COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY OF *Emblica officinalis* (AMLA) AND CURCUMIN IN RATS

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DECLARATION

I hereby declare that the thesis entitled **"COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY OF *Emblica officinalis* (AMLA) AND CURCUMIN IN RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY OF *Emblica officinalis* (AMLA) AND CURCUMIN IN RATS"** is a record of research work done independently by **Dr. Sujith. S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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Introduction

1. INTRODUCTION

Herbal drugs are those traditional medicines that primarily use medicinal plants for therapy of ailments. In India, herbal medicines were in existence for thousands of years. Besides the Vedas, ancient scholars like Charaka, Susruta, Vagabhatta and others brought out texts containing the description of various plants used in several preparations for the treatment of various diseases. The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity, efficacy and economic viability. Several plant extracts have been evaluated for their antimicrobial, antiparasitic, antiviral, hepatoprotective, immunomodulatory, antioxidant properties and so on. Medicinal plants thus play a major role in human health care.

It is widely accepted that free radicals and reactive chemical species can be produced in living tissues and their release increases in pathophysiological states. Oxidative damage to crucial biomolecules like proteins, lipids and DNA due to excess generation of free radicals is a major cause of organ damage. Free radicals play a crucial role in a complex interplay of different mechanisms in normal aging and neuro degenerative diseases.

Free radical can be defined as a chemical species, which is formed by homolytic cleavage of a covalent bond in a molecule, by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. When oxygen is partially reduced, by the addition of one, two or four electrons, it forms the reactive oxygen species (ROS), which reacts readily with a variety of biomolecules (Ray and Hussain, 2002). The oxygen derived free radicals include superoxide ($O2^{\cdot-}$), hydroxyl ($^{\cdot}OH$), hydroperoxyl radical ($HO_2^{\cdot}$), peroxyl (ROO), alkoxyl radical (RO) and non radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and singlet oxygen ($^1O_2$). Nitrogen derived

oxidant species are mainly nitric oxide (NO), peroxy nitrite ($ONOO^-$) , nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) (Irshad and Chaudhari, 2002).

Accumulation of superoxide anion and hydrogen peroxide in the form of hydroxyl radical oxidizes lipids, giving rise to lipid peroxidation. The peroxidation products are highly toxic to the body (Devasagayam and Sainis, 2002).

Chemical compounds and reactions capable of generating toxic oxygen species or free radicals are referred to as prooxidants. On the other hand, compounds and reactions disposing them off, scavenging them, suppressing their formation or opposing their actions are called antioxidants. In a normal cell, there is an appropriate prooxidant: antioxidant balance. However this balance can be shifted towards the prooxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called as oxidative stress and can result in serious cell damage if stress is massive or prolonged (Irshad and Chaudhari, 2002).

The antioxidant system in body comprises of different functional components or lines of defence. The first line comprises of preventive antioxidants that act by quenching of superoxide anion, decomposing hydrogen peroxide and sequestrating metal ions. The antioxidants belonging to this category are enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and non enzymatic molecules which are minerals and proteins (Irshad and Chaudhari, 2002). The superoxide is converted to hydrogen peroxide by superoxide dismutase which is then converted to water and oxygen by catalase (Devasagayam and Sainis, 2002). Glutathione peroxidase contains selenium which catalyses the reduction of hydrogen peroxide and lipid peroxides to water using reduced glutathione as substrate.

The antioxidants belonging to second line of defence include glutathione, Vitamin C, uric acid, β carotene, albumin, bilirubin, Vitamin E, carotenoids, flavonoids and ubiquinol. These are radical scavenging antioxidants. The third line of antioxidants are a complex group of enzymes for the repair of damaged DNA,

damaged proteins, oxidized lipids and peroxides. These include lipase, proteases, DNA repair enzymes, transferases, methionine sulfoxide reductase etc.

One of the therapeutic strategies in Ayurvedic medicine is to increase body's natural resistance to disease causing agents rather than directly neutralizing the agent itself. In practice, this is achieved by using extracts of various plant materials called "rasayanas". Rasayana therapy claims to provide one's longevity, retains youth, increases the strength, sharp intellect, lustrous complexion and freedom from disease. The concept of using rasayanas for health gains a little more credibility when we realize that herbal antioxidants concurrently exhibit significant immunomodulatory property also. A good number of antioxidants are present in nature in various fruits, nuts, grains and a diet, rich in these natural products, seems to be protective against the cumulative effects of oxidative damage in human life span. Several medicinal herbs are also rich in antioxidants and their medicinal properties are perhaps linked to the antioxidative constituents present in them.

