet apparatus, till colourless solvent started returning back to the reservoir. The residue after being completely freed from alcohol by evaporating on a water bath in tarred crucible, was weighed and its percentage extractability was calculated (Pandey, 1980) (Plate 2)

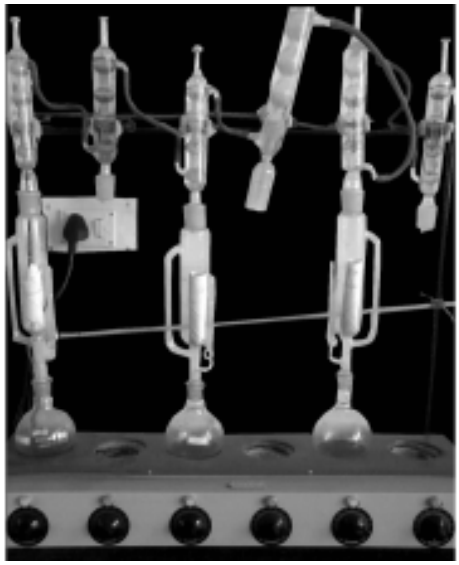


Plate 2 : Soxchlet apparatus showing alcoholic extraction of *Morinda citrifolia*

b. Aqueous extract

It was prepared afresh every time by boiling 1gm powder in 10 ml of distilled water for 5 min. The extract was filtered by cotton plug and the volume was made to 10 ml with distilled water. The left over residue was then completely dried and weighed and its extractability percentage calculated (Pandey, 1980). (Plate 3).

Plate 3 : Water bath used for aqueous extract preparation

Chemical analysis of *M. citrifolia*

The phytochemical study of Noni was undertaken to determine the presence of bioactive ingredients in Noni fruits. The phytochemical study of *Morinda citrifolia* was done as per method described by Tandale *et al.* (1986) (Plate 4).

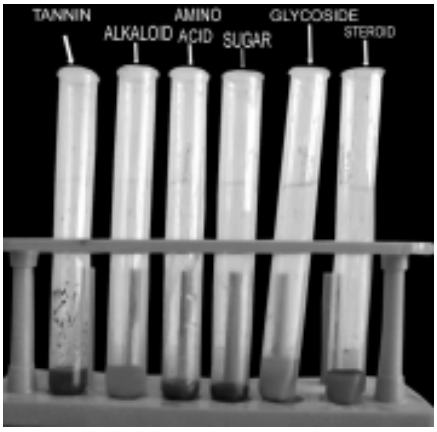


Plate 4 : Test tube showing phytochemical ingredients in *Morinda citrifolia* fruits (aqueous and alcoholic extracts)

The following active principles were determined.

a. Alkaloids

The presence of alkaloid was tested in alcoholic and aqueous extracts of noni fruit with Dragendorff's reagents. A little extract of the fruit was dissolved in N/10 hydrochloric acid and stirred well. The solution was filtered and a few drops of Dragendorff's reagent were added to the filterate. Appearance of red orange precipitate was considered positive for presence of alkaloid.

b. Reducing sugars

The presence of reducing sugar was tested in alcoholic and aqueous extracts of noni fruit. To 2ml of either of the extracts were added 0.5 ml of Benedict's reagent and a few drops of 10% sodium hydroxide solution. This mixture was heated over a water bath for 10 min. Appearance of red precipitate (reduction) indicated presence of reducing sugar.

c. Glycosides

To 2 ml of the alcoholic and aqueous extract of noni fruit in a test tube, 1 ml each of Fehling solution A and B was added. This mixture was heated over a water bath for 10 min .Appearance of yellow-green precipitate indicated presence of glycosides.

d. Tannins

To 0.5 gm alcoholic and aqueous extract of noni fruit, 15 %ferric chloride solution was added. Appearance of green color precipitate after well shaking indicates the presence of hydrolysable tannin and appearance of blue colour precipitate indicates presence of condensed tannin.

e. Proteins

To 2 ml of alcoholic and aqueous extract of noni fruit in the test tube,2 ml of sodium hydroxide was added. After proper mixing, 2-3 drops of .5% copper sulphate solution was added. Appearance of purplish violet or pinkish violet colour indicated presence of proteins.

f. Amino acids

To 2 mg of the alcoholic and aqueous extract of noni fruit 1ml of .2 gmninhydrin reagent was added. The mixture was boiled for 15-20 sec over water bath. Appearance of violet/blue colour indicated presence of amino acids.

g. Anthraquinones

To 0.5 gm of alcoholic and aqueous extract of noni fruit in a test tube, 5 ml of chloroform solution was added. After shaking for 5 min. and filtering 1 ml of 1% ammonia solution was added. Appearance of pink / violet colour in the lower layer indicated presence of anthraquinones.

h. Saponins

To 0.5 gm of the alcoholic and aqueous extract of noni fruit, a few drops of olive oil were added. Appearance of persistent froth on vigarous shaking indicated presence of saponins.

i. Steroids

To 100 mg of the alcoholic and aqueous extract of noni fruit, 2 ml of chloroform was added. On adding a few drops of sulphuric acid from the side of test tube, appearance of reddish brown ring at the interface indicated presence of steroids.

Acute Toxicity Studies

In order to determine the approximate LD50 of aqueous and alcoholic extract of Noni fruit, the extracts were administered orally to the five groups of mice having six mice in each group. Fresh groups of mice were used for each doses (500, 1000, 1500, 2000 and 2500 mg/kg body weight) .The observations were made up to a period of 48 hours and mortality was noted.

During the acute toxicity studies, gross observational effect were observed.

Clinical observation

i) Hair coat
ii) Lacrimation
iii) Salivation
iv) Convulsion
v) Biting
vi) Tremour
vii) Defaecation
viii) Urination

Effect on Nervous system

i) Behaviour
ii) Stimulation
iii) Depression
iv) Respiration

Effect on Locomotor system

i) abnormal gait
ii) ataxia
iii) head position

Chronic toxicity studies

Treatment groups

Groups	Treatment
Group I	Control (normal saline)
Group II	Aqueous extract of Noni fruit.
Group III	Alcoholic extract of Noni fruit.

The aqueous and alcoholic extracts were administered orally at the dose of 1000 mg/kg body weight for a period of 90 days. Animals were observed for abnormal signs, if any.

At the end of study, animals were sacrificed and blood samples were collected directly from heart using ethylene diamine tetra acetic acid (EDTA) (2mg / ml of blood as per the method of Schalm *et al.,* 1975). Pieces of liver and kidney were also collected for histopathological studies. Each carcass was thoroughly examined for any gross abnormality before disposal.

Hematological studies

Blood sample collection

Two ml of blood was collected from the inner canthus of rats in EDTA tubes @ 1 mg/ml of blood for haematological estimation and without anticoagulant for sero-biochemical profile. Blood samples were collected on 0 day, 8th day, 15th day, 21Ist day and 28th day.

a. Total erythrocyte count (TEC)

The red cells were counted in haemocytometer according to the standard procedure (Jain, 1986). Number of cells counted was multiplied by 10,000

due to the dilution of blood @ 1:200. The count was expressed as million / ml of blood.

b. Total leukocyte count (TLC)

Total leukocyte count was done in haemocytometer according to the standard procedure (Jain, 1986). The counts were expressed as thousand / ml of blood.

c. Differential leukocyte count (DLC)

The blood films were prepared on the glass slides and stained with Leishman's stain. The standard procedure was followed for counting the cells (Jain, 1986).

d. Packed cell volume (PCV)

Packed cell volume was estimated by capillary tube according to the standard procedure (Jain, 1986).

e. Haemoglobin concentration (Hb)

Haemoglobin estimation was done by using Sahli'shaemoglobinometer. The results were expressed as g/dl of blood (Jain, 1986).

f. Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) expressed in femtolitre (fl)

$$MCV = \frac{PCV}{RBC / mm3}$$

g. Mean corpuscular haemoglobin (MCH)

Mean corpuscular haemoglobin (MCH) expressed in picogram (pg)

$$MCH = \frac{Hb \text{ in one } mm3}{RBC / mm3}$$

h. Mean corpuscular haemoglobin concentration

(MCHC)Mean corpuscular haemoglobin concentration (MCHC) expressed as g/dl

$$MCHC = \frac{\text{Hb in one mm3}}{\text{PCV}} \times 100 \quad OR \quad \frac{\text{MCH}}{\text{MCV}} \times 100$$

i. Blood clotting time :

Clotting time was recorded by capillary tube method. Capillary tubes with a lumen of about 1mm diameter and length of 5 cm were used. The tubes were then filled with blood and were broken at the intervals of 5 sec till the fibrin thread formed between the tube and the broken end (Jain, 1986).

Sero-biochemical Studies

a. Total protein

Total protein was calculated by Biurette method according to the standard procedure (Tietz, 1970).

b. Albumin

Albumin was estimated by modified Biurette and Doumas method as per standard procedure (Doumas, 1978).

c. Globulin

Globulin was estimated by subtracting albumin from total protein

d. Albumin-globulin ratio

Albumin-globulin ratio was calculated by dividing albumin by globulin.

Gross and Histopathology

Necropsy was done immediately after sacrificing the rats. One or two mm thick tissue pieces from liver and kidney were collected and fixed in 10%

formalin. After proper fixation overnight and washing in gentle tap water, dehydration of tissue was done. The tissue was sampled and processed by conventional method. Paraffin blocks were made and 6 micro meter paraffin sections was stained with haematoxylin and eosin. They were examined under light microscope. All deviation from normal histology were recorded and compared with corresponding controls.

Examination of tissues was done following the standard method described by Lillie (1954).

Gastric ulcers

Gastric ulcers were observed in chronic toxicity groups. The stomach was dissected out, opened along the greater curvature and examined under the dissecting microscope to determine the incident and severity of ulceration. Robert *et al.* (1986).

The presence of

1) Shedding of epithelium
2) Petechial haemorrhages
3) One or two small ulcer
4) Many ulcers and
5) Perforated ulcers

were considered positive. An arbitrary ulcer grading system of 0 to 4 scales, according to severity, was given to determine the ulcer index.

Ulcer index was calculated as per the following formula-

Percent incidence

Ulcer index= ----------+Number of ulcers+ ulcer score

Number of animals

Effect of Noni fruit on motor in-coordination

Treatment groups

Groups	Treatment
Group-I	Aqueous extract of Noni fruit (1000 mg/kg body weight)
Group-II	Alcoholic extract of Noni fruit (1000 mg/kg body weight

Method

a. Rota-rod Method

Motor in coordination as per the technique of Turner (1965). Two groups of rats consisting of six rats in each group were taken in the present study. The rats were trained to maintain on rota rod at least for a period of two minutes and only those rats were selected which completed three successful trials per day.