Turmeric , *Curcuma longa* L. (Zingiberacea) rhizomes, commonly used as a spice, is well known for its medicinal values in the Indian traditional system of medicine and has been a major recipe for several common ailments. Curcumin (diferuloyl methane) gives yellow colour to turmeric rhizomes and is one of the active ingredients responsible for its biological activity. It has proven anti-inflammatory, hepatoprotective, anticancer, antitumor, antiproliferative, immunostimulant, antimicrobial, antiviral and wound healing properties (Srimal,1997). *Emblica officinalis* Gaertn. Syn. *Phyllanthus emblica* Linn. is a commonly used fruit in the Indian system of medicine and is known as Amla in Hindi. Studies have shown that fruits of Amla have got immunostimulating, hypolipidaemic, antioxidant, hepatoprotective and other properties.

The present investigation is undertaken to confirm the antioxidant activity of fruit pulp of *Emblica officinalis* especially in oxidative stress due to high fat diet and to compare its activity with that of curcumin.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PLANTS HAVING ANTIOXIDANT PROPERTY

During normal biochemical reactions in our body, reactive oxygen and nitrogen species are generated. These get enhanced during pathophysiological conditions creating oxidative stress. This can be effectively neutralized by enhancing the cellular defences in the form of antioxidants. There are a number of studies which reveal the beneficial effect of plant extracts having antioxidant properties.

Soudamini *et al.* (1992) studied the effects of curcumin at the rate of 250 mg/kg on tissue lipid peroxidation and cholesterol levels in mice given Carbon tetrachloride, Paraquat and Cyclophosphamide. The treatment group showed decrease in lipid peroxidation, most significant in liver and also a significant decrease of the elevated serum and tissue cholesterol.

Alcoholic extract of *Rubia cordifolia* significantly reduced cumene hydroperoxide induced malondialdehyde formation and the rate of lowering of glutathione content in isolated rat liver homogenate (Pandey *et al.*, 1994).

Sandhika, a compound herbal drug containing *Commiphora mukul, Boswellia serrata, Strychnos nuxvomica and Semicarpus anacardium* was assessed for its antioxidant and anti-inflammatory property by Chaurasia *et al.* (1995). The drug reduced the cumene hydroperoxide induced malondialdehyde formation and oxidation rate of reduced glutathione in a dose dependent manner.

Osawa *et al.*(1995) investigated the antioxidant activity of tetrahydrocurcuminoids (THU1, THU2, THU3) on rabbit erythrocyte membrane and rat liver microsome and found that THU1, one of the major metabolites of curcumin, had the strongest antioxidant activity.

One percent cholesterol diet decreased the concentration of glutathione in tissues and the activity of free radical scavenging enzymes, superoxide dismutase and catalase in liver. Sheela and Augusti (1995) showed that treatment with S-allyl

cysteine sulphoxide at the rate of 200mg/kg/day and Gugulipid at the rate of 50mg/kg/day significantly increased the superoxide dismutase, catalase and glutathione levels.

Reddy and Lokesh (1996) showed that there was an increase in activity of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase in liver homogenates of rats,fed with turmeric containing diet, injected with iron in comparison to those fed with control diet and given iron injection.

The effect of alcoholic extract of Brahmi on lipid peroxidation, induced by cumene hydroperoxide and ferrous sulphate in liver was compared with its hexane fraction, EDTA, Tris and Vitamin E. The reduction in lipid peroxidation was more with alcoholic extract and comparable to EDTA and Vitamin E (Tripathi *et al.*, 1996).

Tripathi *et al.* (1996) studied *Nardostachys jatamanasi*, commonly called Jatamanasi, for its antiperoxidative property by using iron induced lipid peroxidation in five percent rat liver homogenate. The alcoholic and hexane fraction showed protection against lipid peroxidation in a dose dependent manner and it was compared with synthetic antioxidants, Parabenzoquinone and Vitamin E. Parabenzoquinone provided most protection followed by Vitamin E, hexane and alcoholic fractions of Jatamanasi.

The antioxidant activity of glycowithanolides of *Withania somnifera* at doses of 10 and 20 mg/kg body weight i/p on rat brain frontal, cortical and striatal areas was studied and the results were compared with Deprenyl (2mg/kg/day i/p) by Bhattacharya *et al.*(1997). The active principles induced a dose related increase in the activity of superoxide dismutase, catalase and glutathione peroxidase except for a low dose of glycowithanolides on glutathione peroxidase activity and the antioxidant activity was comparable to the effect of Deprenyl.

Khan *et al.* (1997) investigated the status of lipid peroxidation in rats fed with high fat diet and the antioxidant effects of *Murraya koenigii* and *Brassica juncea* on

them. The spices at the rate of 10 percent in diet increased the activity of antioxidant enzymes like superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase, glutathione peroxidase as well as glutathione.