Plate 5 : Rota-rod used for study of motor incordination

After training, the extracts were immediately administered to rats. The rats were again placed on the rota rod after 60,120,180 and 240 minutes of drug administration and were required to remain on rota rod for a period

of 2 minutes. The number of rats falling off within two minutes at each time interval from each group was recorded. Rats falling off the rota rod within two minutes were considered as having motor inco-ordination (Plate 5).

b. Actophotometer

One of the most obvious signs of sedation in animals is reduction in their motor activity and this change in motor activity is measured by a photoelectric activity box known as actophotometer. In actophotometer photocells are activated when the rays of light falling on photo cells are cut off by animals crossing the path of the light beam. The cumulative count is therefore recorded on the counter.

The rats were placed in the actophotometer and the movements were recorded for a period of ten minutes at a time and the cumulative count is recorded. The movements of rats were recorded at 0 hour and at the end of 1,2,3 and 4 hours after drug administration (Plate 6).

Plate 6 : Actophotometer - Used for Study of Motor incordination

Effect of Noni fruit on pentobarbitone induced sleeping time

Treatment groups

Groups	Treatments
Group -I	Pentobarbitone sodium (20 mg/kg bwt. I/P)
Group -II	Pentobarbitone sodium (20 mg/kg bwt. I/P) + Aqueous extract of Noni fruit (1000 mg/kg bwt. orally).
Group -III	Pentobarbitone sodium (20 mg/kg bwt,I/P) + Alcoholic extract of Noni fruit (1000 mg/kg bwt. orally)

Method

The sleeping time was recorded as per the method of Brodi *et.al.* (1955).Sleeping time was measured at the interval between the loss and recovery of the rightening reflex. The loss of rightening reflex was considered to be present if the animals were placed on their ventral side and they failed to regain their normal posture within one minute.

The alcoholic and aqueous extracts of Noni fruit were administered orally half an hour prior to the intraperitoneal injection of pentobarbitone sodium.

Anti-inflammatory

The experiment was conducted on 240 healthy adult albino rats of either sex, weighing between 120-150g.

The following materials were used for anti-inflammatory study

1) Noni fruit-Aqueous and alcoholic extract
2) Phenylbutazone
3) Promethazine
4) Cimetidine
5) Cyproheptadine
6) Paracetamol

7) Carrageenin
8) Hot plate analgesiometer
9) 15% dried Brewers yeast
10) 2% gum acacia
11) Plethysmometer
12) Digital tele thermometer
13) Cotton balls (10 mg)

Treatment groups

The model of experiment on inflammation was as follows :

S.No.	Groups	Treatment
1.	Group I	Aqueous extract of Noni
2.	Group II	Alcoholic extract of Noni
3.	Group III	Phenylbutazone
4.	Group IV	Phenylbutazone + aqueous extract
5.	Group V	Phenylbutazone + alcoholic extract
6.	Group VI	Cyproheptadine + aqueous extract
7.	Group VII	Cyproheptadine + alcoholic extract
8.	Group VIII	Promethazine + aqueous extract.
9.	Group XI	Promethazine + alcoholic extract
10.	Group X	Cemitidine + aqueous extract
11.	Group XI	Cemitidine + alcoholic extract.
12.	Group XII	Paracetamol + aqueous extract
13.	Group XIII	Paracetamol + alcoholic extract
14.	Group XIV	Control (normal saline)

Method

Aqueous extract (1000 mg/kg) and alcoholic extract (1000 mg/kg) of Noni were administered orally to six rats in each group .Simultaneously, standard anti-inflammatory drug, phenylbutazone (100 mg/kg body weight) was also administered orally (Antarkar *et al.,* 1983).One group of rats were administered saline orally and kept as control. Standard blockers of mediators of inflammation were administered intra-peritoneally half an hour before the administration of aqueous and alcoholic extract of Noni viz. cyproheptadine @ 10 mg/kg body wt. (Curzen *et al.,* 1980), promethazine @ 10 mg/kg body wt. (Acharia & Sinha, 1967), cemitidine @ 20 mg/kg body wt. (Mahajan *et al*., 1984) and paracetamol @ 100 mg/kg body wt. (Srivastava *et al.,* 1978) in similar groups of rats.

Acute inflammation

Carrageenin induced rat paw edema

Rat paw edema was produced by the method described by Winter *et al.* (1962). Freshly prepared 0.05 ml suspension of carageenin (1.0% in 0.9% saline) was injected under the plantar aponeurosis of the right hind paw of

Plate 7 : Fabricated Plethysmometer used for acute inflammation measurement

each rat. The paw volume was measured before and at 1 hour interval for 4 hours after the injection of carageenin by the method of Bhatt *et al.* (1977). The alcoholic and aqueous extracts of Noni were administered half an hour before carageenin injection. Cyproheptadine, promethazine, cimetidine and paracetamol were however, given half an hour before Noni (Plate 7).

Chronic inflammation

Cotton pellet implantation method

The pellets of surgical cotton wool weighing 10 mg were sterilized in an autoclave at 15 lbs pressure for 30 minutes and handled with sterilized instruments. These sterilized pellets were aseptically implanted subcutaneously in both the groins of each rat under ether anesthesia as per method described by Meier *et al.* (1950). Procedure of Cotton pellet implantation shown in photograph 8. Extracts and drugs were administered orally daily for seven days and on eighth day the rats were sacrificed and the pellets were dissected

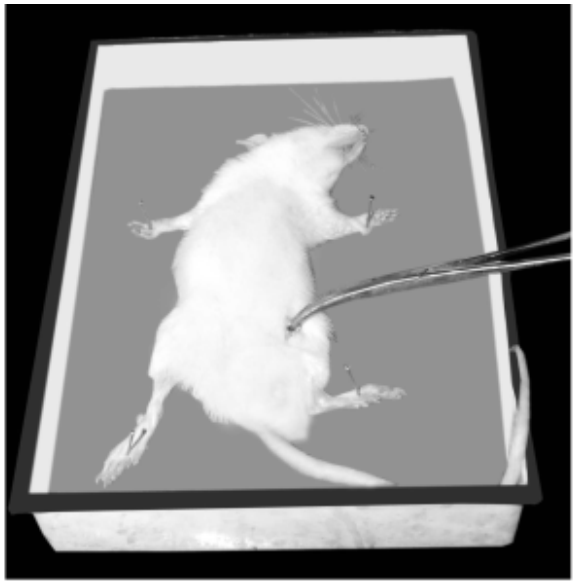


Plate 8 : Rat in dissecting tray showing cotton ball implantation in groin region.

out. After removal of fat and extraneous tissue, the pellets were dried over night at 60°C in hot air oven and weighed. The amount of granulation tissue under various treatments was determined by subtracting the original weight of cotton pellets. The mean weight of granulation tissue of treated groups was compared with that of control. The percent inhibition of granulation tissue was also calculated (Plate 8).

Percent anti inflammatory activity = 1- T/C × 100.

Where T = mean volume of edema in drug treated groups
C = mean volume of edema in control group.

Analgesic activity of Noni

Treatment groups

The dose response study of aqueous and alcoholic extracts of Noni was undertaken in following groups

	Groups	Treatments
1.	Group-I	Aqueous extract of Noni (250 mg /kg bwt. orally)
2.	Group-II	Aqueous extract of Noni (500 mg/kg bwt. orally)
3.	Group-III	Aqueous extract of Noni (1000 mg/kg bwt. orally)
4.	Group-IV	Alcoholic extract of Noni (250 mg /kg bwt. orally)
5.	Group-V	Alcoholic extract of Noni (500 mg/kg bwt. orally)
6.	Group-VI	Alcoholic extract of Noni (1000 mg/kg bwt. orally)

Method

The experiment was conducted in six groups of rats consisting of six rats in each group. The rats were administered the aqueous and alcoholic extracts of Noni orally in graded doses of 250, 500, 1000 mg/kg body weight. One group was kept as control.

The analgesic activity of Noni was studied by the method of Curzen *et al.* (1980) using the hot plate analgesiometer (photograph 9). The licking of hind paw in hot plate was taken as the index of analgesia. The time between putting the rat on hot plate and start of licking the paw was the latent period or reaction time for analgesic activity of drug (Plate 9).

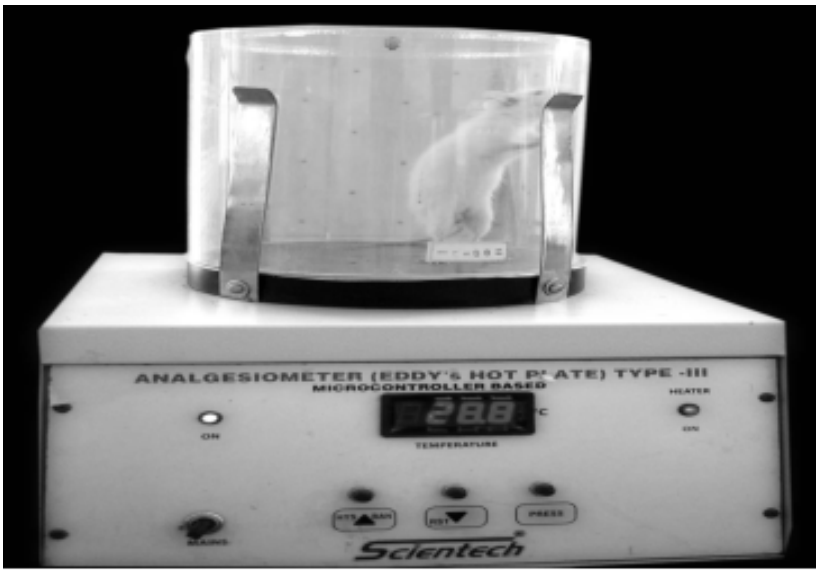


Plate 9 : Hot Plate used for Analgesic activity measurement

Percent analgesic scope (PAS) = 1-T2/T1 × 100.

Where T1 = reaction time (in sec.) before drug administration,

T2 = reaction time (in sec.) after drug administration

Antipyretic activity of Noni

Treatment groups

In pyretic rats, aqueous and alcoholic extracts of Noni was administered orally in following groups

S.No.	Groups	Treatment
1.	Group-I	Aqueous extract of Noni (250 mg /kg bwt. orally)
2.	Group-II	Aqueous extract of Noni (500 mg/kg bwt. orally)
3.	Group-III	Aqueous extract of Noni (1000 mg/kg bwt. orally)
4.	Group-IV	Alcoholic extract of Noni (250 mg /kg bwt. orally)
5.	Group-V	Alcoholic extract of Noni (500 mg/kg bwt. orally)
6.	Group-VI	Alcoholic extract of Noni (1000 mg/kg bwt. orally)

Method

The study was conducted in six groups of rats consisting of six rats in each group. The rats were individually caged in a room maintained at the constant temperature of 25°C. Normal rectal temperature was recorded by a Telethermometer and its hourly variation was noted over a period of 4 hours at the beginning of the experiment.

In above said rats, pyrexia was produced according to the method of Gujral *et al.* (1956). A suspension of 15% dried Brewers yeast and 2% gum acacia in normal saline was injected subcutaneously. The volume injected was 1.0 ml of suspension per 100 gm of body weight. A stabilized temperature was recorded in 18 hours. In pyretic rats, the rectal temperature was recorded at hourly interval for a period of 4 hours after administration of the drug.

Anti bacterial activity of Noni

36 albino rats of 150 -200 gms weight and of either sex with six rats in each group were examined for the effect of alcoholic and aqueous extracts of *Morinda citrifolia* on immune modulation and as anti bacterial. Rats were fed the under mentioned drug candidate indigenous combinations.

S.No.	Groups	Treatments
1.	Group-I	Control
2.	Group-II	Aqueous extract of Noni
3.	Group-III	Alcoholic extract of Noni

List of bacteria used for *in vitro* antibacterial study

S.No.	Bacteria	MTCC Cat. No.
1.	*Bacillus subtilis*	121
2.	*Escherichia coli*	723
3.	*Klebsiella pneumoniae*	109
4.	*Salmonella typhimurium*	98
5.	*Staphylococcus aureus*	1144
6.	*Bacillus cereus*	1272
7.	*Listeria monocytogens*	893

Both *in vitro* and *in vivo* antibacterial activities of different treatments were studied using the various bacterial cultures procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh in freeze dried form. The cultures were subcultured on Nutrient agar tubes for obtaining more bacterial population.

In vitro study

a. Preparation of bacterial broth

The above cultured bacteria were inoculated in Brain Heart infusion nutrient broth tubes and incubated at 37°C for 24 hours for the experiment.

b. Preparation of extract of *Morinda citrifolia*

For the experiment, the dried extract was re-suspended in distilled water to obtain required concentration. Alcoholic and aqueous extracts of Noni were prepared as per the method described by Pandey (1980) with slight modification.

c. Preparation of discs (disc impregnation method):

Different dilutions of alcoholic extracts of Noni and aqueous extracts of Noni, were prepared. Sterile discs (Hi-media) were soaked in these dilutions

in a sterile petridish for 24 hour and were dried in laminar air flow. After drying, the discs were used immediately for disc impregnation in the inoculated plates as described by Kirubaharan *et al.* (1999) with slight modifications.

d. Antibacterial sensitivity and Determination of antibacterial effect (Paper disc diffusion method)

The above prepared bacterial inocula were evenly spread on sterile Muller Hinton Agar plates (Hi-media) as per the method described by Bauer *et al.* (1969) and antibacterial effect was studied by the paper disc diffusion method in these plates. The known antibiotic Ciprofloxacin (Ci) was simultaneously placed as control for antibiotic sensitivity. The dried discs were immediately used and incubated at 37° c for 24 hours and result was recorded as positive (growth) or negative (no growth) and zone of inhibition of growth exerted by these impregnated discs of various dilutions.

In vivo study

Microbial challenge with *E. coli* was done on 60th day of experimentation. This experimentation was conducted in six rats each of six groups. The bacterial culture was procured from the Institute of Microbial Technology (IMTECH), Chandigarh.

a. Preparation of bacterial suspension

Mc-Farland's nephlometer for the determination of bacterial concentration was prepared as described by Henric *et al.* (1956).

b. Dose rate and route

One ml *E.coli* bacterial culture containing 3.0x109cfu/ml as determined by Mc-Farland's nephlometer was used to challenge orally in six rats each group on the 60th day of experiment.

c. Clinical symptoms

The experimental rats of all the six groups were carefully examined for the clinical symptoms following oral inoculation of *E. coli.* Morbidity and mortality rate in the groups were recorded.

Studies of immune response

Cell mediated immune response (CMI)

a. Preparation of Sheep Red Blood Cells (SRBCs) Antigen

SRBCs were collected from jugular vein of sheep and stored in a cold sterile Alsever's solution for immunization and challenge, at required time schedule. Stored sheep red blood cells were centrifuged and washed three times with pyrogen free sterile normal saline (0.9% NaCl w/v) and adjusted to a required concentration of 0.5X 109 and 0.025 X 109 SRBC/ml.

b. Delayed Type Hypersensitivity (DTH) response to SRBC

Delayed type hypersensitivity (DTH) response to SRBC was carried out as per the method described by Doherty (1981) on 90th day. Six rats per group were immunized on 90th day by intra-peritoneal administration of 0.5ml of 5X109 SRBC/rat, and referred as day '0'.The rats were then challenged by subcutaneous administration of 0.025ml of 5X109 SRBC/ml into the right hind footpad on 14th.day. Delayed type hypersensitivity (DTH) response was measured at 24 hour after the SRBC Challenge on day 14 and expressed as mean percent increase in paw volume (Plethysmometrically) (Photograph 10).

Humoral Immune (HI) response:

a. Vaccination (NDV) and collection of serum:

Six rats each in Group-1(Control), Group-2 (Aqueous extract of Noni),Group-3(Alcoholic extract of Noni), Group-4(Syrup Immu-plus) treated up to day 90th and each rat was then vaccinated against New castle disease by F1 strain including the control rats. The day of first immunization was referred to as day '0'. The booster was given on day '3'. The rats were treated according to the scheduled group. The blood samples collected from each rat on day '0', day '7' for primary immune response and day '14' for secondary immune response. The serum samples were separated and centrifuged at 3000 rpm for 10 minutes at room temperature and stored at -20 degree Celsius till further analysis.

b. Haemagglutination inhibition test (HI) titre

Humoral immune response was evaluated by determining the HI titre of rats against Newcastle disease virus as per the procedure described by Fulzele *et al.* (2002).

5. Experimental findings

Phytochemical study

Aqueous and alcoholic extract of fruit of *Morinda citrifolia* (Noni) were prepared and the extractabilities of the fruit was calculated.

Extractability

The percent extractability of Noni fruit in alcohol was 20.40%. The percent extractability of Noni fruit in distilled water was 35%. The alcoholic extract of Noni fruit possessed semi solid consistency and dark brown colour. The aqueous extract of Noni fruit possessed semi solid consistency and light brown colour

Chemical constituents

Chemical tests of aqueous extracts of Noni fruit were undertaken to find out presence of active principles. The results indicated presence of alkaloids, amino acids, reducing sugar, glycosides, tannin and steroid in aqueous extracts of Noni fruit.

Similarly, the alcoholic extract of Noni fruit was also subjected to chemical test for presence of active principles. The results indicated presence of alkaloid, protein, amino acid, reducing sugar, tannin, anthraquinone and steroid in alcoholic extracts of Noni fruit.

Acute toxicity studies

The aqueous and alcoholic extract of Noni fruit did not show any lethal effect in rats up to 2000 mg/kg body wt. orally. The extract was administered orally in graded doses of 500, 1000, 1500, 2000 and 2500 mg/kg body weight.

No mortality was recorded up to 48 hrs after the administration of aqueous and alcoholic extracts. Therefore, oral approx. LD50 of aqueous and alcoholic extract of Noni fruit was more than 2000 mg/kg body wt.

The gross effects observed were negligible at the dose of 2000 mg/kg body weight with aqueous and alcoholic extract of Noni fruit. However there was initial excitement in rats followed with depression and dullness. The extracts at the same dose level produce scrouching of nose. There was no apparent disturbance in locomotion, excretion and secretion. The rectal temperature remains unchanged.

Chronic Toxicity studies

The aqueous and alcoholic extract of Noni fruit administered, orally, at the dose of 1000 mg/kg body weight once daily for a period of three months, did not show any untoward effect in the behavior and body weight of albino rats.

Haematological studies

The rats were sacrificed and the blood was collected for routine haematological studies on 90th day of treatment with aqueous and alcoholic extract of Noni fruit.

a. Effect of Noni (aqueous and alcoholic extract) on TEC (million/cu.mm.)

The mean values of effect of Noni on TEC (million/cu.mm.) in groups with control, aqueous and alcoholic extract were 7.55 ± 0.04, 7.56 ± 0.05 and 7.83 ± 0.05respectively (Table 3 and Fig. 1). Analysis of variance reveals that there is not much difference in TEC in aqueous extract as compared to control but significant increase is seen in alcoholic extract.

b. Effect of Noni (aqueous and alcoholic extract) on Haemoglobin (gm%)

The mean values of effect of Noni on haemoglobin (gm%) in groups with control, aqueous and alcoholic extract were 15.10±0.10, 15.12±0.11 and 15.66 ± 0.11 respectively (Table 4 and Fig. 2). Analysis of variance reveals that there is not much difference in haemoglobin in aqueous extract as compared to control but significant increase is seen in alcoholic extract.

c. Effect of Noni (aqueous and alcoholic extract) on TLC (thousand / cu.mm.)