Adegoke *et al.* (1998) demonstrated the effect of *Garcinia kola* on lipid peroxidation in rat liver homogenate. It was found to inhibit lipid peroxidation in a dose dependent manner and was equivalent to the inhibitory effect of 10μM Butylated Hydroxy Anisole (BHA), a standard antioxidant.

The effect of consumption of glabridin, an isoflavon from *Glycyrrhiza glabra* root, on the susceptibility of LDL to oxidation was investigated by Belinky *et al.* (1998) and was compared with quercetin and cataechin. Glabridin inhibited the formation of thiobarbituric acid reacting substances, lipidperoxide and cholesteroyl linoleate hydroperoxide at all concentrations, reduced the copper ion and 2, 2'azo bis (2 amidino – propane) dihydrochloride (AAPH) induced LDL oxidation.

The two flavonoids from the bark of *Ficus bengalensis* restored the activity of glutathione peroxidase, superoxide dismutase, catalase and glutathione and decreased lipid peroxidation in rats fed with two percent cholesterol diet. The activity of quercetin was compared with these flavonoids and was found to be more active than these flavonoids (Daniel *et al.*, 1998).

Dhuley (1998) studied the effect of Ashwagandha on lipid peroxidation induced by the administration of lipopolysaccharide (LPS) endotoxin from *Klebsiella pneumoniae* and peptidoglycan (PGN) from *Staphylococcus aureus* in rabbits and mice. Simultaneous oral administration of Ashwagandha at the rate of 100mg/kg with LPS and PGN significantly reduced lipid peroxidation.

The water extract of *Boehmeria nivea var nivea* and *B. nivea var tenacissima* at the rate of 10 mg/ml significantly inhibited lipid peroxidation on rat liver homogenate and showed enhanced superoxide radical scavenging activity (Lin *et al.*, 1998).

Rajasree and Rajamohan (1998) reported the increase in the activity of antioxidant enzymes like superoxide dismutase and catalase along with a decrease in malondialdehyde, hydroperoxide and diene conjugates, glutathione peroxidase and glutathione –S- transferase in rats, which were fed with garlic protein, at the rate of 500g/kg/day and alcohol simultaneously. The antioxidant effects were comparable to Gugulipid at the rate of 50mg/kg/day.

The antioxidant activity of tannoid principles of *Emblica officinalis* on rat brain frontal, cortical and striatal areas was studied by Bhattacharya *et al.* (1999). The tannins at the rate of 5 mg/kg and 10 mg/kg i/p induced a dose related increase in activity of superoxide dismutase, catalase and glutathione peroxidase in seven days and lipid peroxidation was found to be decreased in treatment group. The activities were comparable to the effect of Deprenyl.

Rats fed with high fat diet were in a state of oxidative stress as indicated by an increase in thiobarbituric acid reacting substances and a decrease in glutathione and glutathione reductase activity. The coadministration of cinnamon bark and greater cardamom seeds at the rate of 10 percent in diet increased the glutathione content, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase activities and decreased thiobarbituric acid reacting substances (Dhuley, 1999) .

Prince and Menon (1999) found that oral administration of the aqueous extract of *Tinospora cordifolia* roots at the rate of 2.5 to 7.5 g/kg for six weeks decreased the level of thiobarbituric acid reacting substances, cerulloplasmin, α – tocopherol and increased the level of glutathione and Vitamin C in alloxan diabetic rats. Administration of Glibenclamide at the rate of 0.6 mg/kg also showed same results.

Ginger (*Zingiber officinale* Rosc.) significantly reduced lipid peroxidation by maintaining the activities of antioxidant enzymes-superoxide dismutase, catalase and glutathione peroxidase and by increasing glutathione content (Ahamed *et al.*, 2000).

Bacopa monniera extract was assessed for its activity on brain frontal, cortical, striatal and hippocampal superoxide dismutase, catalase and glutathione peroxidase activities (Bhattacharya *et al.*, 2000). A dose related increase in all parameters were noticed confirming antioxidant property.

Sanchez *et al.* (2000) compared the protection offered by *Mangifera indica* stem bark extract (vimang), mangiferin, Vitamin C, Vitamin E and β-carotene against 12-O-tetra decanoyl phorbol-13-acetate (TPA) induced oxidative damage and hyper production of reactive oxygen species by peritoneal macrophages.

The antioxidant activity of glycowithanolides of *Withania somnifera* in chronic foot shock induced stress in rat brain frontal, cortex and striatum was proved by Bhattacharya et *al.* (2001).

Chatterjee *et al.* (2001) reported that *Terminalia arjuna* bark extract at the rate of 250 and 500 mg/kg effectively antagonized various reactive oxygen species like superoxide radical, hydroxyl radical, lipid peroxide formation, singlet oxygen and hydrogen peroxide induced membrane damage.