The mean values of effect of Noni on TLC (thousand/cu.mm.) in groups with control, aqueous and alcoholic extract were 7.94 ± 0.05, 7.95 ± 0.05 and 7.96 ± 0.05 respectively. Analysis of variance reveals that there is no much difference seen in TLC in alcoholic and aqueous extracts as compared to control.

d. Effect of Noni (aqueous and alcoholic extract) on PCV (%)

The mean values of effect of Noni on PCV (%) in groups with control, aqueous and alcoholic extract were 45.3 ± 0.33, 45.38±0.36 and 47±0.36respectively (Table 6 and Fig. 4). Analysis of variance reveals that there is not much difference in PCV in aqueous extracts as compared to control but significant increase is seen in alcoholic extract.

e. Effect of Noni (aqueous and alcoholic extract) on Neutrophil count (%)

The mean values of effect of Noni on neutrophil count (%) in groups with control, aqueous and alcoholic extract were 33.33 ± 0.66, 32.5 ± 0.76 and 33.32 ± 0.77 respectively (Table 7 and Fig. 5). Analysis of variance reveals that there was no significant difference in three treatments for neutrophilcount.

f. Effect of Noni (aqueous and alcoholic extract)on Lymphocyte count (%)

The mean values of effect of Noni on lymphocyte count (%) in groups with control, aqueous and alcoholic extract were 78.33±1.4, 78.34±1.1 and 78.35 ±0.44 respectively.Analysis of variance reveals that there was no significant difference in three treatments for lymphocyte count

g. Effect of Noni (aqueous and alcoholic extract) on monocyte count (%)

The mean values of effect of Noni on monocyte count (%) in groups with control, aqueous and alcoholic extract were 8.16 ± 0.47, 8.17 ± 0.30 and 8.16 ± 0.36 respectively. Analysis of variance revealed that there was no significant difference in three treatments for monocyte count.

h. Effect of Noni (aqueous and alcoholic extract) on eosinophil count (%)

The mean values of effect of Noni on eosinophil count (%) in groups with control, aqueous and alcoholic extract were 1.33 ±0.43, 1.35±.0.56 and 1.36±0.58respectively.Analysis of variance revealed that there was no significant difference in three treatments for eosinophil count.

i. Effect of Noni (aqueous and alcoholic extract) on basophil count (%)

The mean values of effect of Noni on basophil count (%) in groups with control, aqueous and alcoholic extract were 0.166±0.39, 0.170±0.54 and 0.166±.52 respectively. Analysis of variance revealed that there was no significant difference in three treatments for basophil count.

j. Effect of Noni (aqueous and alcoholic extract) on MCV (fl)

The mean values of effect of Noni on MCV (fl) in groups with control, aqueous and alcoholic extract were 60.02 ± 0.01,60.02 ± 0.01 and 61.02 ± 0.01 respectively (Table 12 and Fig. 10). Analysis of variance revealed that there was slight change in MCV in alcoholic extracts as compared to control.

k. Effect of Noni (aqueous and alcoholic extract) on MCH (pg)

The mean values of effect of Noni on MCH (pg) in groups with control, aqueous and alcoholic extract were 200.06 ± 0.02, 200.04 ± 0.02 and 202.04 ± 0.02respectively. Analysis of variance revealed that there was slight change in MCH in alcoholic extracts as compared to control.

l. Effect of Noni (aqueous and alcoholic extract) on MCHC (%)

The mean values of effect of Noni on MCHC (%) in groups with control, aqueous and alcoholic extract were 33.32 ± 0.14, 33.02 ± 0.14 and 35.32 ± 0.14 respectively. Analysis of variance reveals that there was slight change in MCH in alcoholic extracts as compared to control.

m. Effect of Noni (aqueous and alcoholic extract) on Blood clotting time (Sec)

The mean values of effect of Noni on blood clotting time (Sec) in groups with control, aqueous and alcoholic extract were 18.84 ± 0.18 , 20.11 ± 0.17 and 20.93 ± 0.32, respectively. Analysis of variance reveals that there is significant increase in blood clotting time in both aqueous and alcoholic extracts of Noni as compared to control.

Biochemical studies

a. Effect of Noni (aqueous and alcoholic extract) on Total Protein (gm/dl)

The mean values of effect of Noni on total protein (gm/dl) in groups with control, aqueous and alcoholic extract were 6.54 ± 0.12, 6.11 ± 0.03 and 6.49± 0.13 respectively. Analysis of variance reveals that there is significant decrease in total protein in aqueous extracts as compared to control but no significant decrease is seen in alcoholic extract.

b. Effect of Noni (aqueous and alcoholic extract) on Albumin (gm/dl)

The mean values of effect of Noni on albumin (gm/dl) in groups with control, aqueous and alcoholic extract were 3.78 ± 0.05, 3.48 ± 0.10 and 3.75 ± 0.05 respectively. Analysis of variance reveals that there is significant decrease in albumin in aqueous extracts as compared to control but no significant decrease is seen in alcoholic extract.

c. Effect of Noni (aqueous and alcoholic extract) on Globulin (gm/dl)

The mean values of effect of Noni on globulin (gm/dl) in groups with control, aqueous and alcoholic extract were 2.75 ± 0.08, 2.63 ± 0.11 and 2.73 ± 0.07respectively. Analysis of variance revealed that there was no significant effect on globulin in either aqueous or alcoholic extracts as compared to control.

d. Effect of Noni (aqueous and alcoholic extract) on Albumin-Globulin ratio (gm/dl).

The mean values of effect of Noni on albumin-globulin ratio (gm/dl) in groups with control, aqueous and alcoholic extract were 1.37 ± 0.04, 1.34 ± 0.10 and 1.36 ± 0.01 respectively. Analysis of variance revealed that there was no significant effect on albumin-globulin ratio in either aqueous and alcoholic extracts as compared to control.

Gross and Histopathological studies of Liver and Kidney of rats

The rats were sacrificed on 90 th day of treatment with aqueous and alcoholic extracts of Noni. Liver and kidney were collected for gross and histopathological examination.

The gross examination of liver and kidney did not show any significant pathological alteration

Liver

The histopathological studies of liver from group of rats treated with alcoholic and aqueous extracts of Noni showed swollen hepatocytes and mild fatty changes. There was congestion and degeneration in hepatic cells. The lesions were mild in nature in alcoholic extract as compared to aqueous extract (Plate 10, 11, 12)

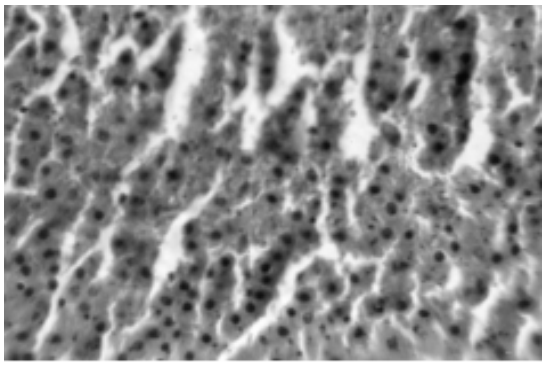


Plate 10 : Photomicrograph showing liver parenchyma and hepatocytes arranged in cords and sinusoidal spaces.

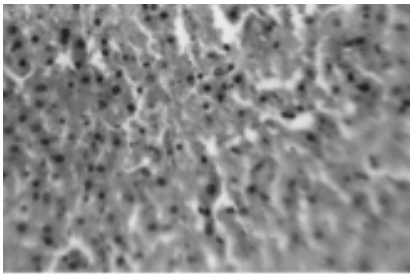


Plate 11 : Photomicrograph showing swollen Hepatocytes with mild degenerative changes in liver with alcoholic extract

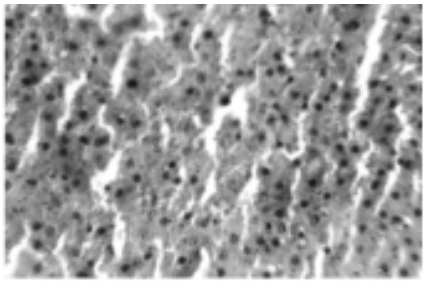


Plate 12 : A Photomicrograph showing swollen hepatocytes and fatty changes in liver with aqueous extract

Kidney

The histopathological studies of kidney from group of rats treated with alcoholic extract of Noni produced leucocytic infiltration and mild congestion in glomerulus of kidney whereas aqueous extract showed degenerative changes with slight haemmorhages in interstitial tissue. The pathological changes in the sections indicated that aqueous extract produced more degenerative changes as compared to aqueous extract. (Plate 13, 14, 15)

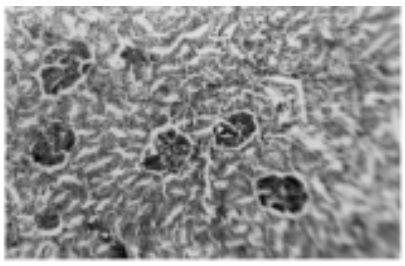


Photo 13: Photomicrograph showing parenchyma of kidney and tubules arranged normally

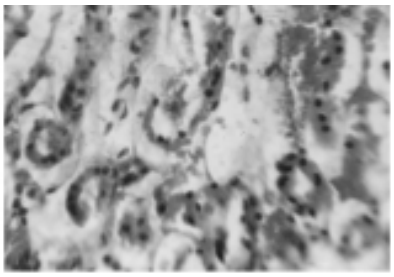


Photo 14: Photomicrograph showing congestion, haemmorhages and degenerative changes in kidney treated with aqueous extract

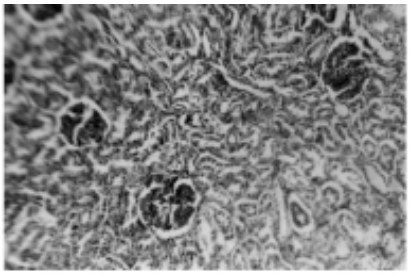


Plate 15 : Photomicrograph showing leucocytic infilteration and mild congestion in glomerulus of kidney interstitial tissue treated with alcoholic extract

Gastric ulcers

No ulcers were found on gastric mucosa. The epithelium was found red in colour and with intact cells. There are no haemorrhages seen.

Motor incoordination

a. Effect of Noni (aqueous and alcoholic extract) on motor incordination by Rota rod

Percent reduction in activity of effect of Noni on motor in coordination by rota rod in group with aqueous extract were 50, 33, 16.66 and 0 in 1st, 2nd, 3rd and 4th hour respectively .The mean values of effect of Noni on motor in-coordination by rota rod in group with alcoholic extract were 16.66, 16.66, 0 and 0 in 1st, 2nd, 3rd and 4th hour respectively. Aqueous extract reduced the motor activity more then alcoholic extract.

b. Effect of Noni (aqueous and alcoholic extract) on motor incordination by Actophotometer.

Percent reduction in activity of effect of Noni on motor incoordination by actophotometerin group with aqueous extract were 13.15, 21.05, 5.26 and 7.81 in 1st , 2nd , 3rd and 4 th hour respectively .The mean values of effect of Noni on motor incoordination by actophotometer in group with alcoholic extract were found to be 0, 5, 2.5 and 2.5 in 1st, 2nd, 3rd and 4th hour respectively. Aqueous extract reduced the motor activity more then alcoholic extract.

Pentobarbitone induced sleeping time

Effect of Noni (aqueous and alcoholic extract) on Pentobarbitone induced sleeping time in rats.

The mean values of effect of Noni (aqueous and alcoholic extract) on sleeping time (min) in groups with control with pentobarbitone, aqueous extract with pentobarbitone and alcoholic extract with pentobarbitone were 31.62 ± 0.55 , 32.66 ± 0.24 and 30.73 ± 0.22 respectively. Aqueous extract of Noni with pentobarbitone produced 3.28% prolongation in sleeping time and

alcoholic extract with pentobarbitone produced 2.81% diminution in sleeping time.

Anti-inflammatory activity of Noni

Acute inflammation

a. Anti-inflammatory activity of aqueous and alcoholic extracts of Noni on carrageenin induced acute rat paw edema.

The results for mean edema volume (ml) in rats recorded at different hours for different treatments (control, aqueous extract and alcoholic extract of Noni.

The anti-inflammatory activity of aqueous and alcoholic extracts of Noni was studied in groups of rats on carrageenin induced rat paw edema, an acute inflammatory process. Aqueous and alcoholic extracts of Noni were administered orally, at the dose of 1000 mg/kg body weight, half an hour before carrageenin injection .One group of rat administered normal saline and was treated as control. The edema volume was measured at hourly intervals for four hours.

The mean edema volume (ml) in control group were 1.1±0.11, 3.71±0.07, 5.0 ± 0.07 and 5.3±0.08, in aqueous extract of Noni were 0.84 ±0.04, 1.6±0.12, 1.1 ±0.09 and 0.68 ±0.07 and in alcoholic extracts of Noni were 0.81 ±0.01, 1.3 ±0.09, 0.83 ±0.03 and 0.5 ± 0.03 at the interval of 1st, 2nd, 3 rdand 4th hour of administration respectively.

The percent anti-inflammatory activity was calculated at hourly interval for 4 hours against carrageenin induced acute inflammation .The aqueous extract of Noni produced 23.6, 56.87, 78.0 and 87.16%. anti- inflammatory activity after 1st, 2nd, 3rd and 4th hour of administration respectively. The alcoholic extract of Noni produced 26.3, 64.95, 83.4 and 90.56% anti-inflammatory activity after 1st, 2nd, 3rd and 4th hour of administration respectively. The analysis of variance revealed that there was significant ($P< 0.01$) difference in mean edema volume observed at different time intervals in a treatment .Both alcoholic and aqueous extract had significant anti-inflammatory action as compared to control.

b. Interaction of phenylbutazone with aqueous and alcoholic extracts of Noni on carrageenin induced acute rat paw edema.

The results for mean edema volume (ml) in rats recorded at different hours for different treatments (phenylbutazone alone,phenylbutazone with aqueous extract and phenylbutazone with alcoholic extract of Noni).

The interaction between phenylbutazone and aqueous extract and alcoholic extract of Noni were studied in groups of rats on carrageenin induced rat paw edema, an acute inflammatory process. Phenybutazone, aqueous extract and alcoholic extract of Noni were also tested for their *per se* anti-inflammatory activity.

The mean edema volume (ml) in phenylbutazone group were 0.73 ±0.01, 0.83 ± 0.03, 0.66 ±0.07 and 0.58 ± 0.05, in phenylbutazone with aqueous extract were 0.7 ±0.02, 0.66 ± 0.02, 0.58 ± 0.02 and 0.50 ± 0.01 and in phenylbutazone with alcoholic extract were 0.66 ±0.04, 0.58 ±0.02, 0.41 ± 0.03 and 0.33 ± 0.02 at the interval of 1st, 2nd, 3rd and 4th hour of administration respectively.

The results indicated that phenylbutazone at the dose of 100 mg/kg body weight orally produced significant anti-inflammatory activity after 2nd, 3rd and 4th hours of treatment, which was 77.62, 86.80, and 89.05% respectively. The combination of phenylbutazone (100 mg/kg body weight, orally) and aqueous extract of Noni (1000 mg/kg body weight ,orally) produced 82.21, 88.4 and 90.56% inhibition of inflammation after 2nd, 3rd and 4th hours of treatment respectively, which was more then per se anti- inflammatory effect of aqueous extract of Noni and phenylbutazone. However, phenylbutazone (100 mg/kg body weight, orally) and alcoholic extract of Noni (1000 mg/kg body weight, orally) produced 84.36, 91.80 and 93.77 percent inhibition of inflammation after 2nd, 3rd and 4th hours of treatment, respectively which was more then per se anti-inflammatory effect of alcoholic extract of Noni and phenylbutazone. The analysis of variance revealed that there was significant ($P < 0.01$) difference in mean edema volume observed at different time intervals in a treatment. Phenylbutazone showed highest anti-inflammatory activity with alcoholic extract of Noni.

c. Interaction of different antagonists of mediators of inflammation with aqueous extracts of Noni on carrageenin induced acute rat paw edema.

The anti-inflammatory activity of aqueous extract of Noni (1000 mg/kg, orally) alone and in combination with antagonists of mediators of inflammation, namely, cyproheptadine (10 mg/kg body wt, I/P), promethazine (10 mg/kg body wt., I/P), cemitidine (20 mg/kg body wt., I/P) and paracetamol (100 mg/kg body wt., I/P) were studied by carrageenin induced rat paw edema in albino rats .The antagonist were administered, intraperitoneally, half an hour before the administration of aqueous extract of Noni. In all groups of rats, carrageenin was injected 30 minutes after aqueous extract administration and mean edema volume was measured at hourly intervals for four hours.

The mean edema volume (ml) in promethazine with aqueous extract of Noni were 0.76 ±0.01, 2.0 ± 0.25, 1.6 ± 0.17 and 1.0 ± 0.11, in cimetidine with aqueous extract of Noni were 0.79 ±0.02, 0.81± 0.01, 0.40 ± 0.01 and 0.16± 0.02 , in cyproheptadine with aqueous extract of Noni were 0.81±.01, 1.95 ± 0.23, 1.63 ± 0.15 and 0.83 ± 0.17 and in paracetamol with aqueous extract of Noni were 0.70 ± 0.02, 0.73± 0.01, 0.49 ± 0.02 and 0.30 ± 0.01 at the interval of 1st,2nd ,3rd and 4th hour of administration respectively.

The results indicated that all antagonists of mediators of inflammation used in the study showed a significant increase in anti-inflammatory activity of aqueous extract of Noni. Cemitidine (20 mg/kg body wt., I/P) increased the anti -inflammatory activity of aqueous extract of Noni by 5.95, 49.37, 63.63 and 76.47% after 1st, 2nd, 3rd and 4th hours of treatment, respectively as compared to aqueous extract of Noni alone. Paracetamol(100 mg/kg body wt., I/P) also increased the anti-inflammatory activity of aqueous extract of Noni by 16.66, 54.37, 55.45 and 55.88% after 1st, 2nd, 3rd and 4th hours of treatment, respectively. Promethazine (10 mg/kg body wt., I/P), increases the anti -inflammatory activity of aqueous extract of Noni by 9.52, 25, 45.45 and 47.05% after 1st, 2nd, 3rd and 4th hours of treatment, respectively. Cyproheptadine(10 mg/kg body wt, I/P) did not produce significant increase in anti-inflammatory activity of aqueous extract of Noni in 2nd, 3rd and 4th hours of treatment which were 21.87, 21.87 and 22.05, respectively.

The results indicated that the maximum increase in anti-inflammatory activity of aqueous extract of Noni was produced by cemitidine which was persisted for 4 hours followed by paracetamol and promethazine. The analysis of variance revealed that there was significant ($P< 0.01$) difference in mean edema volume observed at different time intervals in a treatment. Aqueous extract of Noni showed significant anti-inflammatory activity with cimitidine, paracetamol and promethazine but not with cyproheptadine.

d. Interaction of different antagonists of mediators of inflammation with alcoholic extracts of Noni on carrageenin induced acute rat paw edema.

The anti-inflammatory activity of alcoholic extract of Noni (1000 mg/kg, orally) alone and in combination with antagonists of mediators of inflammation, namely, cyproheptadine (10 mg/kg body wt, I/P), promethazine (10 mg/kg body wt., I/P), cemitidine (20 mg/kg body wt. I/P) and paracetamol (100 mg/kg body wt., I/P) were studied by carrageenin induced rat paw edema. The antagonists were administered, intraperitoneally, half an hour before the alcoholic extract of Noni. In all groups of rats,carrageenin was injected 30 minutes after alcoholic extract administration and mean edema volume was measured at hourly intervals for four hours.

The mean edema volume (ml) in promethazine with alcoholic extract of Noni were 1.0 ± 0.01, 2.0 ± 0.15, 1.58 ± 0.18 and 0.01 ± 0.08, in cimetidine with alcoholic extract of Noni were 0.65 ± 0.01 , 2.0 ± 0.22, 1.34 ± 0.20 and 0.82 ± 0.11, in cyproheptadine with alcoholic extract of Noni were 0.92 ±0.02 , 1.91±0.36 , 0.26 ±0.02 and 0.15 ±0.02 and in paracetamol with alcoholic extract of Noni were 0.91 ±0.03 , 1.9 ± 0.27, 0.33 ± 0.01 and 0.16 ± 0.06 at the interval of 1st, 2nd ,3rd and 4th hour of treatment respectively.

The administration of promethazine and alcoholic extract of Noni significantly reduced the paw volume in the carrageenin induced rat paw edema as compared to alcoholic extract of Noni administered alone. The increase in anti-inflammatory activity was 53.84, 90.36 and 98% after 2 nd,3 rd and 4 th hours of treatment respectively. Similarly, paracetamol produced 12.34 46.15 , 60.24 and 68% increase in anti- inflammatory activity of alcoholic

extract of Noni after 1st, 2nd, 3rd and 4th hour of treatment respectively. Cyproheptadineand alcoholic extract of Noni produced 13.58, 46.92, 68.67 and 70% increase in anti-inflammatory activity after 1st, 2nd, 3rd and 4th hours of treatment respectively. Cimetidine and alcoholic extract of Noni produced 19.75, 53.84, 61.44 and 64% increase in anti- inflammatory activity after 1st, 2nd, 3rd and 4th hour of treatment respectively.

The results indicated that the maximum increase in anti-inflammatory activity of alcoholic extract of Noni was produced by promethazine which was found to persist for 4 hours followed byparacetamol, cyproheptadine and cimetidine. The analysis of variance revealed that there was significant ($P< 0.01$) difference in mean edema volume observed at different time intervals in a treatment .Alcoholic extract of Noni showed significant anti-inflammatory activity with promethazine, cyproheptadine , cimetidine and paracetamol.

Chronic inflammation

a. Anti-inflammatory activity of aqueous and alcoholic extracts of Noni on cotton pellet induced chronic inflammation

The anti-inflammatory activity of aqueous and alcoholic extracts of Noni was studied in groups of rats on cotton pellet induced chronic inflammation. Aqueous and alcoholic extracts of Noni were administered orally, at the dose of 1000 mg/kg body weight to a group of rats for seven days after implantation of cotton pellet (10 mg).The weight of granulation tissue was calculated after removing the cotton pellets on the eight day, by subtracting the original weight of cotton pellet. One group of rat was administered normal saline and was treated as control.

The mean weight of granulation tissue in control, aqueous and alcoholic extracts of Noni was 21.1±1.22, 14.6±1.70 and 10±1.41 mg respectively. Both the aqueous and alcoholic extracts of Noni showed significant anti-inflammatory response and percent anti-inflammatory activity was found to be 30.80 and 52.60% respectively. The results indicated that alcoholic extracts of Noni was more effective anti-inflammatory as compared to aqueous extracts of Noni on chronic inflammatory process induced by cotton pellet

implantation .The analysis of variance revealed that there was significant ($P< 0.01$) difference in cotton pellet inflammation in different treatment . Both alcoholic and aqueous extract of Noni showed significant anti-inflammatory activity as compared to control.

b. Interaction of phenylbutazone with aqueous and alcoholic extracts of Noni oncotton pellet induced chronic inflammation.

The interaction between phenylbutazone with aqueous extract and alcoholic extract of Noni were studied on cotton pellet induced chronic inflammation in albino rats.

Phenybutazone, aqueous extract and alcoholic extract of Noni were also tested for their per seanti-inflammatory activity .In cotton pellet implantation method, phenylbutazone at the dose of 100 mg/kg body weight, orally, produce significant anti-inflammatory activity. The mean weight of granulation tissue in phenylbutazone treated group were 11.8±1.49, in phenybutazone with aqueous extract of Noni were 7.5±1.1 and in phenybutazone with alcoholic extract of Noni were 3.66±0.49 mg.

The anti-inflammatory activity for phenylbutazone alone was 44.07%. But the anti-inflammatory activity for combination of phenylbutazone and aqueous extract of Noni was 64.45 which was more then that of effects of either drug when used alone. Similarly, combination of phenylbutazone and alcoholic extract of Noni produced 82.65% inhibition of granulation tissue as compared to effects of either drug when used alone. The analysis of variance revealed that there was significant ($P< 0.01$) difference in weight of granulation tissue in different treatment. Phenylbutazone showed significant anti-inflammatory activity with alcoholic extract of Noni.

c. Interaction of different antagonists of mediators of inflammation with aqueous extracts of Noni on cotton pellet induced chronic inflammation.

The anti-inflammatory activity of aqueous extract of Noni (1000 mg/kg, oral) alone and in combination with antagonists of mediators of inflammation, namely, cyproheptadine (10 mg/kg body wt, I/P), promethazine (10 mg/kg body wt., I/P), cemitidine (20 mg/kg body wt. I/P) and paracetamol (100

mg/kg body wt., I/P) was studied by cotton pellet induced chronic inflammation in albino rats. The antagonist was administered, intraperitoneally, half an hour before the administration of aqueous extract of Noni.

The mean weight of granulation tissue in paracetamol and aqueous extract treated group were 10.00 ±1.23, in cemitidine and aqueous extract treated group were 12.00.±1.61, incyproheptadineand aqueous extract treated group were13.66 ±1.01 and in promethazine and aqueous extract treated group were 14.16±1.53 mg.

The administration of paracetamoland aqueous extract of Noni combination significantly reduced the granulation tissue in cotton pellet implantation method as compared to effects on aqueous extract of Noni alone. The anti-inflammatory activity was 31.50%. Cemitidine and aqueous extract of Noni produced 17.80% anti-inflammatory activity. Cyproheptadine and promethazine do not produce any significant anti- inflammatory activity which was 4.79 and 3.01% respectively.

The results indicated that the maximum increase in anti-inflammatory activity of aqueous extract of Noni has been produced by paracetamol followed by cemitidine, cyproheptadine and promethazine. The analysis of variance revealed that there was significant ($P< 0.01$) difference in weight of granulation tissue in different treatment. Aqueous extract of Noni showed significant anti - inflammatory activity with paracetamol and cimitidine.

d. Interaction of different antagonists of mediators of inflammation with alcoholic extracts of Noni on cotton pellet induced chronic inflammation.

The interaction between alcoholic extract of Noni (1000 mg/kg oral) and antagonists of mediators of inflammation, namely, cyproheptadine (10 mg/ kg body wt, I/P), promethazine (10 mg/kg body wt., I/P), cemitidine (20 mg/kg body wt., I/P) and paracetamol (100 mg/kg body wt., I/P) was studied on cotton pellet induced chronic inflammation. The antagonist was administered, intraperitoneally, half an hour before the alcoholic extract of

Noni. In all groups of rats, carrageenin was injected 30 minutes after alcoholic extract administration.

The mean weight of granulation tissue in combination of promethazine with alcoholic extract of Noni was9.5 ±1.02, in cemitidine with alcoholic extract of Noni was 7.0 ±0.93, in cyproheptadine with alcoholic extract of Noni was 8.66 ±1.3 and in paracetamol with alcoholic extract of Noni was 4.5 ±0.80 mg.

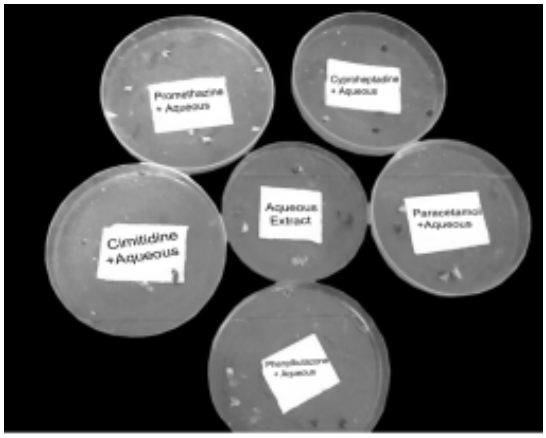


Plate 16 : Cotton Pellets (Aqueous)

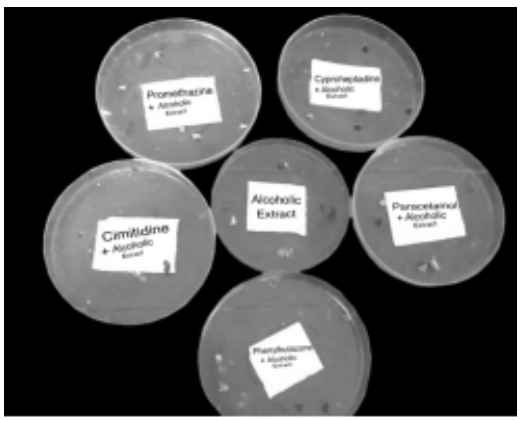


Plate 17 : Cotton Pellets (Alcoholic)

The administration of paracetamoland alcoholic extract of Noni combination significantly reduced the granulation tissue in cotton pellet implantation method as compared to effects on alcoholic extract of Noni alone. The increase in anti- inflammatory activity was 55%. Cemitidine and alcoholic extract of Noni produce 30% anti-inflammatory activity. Similarly cyproheptadine produces 13.4% anti-inflammatory activity. Promethazine with alcoholic extract of Noni do not show significant anti- inflammatory activity which was only 5.0%.

The results indicated that the maximum increase in anti-inflammatory activity of alcoholic extract of Noni was produced by paracetamol followed by cemitidine, cyproheptadine and promethazine. The analysis of variance revealed that there was significant ($P< 0.05$) difference in weight of granulation tissue in different treatment and alcoholic extract of Noni showed significant anti-inflammatory activity with paracetamol and cimetidine (Plate 16, 17)

Analgesic activity of orally administered aqueous and alcoholic extracts of Noni by hot plate method :

The analgesic effects of aqueous and alcoholic extracts of Noni were studied after administering the drug orally, to groups of rats, in graded doses of 250, 500 and1000 mg/kg body weight. The analgesic effect was studied by hot plate method and licking of the hind paw response was taken as criterion of no ciception and was noted before and at hourly intervals for four hours after administration of extracts.

Aqueous extracts of Noni in doses of 250 mg/kg body weight did not show any significant increase in the reaction time except after 2nd and 3rd hours, where the increase being from 3.26 ±0.17 to 4.32 ± 0.31 and 4.85 ± 0.31 seconds. The aqueous extracts of Noni in doses of 500 mg/kg body weight showed significant increase in the reaction time, the increase being from 3.08 ±0.24 to 4.84 ± 0.31and 5.0 ± 0.30 respectively at 2nd and 3rd hours of treatment. The dose of 1000 mg/kg body weight of aqueous extracts of Noni significantly increase the reaction time from 3.14 ± 0.11 to 5.21± 0.40 , 6.48 ± 0.30 and 6.51 ± 0.33 in 1st , 2nd and 3rd hours of treatment.

Alcoholic extracts of Noni in doses of 250 mg/kg body weight did not show any significant increase in the reaction time except after 3rd and 4th hours, where the increase being from 3.15 ± 0.37 to 4.71 ± 0.38 and 4.74 ± 0.36 seconds. The alcoholic extracts of Noni in doses of 500 mg/kg body weight showed significant increase in the reaction time, the increase being from 3.22 ± 0.13 to 5.61 ± 0.14 and 5.82 ± 0.20 respectively at 2nd and 3rd hours of treatment respectively. The dose of 1000 mg/kg body weight of alcoholic extracts of Noni significantly increase the reaction time from 3.28 ± 0.17 to 5.5 ± 0.12, 6.90 ± 0.10 and 7.20 ± 0.10 in 1st , 2nd and 3rd hours of treatment respectively.

The peak analgesic effect of alcoholic and aqueous extracts of Noni after its oral administration was observed after two hours of its administration and both the extracts showed the dose dependent analgesic activity. Percent analgesic score for aqueous and alcoholic extracts of Noni were 75.47 and 89.02% respectively at 3 rd hour of treatment. The alcoholic extracts of Noni with 1000 mg/kg, however had better analgesic action which persisted for four hours. The analysis of variance revealed that there was significant ($P< 0.01$) difference in analgesic activity at different time intervals in different treatment .Alcoholic and aqueous extracts of Noni at graded doses showed significant analgesic action.

Antipyretic activity of orally administered aqueous and alcoholic extracts of Noni on yeast induced pyrexia

The antipyretic activity of aqueous and alcoholic extracts of Noni was studied after administering the drugs, orally to a group of rats in graded doses of 250,500 and1000 mg/kg body weight .The antipyretic effect was studied on yeast induced pyrexia in rats. Both aqueous and alcoholic extracts of Noni at doses 250 mg / kg did not showed any significant antipyretic activity. However aqueous extracts of Noni at doses 500 mg / kg showed a significant decrease in pyretic temperature, the decrease being from 102.73°F ± 0.09 to 101.21°F ± 0.02, 100.13°F ± 0.01 and 100.06°F ± 0.02 in 2nd, 3rd and 4th hours of treatment respectively. Aqueous extracts of Noni at doses 1000 mg / kg also showed a significant decrease in pyretic temperature, the decrease being from 102.82 °F ±0.05 to 100.47°F ± 0.09 and 100.07°F ± 0.01 in 2nd and 3rd hours of treatment respectively.

Alcoholic extracts of Noni at doses 500 mg / kg also showed a significant decrease in pyretic temperature, the decrease being from 102.70°F ± 0.09 to 100.38°F ± 0.04 and 100.03°F ± 0.01 in 2nd and 3 rd hours of treatment respectively. Alcoholic extracts of Noni at doses 1000 mg / kg showed a significant decrease in pyretic temperature from first hour onwards, the decrease being from 102.76°F ±0.09 to 101.14°F ± 0.02, 100.06°F± 0.02 and 100.01°F ± 0.25 in 2nd, 3rd and 4th hours of treatment respectively. The analysis of variance revealed that there was significant ($P< 0.01$) difference in antipyretic activity at different time intervals in different treatment. .Alcoholic and aqueous extracts of Noni at graded doses showed significant antipyretic action.

Anti bacterial activity of Noni

In vitro tests

a. Gram positive bacteria

Antibacterial activity on two accounts in respect of the growth and antibiotic sensitivity / inhibition zones exerted due to use of various dilutions and combinations of *Morinda citrifolia* extracts and known antibiotic Ciprofloxacin (Ci) was studied for gram positive bacteria viz. *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogens* and *Bacillus cereus*. Discs impregnated with different dilutions were used. These discs of aqueous and alcoholic extracts of Noni (*Morinda citrifolia*) were used along with control drug Ciprofolxacin (Ci) on theinoculated plates of Muller-Hinton agar media and incubated at 37 degree Celsius.

The result showed that the growth of *Bacillus subtilis* up to 30%, 30%, *Staphylococcus aureus* up to 30%,40%, *Bacillus cereus* up to 40%,40%, and Listeria monocytogens up to 30%,30% in various dilutions of aqueous and alcoholic extract of Noni. These discs were infused into the inoculated plates of Muller- Hinton agar.

b. Gram negative bacteria

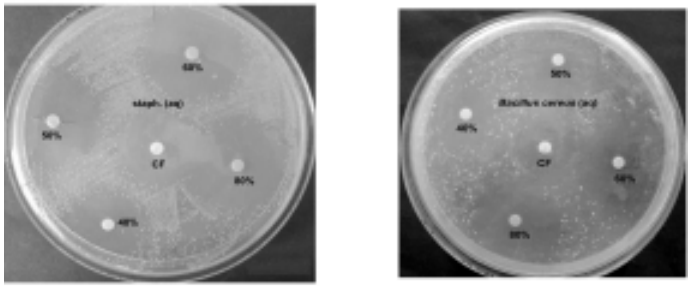
Antibacterial activity on two accounts in respect of the growth and antibiotic sensitivity / inhibition zones exerted due to use of impregnated discs of

various dilutions and combinations of *Morinda citrifolia* extracts and known antibiotic Ciprofloxacin (Ci) was studied for gram negative bacteria viz. *Escherichia coli*, *Salmonella yyphimurium* and *Klebsiella pneumoniae* using different dilutions of aqueous extract of Noni ,alcoholic extract of Noni , showed growth up to 20%, 30%, 20 %,20% & 40% for *K. pneumoniae,* 30%, 20%, 30%,30% & 30% for E. coli and 30%, 20%, 30% ,30% & 30% for *Salmonella typhimurium*, respectively.

c. Antibiotic sensitivity of Ciprofloxacin (Ci):

Ciprofloxacin (Ci) is a prevalent antibiotic in use in veterinary practice and the results indicated that, it was effective in creating a marked zone of inhibition for the entire range of gram positive and gram negative bacteria used for the experiment. The sensitivity effect of the combination of aqueous and alcoholic extracts of Noni (*Morinda citrifolia*) and cow urine ark at a dilution of 40% and above had antibacterial effects comparable to Ciprofloxacin (Ci). All the bacteria were sensitive. (Plate 18, 19, 20)

Plate 18 : In Vitro Antibacterial Effect of Different Dilutions of Aqueous Extract of Noni Fruit Against Gram Positive Bacteria



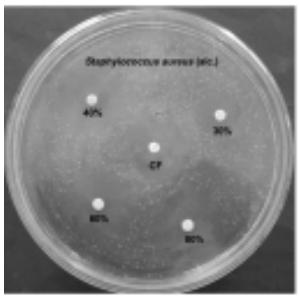
Staphylococcus aureus

(3) *Bacillus cereus*

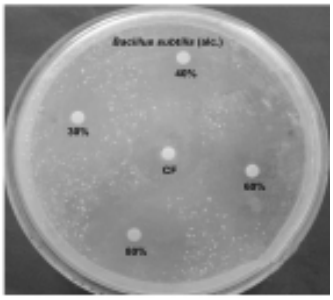
(2) *Bacillus subtilis*

(4) *Listeria monocytogenes*

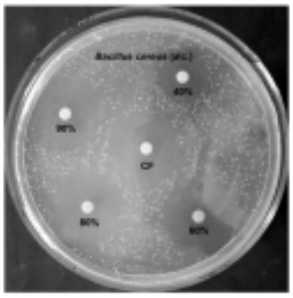
Plate 19 : *In Vitro* Antibacterial Effect of Different Dilutions of Alcoholic Extract of Noni Fruit Against Gram Positive Bacteria



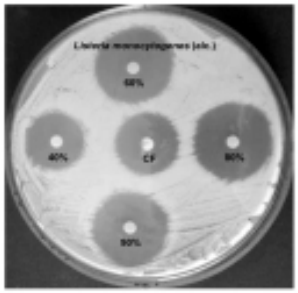
Staphylococcus aureus



Bacillus subtilis

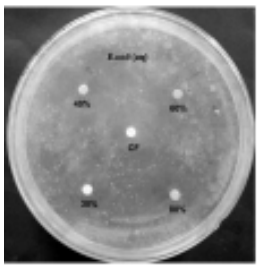


Bacillus cereus

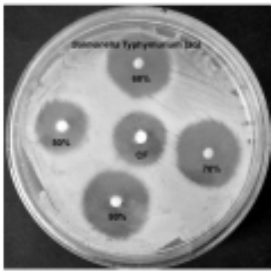


Listeria monocytogenes

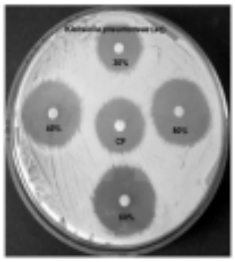
Plate 20 : *In Vitro* Antibacterial Effect *of different dilutions* Aqueous of Extract of Noni Fruit Gram Negative Bacteria



E.coli



Salmonella Typhymurium



Klebsiella pneumoniae

In vivo tests

Clinical symptoms :

Following oral administration of *E. coli* in albino rats of each group on 90th day of experimentation, the observed mortality percentage and clinical symptoms are as follows:

a. Group I (Control)

The rats of this group were extremely dull, depressed and off feed. Diarrhoea started from 12 hours post infection which was profuse and semi watery. Initial symptoms of pyrexia were followed by subnormal temperature after onset of diarrhoea. Pulse and respiration rates were increased. In terminal stage dyspnoea with recumbence was marked. Out of six rats of this group, three rats died on 4th day and one died on 5th day post infection. The others recovered in due course of time.

b. Group II (Aqueous extract of Noni)

The rats of this group were dull and depressed. Diarrhoea started from 16 hours of post infection. Initial symptoms of pyrexia were followed by subnormal temperature after onset of diarrhoea as in group 1. All six rats showed diseased symptoms. No mortality was found in post infection. All the rats recovered and remained healthy.

c. Group III (Alcoholic extract of Noni)

The rats of this group were also dull and depressed. Diarrhoea started from 18 hours of post infection. Initial symptoms of pyrexia were followed by subnormal temperature after the onset of diarrhoea as in group 1 and 2. All six rats showed diseased symptoms. All recovered and remained healthy. No mortality was seen in the group.

d. Group IV (ImmuPlus)

Rats of this group were dull, depressed and off feed for one day after infection. No other overt symptoms were seen. None of the rats died. Only slight fever in one rat was observed. Rest of the rats remained healthy.

Immunological observations

Cell mediated immune response

CMI response, assessed by delayed type of hypersensitivity response, which had a direct correlation to cell mediated immunity (CMI), was increased in the treatment groups as compared to control.

The mean values of paw volume in the rats were measured plethysmometrically of group I(Control), group II (aqueous extract of Noni), group III (alcoholic extract of Noni), group IV ((ImmuPlus) are given in Table-37(Fig 37).

The mean values of paw volume were 3.1 ± 0.052 in group I(Control), 6.033 ± 0.67mm in group II (aqueous extract of Non), 6.45 ±0.056 mm in group III (alcoholic extract of Noni), 11.067±0.368 in group IV (ImmuPlus) at 24 hour post challenge on day '14' of experiment respectively. (Plate 22).

Photo 22: Plethysmometer used for showing cell mediated immunity

Humoral immune response

Humoral response was carried out by assessing the Haemagglutination Inhibition (HI) titre of Newcastle disease vaccination (F1 Strain) in rats. The mean value of HI titre in group I(Control), group II (aqueous extract of Noni), group III (alcoholic extract of Noni), group IV (Immu Plus) rats after vaccination with F1 strain is given in Table 38.

The mean values of HI titre were 33.33±10.20 in group I (Control), 40.0 +8 in group II (aquous extract of Noni), 32 +7.15 in group III (alcoholic extract of Noni), and 52.0+ 17.97 group IV (Immu Plus) at day 0 of vaccination with F1 strain.

On 7th day of vaccination, The mean values of HI titre were 42.66±9.834in group I(Control), 192+70.108 in group II (aqueous extract of Noni), 501.33 +176.75 in group III (alcoholic extract of Noni) and 640+ 128 in group IV(Immu plus).

The mean values of HI titre on 14th day of vaccination were 45.3±8.682 in group I(Control), 533.3+163.308 in group II (aqueous extract of Noni), 725.33+138.91in group III (alcoholic extract of Noni) and 1621.33+ 277.825 in group IV (Immu plus) (Plate 23).

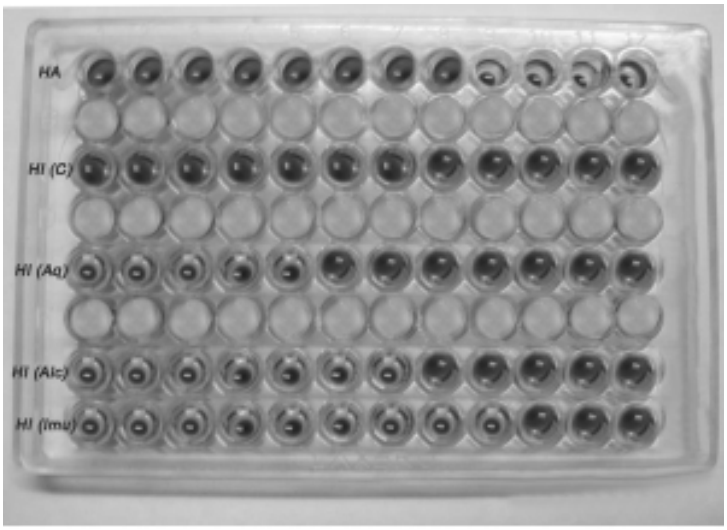


Plate 23 : Haemagglutination test for showing humoral immunity

The analysis of variance revealed highly significant ($P<0.01$) increase in HI titre within group in between intervals.

6. Summary and conclusion

This experimental investigation was carried out in albino rats of either sex weighing 120-150 g. The alcoholic and aqueous extracts of *Morinda citrifolia* (Noni) were prepared and used to find out the chemical constituents. The extractability of Noni was recorded more in water as compared to alcohol. The phyto chemical test revealed the presence of alkaloid, amino acid, reducing sugar, glycosides, tannin and steroid in aqueous extracts of Noni fruit and alkaloid, protein, amino acid, reducing sugar, tannin, anthraquinone and steroid in alcoholic extracts of Noni fruit.

Morinda citrifolia (Noni) has been found to be safe drug as the oral LD50 was found to be more than 2000 mg/kg body weight. Moreover it did not show any significant toxic symptoms. However on chronic exposure for three months the alcoholic extract administered, orally, at the dose rate of 1000 mg/kg body weight produced significant increase in TEC, haemoglobin and PCV. A significant increase in blood clotting time in both aqueous and alcoholic extracts of Noni was produced.

The histopathological study of liver and kidney on chronic exposure for three months showed swollen hepatocytes and mild fatty changes. There was congestion and degeneration in hepatic cells. The histopathological studies of kidney from group of rats treated with alcoholic extract of Noni produced leucocytic infiltration and mild congestion in glomerulus of kidney whereas aqueous extract showed degenerative changes with slight haemmorhages in interstitial tissue. Aqueous extract of Noni produced more degenerative changes in kidney and liver as compared to alcoholic extracts suggesting that aqueous extract is more toxic.

The interpretations of results suggest that though, Noni is a quite safe drug, it produces toxicity during its metabolism and elimination.

This experimental investigation was carried out in albino rats of either sex weighing 120-150g, to elucidate the mechanism of action of *Morinda citrifolia* (Noni). In this study, the aqueous and alcoholic extracts of Noni were used for study on inflammation, pain and pyrexia.

The anti-inflammatory activity was studied against acute and chronic inflammations by using carrageenin induced hind paw edema model and cotton pellet induced granuloma model respectively. Analgesic activity was studied by hot plate method and antipyretic activity was studied against yeast induced pyrexia.

The results of the present experimental investigation are summarized as follows:

The alcoholic extract of Noni has been found to produce significant anti-inflammatory activity after 2nd, 3rd and 4th hours of administration on carrageenin induced rat paw edema. Phenylbutazone has been found to significantly increase the anti-inflammatory activity of both aqueous and alcoholic extracts of Noni in acute and chronic process, suggesting similar mechanism of action of these drugs. The anti-inflammatory action is more significant with alcoholic extract and phenylbutazone.

The possible role of mediators of inflammation in anti-inflammatory activity of Noni was studied by pretreating the animals with number of antagonist of mediators namely, cyproheptadine, promethazine, cimetidine and paracetamol both on acute and chronic inflammatory models.

The results of the study showed that during acute phase, the maximum increase in anti-inflammatory activity of aqueous extract of Noni was produced by cemitidine which was found to persist for 4 hours followed by paracetamol and promethazine. Aqueous extract of Noni showed significant anti-inflammatory activity with cimetidine, paracetamol and promethazine but not with cyproheptadine. Thus anti-inflammatory effect of aqueous extract of Noni might be due to the inhibition of endogenous histamine which increased by pretreatment with cimetidine. The results further revealed that besides histamine, prostaglandins also play a role in controlling anti-inflammatory activity of Noni. Thus anti-inflammatory activity of Noni on transudative and proliferative stages of inflammation may be due to its blocking effects on H1 receptors, H2 receptors and prostaglandins.

The results of the study showed that the maximum increase in anti-inflammatory activity of alcoholic extract of Noni was produced by promethazine during

acute phase which was found to persist for 4 hours followed by paracetamol, cyproheptadine and cimetidine. Thus anti-inflammatory effect of alcoholic extract of Noni might be due to the inhibition of endogenous histamine, prostaglandins and 5 HT.

The alcoholic extract of Noni was more effective as compared to its aqueous extract on chronic inflammatory process. The maximum increase in anti-inflammatory activity of aqueous extract on cotton pellet induced granuloma was produced by paracetamol. These results indicate that prostaglandins mainly are responsible for anti-inflammatory action of aqueous extract of Noni in chronic process against proliferative phase of inflammation and this activity may be due to blockade of endogenous prostaglandins .Noni has also been found to produce anti- inflammatory activity on chronic proliferative stage by its antihistaminic effect as it is potentiated by administration of cimetidine.

The maximum increase in anti-inflammatory activity of alcoholic extract on cotton pellet induced granuloma was produced by paracetamol. These results indicate that prostaglandins mainly are responsible for anti- inflammatory action of alcoholic extract of Noni in chronic process against proliferative phase of inflammation and this activity may be due to blockade of endogenous prostaglandins. Noni has also been found to produce anti- inflammatory activity on chronic proliferative stage by blocking 5-hydroxytryptamine.

The aqueous and alcoholic extracts of Noni have also shown dose related antipyretic and analgesic activity. The percent analgesic score of both extracts persisted up to 3 hour of administration. The alcoholic extract @ 1000 mg/kg produced better analgesic action as compared to aqueous extract at the same dose rate.

Both the aqueous and alcoholic extracts of Noni significantly reduced the yeast induced pyrexia starting from 1 hour post administration and it was completely brought back to normal within 4 hour post administration . The alcoholic extract @ 1000 mg/kg produced better antipyretic action as compared to aqueous extract at the same dose rate up to 3 rdhr of administration.

The present study was aimed to elucidate the usefulness of Aqueous extract of Noni, Alcoholic extract Noni, as antibacterial and immunomodulatory agent, affecting haematological and sero biochemical profile in albino rats.

Antibacterial activity of above treatments (*in vitro*) against *Bacillus subtilis, Escherichia coli, Klebsiellap neumoniae, L.monocytogens, Salmonella typhimurium, Staphylococcus aureus* and *Bacillus cerius* was assessed as compared to Ciprofloxacin, (Ci) the standard drug.

It was interesting to note that aqueous extract of Noni and alcoholic extract of Noni alone did not reveal growth of any bacteria at 40% dilution. This indicates similar antibacterial effect of aqueous extract of Noni and alcoholic extract of Noni at higher concentration.

Antibacterial activity of above treatments (*in vivo*) against *E. coli* was also studied following oral administration of one ml broth culture containing 3x 109 cfu/ ml. The clinical symptoms noticed were dull, depression, anorexia, diarrhoea, pyrexia, increased pulse dyspnoea and prostration in later stage of infection. The overt clinical symptoms in rats of group I (Control) were most severe than other treatment groups. The mortality rate in group I (50%), other groups did not shown any mortality.

The DTH response, which has a direct correlation of cell mediated immunity (CMI), showed highly significant ($P<0.01$) increase in mean paw volume .The order of significance of mean paw volume was as follows group IV (IP), group III (Alc), group II(Aq) and group I (C) rats.

The humoral immune response was evaluated by Haemagglutination inhibition (HI) titre against Newcastle disease virus. HI titre showed highly significant increase in group IV (IP) followed by group III (Alc), group II (Aq) and group I (C) rats ($P<0.01$). Increase in both, HI titre and DTH response indicated that the use of Alcoholic and aqueous extract of Noni has humoral as well as cellular immunity.

In recent years, there has been an upsurge in the clinical use of natural and indigenous drugs. Side effects and operating cost associated with allopathic drugs have forced the need for research into drugs, which are without the side effects. There is an increasing interest in the search for potential drugs,

especially of plant origin, that are capable of masking the untoward side effects of steroidal and non-steroidal drugs. Medicinal plants have many traditional claims including the treatment of ailments of infectious origin. In the evaluation of traditional claims, scientific research is important. The objectives of the study were to determine the presence of antibacterial andimmuno modulatory activity in the extracts of Noni fruits.

Looking to all parameters that were envisaged to study the herbal plant, *Morinda citrifolia* (Noni), it can be concluded that both aqueous and alcoholic extracts of Noni fruit contain several phytochemicals viz., alkaloid, amino acid, reducing sugar, glycosides, tannin , steroid, , anthraquinone and protein. All these components are responsible for its pharmacological activity.

The chronic intake of both aqueous and alcoholic extracts of Noni fruit did not produce much damage to vital organs but in turn the alcoholic extract showed a increase in TEC, haemoglobin and PCV indicating its stimulating effect on the haemopoietic system.

Further studies concluded that alcoholic extract was better as anti-inflammatory, analgesic and antipyretic agent with no side effects. Noni interact well with other anti-inflammatory drugs such as phenylbutazone. In acute phase of inflammation Noni mainly works via blocking histamine, 5-HT and prostaglandins and in chronic phase of inflammation Noni mainly works via blocking prostaglandins, histamine and 5 HT. Noni besides these functions is also useful in diabetes, hypertension, arthritis, asthma and stones. All parts of Noni plant whether roots, barks, leaves and fruits can be utilized in medication.

A logical conclusion from the present study indicates with certainty that Aqueous extract of Noni, Alcoholic extract Noni distinctly have both antibacterial and immunomodulatory action. Based on scientific deduction and accessible literature indicates that there may be certain metabolites or bio-molecules present in Noni, which possess protective properties i.e. antibacterial action. Aqueous extract of Noni and Alcoholic extract Noni are found to consist of certain alkaloids and resins which are responsible for their antibacterial and immunomodulatory action.

HI titre and DTH response indicates that aqueous and alcoholic extract of Noniare potentimmuno-modulatory agents enhancing the humoral and cell mediated immune response. One of the explanations forwarded to justify the beneficial effect of aqueous and alcoholic extract of Noni, is non specific enhancement of immune status. In summation, aqueous and alcoholic extract of Noni are natural medicinal agents and God's gift to Mankind.

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