Tripathi *et al.* (2001) demonstrated that the ethanolic fraction of *Smilex china* inhibited ferrous sulphate induced lipid peroxidation as well as scavenged superoxide and hydroxyl radicals in a dose dependent manner.

Trivedi and Rawal (2001) showed that aqueous extract of *Andrographis paniculata* (nees) at the rate of 12 mg/kg body weight /day increased the activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione which were decreased by the administration of Benzenehexachloride at the rate of 500ppm /kg and consequent liver damage in mice.

Liposem- a polyherbal preparation containing 17 medicinal plants scavenged superoxide anions and inhibited the generation of hydroxyl radicals and lipid peroxidation. It also reduced LDL cholesterol, total cholesterol and total lipids (Mary *et al.*, 2002).

Methanolic extract of Pomegranate peel at the rate of 50mg/kg preserved the activity of catalase, glutathione peroxidase and superoxide dismutase and decreased lipid peroxidation in rats fed Carbontetrachloride (Murthy *et al.*, 2002).

Raphel *et al.* (2002) demonstrated that methanolic extract of *Phyllanthus amarus* scavenged superoxide anion, inhibited hydroxyl radical generation and lipid peroxidation.

2.2 OTHER PHARMACOLOGICAL PROPERTIES OF PLANTS UNDER STUDY

2.2.1 *Emblica officinalis*

Fruits of *Emblica officinalis* Gaertn. (Amla) has been used in Ayurveda as a potent Rasayana and also as a treatment of diseases of diverse etiology. It is a rich source of Vitamin C. Unlike other citrus fruits, the fruits of Amla have ascorbic acid conjugated to gallic acid and reducing sugars, forming a tannoid complex, which makes it more stable.

Mishra *et al.* (1981) showed that *Emblica officinalis* at the rate of one gram could reduce the elevated mean serum cholesterol levels in rabbits fed with 0.5g cholesterol and one gram clarified butter.

Jacob *et al.* (1988) studied the effect of diet, supplemented with Amla, on the total serum cholesterol and its lipoprotein fractions in normal and hypercholesterolaemic men of 35-55 years for a period of 28 days. There was a decrease in cholesterol levels in both groups. Two weeks after the withdrawal of Amla, serum cholesterol levels of hypercholesterolaemic subjects rose to almost initial levels.

The protection offered by *Phyllanthus emblica* fruit and ascorbic acid on clastogenicity induced by intraperitoneal injection of lead and aluminium salts on mouse bone marrow chromosomes was studied by Dhir *et al.*(1990). Oral administration of the fruit extract seven days prior to the exposure to metals increased the frequency of cell division and decreased the frequency of chromosome

breaks significantly. Comparable doses of synthetic ascorbic acid could protect against effects of aluminium and low doses of lead only.

Dhir *et al.* (1991) investigated the effect of aqueous extract of edible dried fruits of *Phyllanthus emblica* on the chromosomal aberrations and frequency of micronuclei production induced by different doses of nickel chloride (10, 20 and 40 mg/kg) in *Mus musculus*. The fruit extract significantly reduced the frequency of chromosomal aberrations per cell, percentage of aberrant cells and the frequency of micronuclei production.

Pretreatment with aqueous extract of *Phyllanthus emblica* and an equivalent amount of Vitamin C orally, reduced the frequency of chromosomal aberrations induced by Cesium chloride in *Mus musculus* (Ghosh *et al.*, 1992).

Asmawi *et al.* (1993) examined the effect of water fraction of the ethanolic extract of *Emblica officinalis* leaves on carrageenan and dextran induced hind paw edema and on synthesis of mediators of inflammation in rats. The extract inhibited migration of human polymorpho nuclear cells in relatively low concentration, but did not inhibit LTB_4, PAF and TXB_2 synthesis.

Aqueous extract of *Allium sativum* bulb (10ml/kg orally), fruit pulp of *Emblica officinalis* (1g/kg orally), *Emblica officinalis* aqueous extract (1g pulp in 10 ml water/kg orally) and Gemfibrozil (120mg/kg orally) were compared for their hypolipidaemic effect in rabbits (Mini, 1993). All of them reduced the elevated plasma levels of cholesterol and triglycerides significantly ($P< 0.01$). The degree of fatty change in liver was reduced in treatment groups and the lining of the wall of aorta was also intact.

Suresh and Vasudevan (1994) found that *Phyllanthus emblica* enhanced Natural Killer (NK) cell activity and antibody dependent cellular cytotoxicity (ADCC) in mice bearing Daltons lymphoma ascites tumor. An increase in life span of 35 percent was also recorded in these tumor bearing mice.

Fifty percent alcoholic extract of *Phyllanthus emblica* at the rate of 100mg/100g body weight p.o and quercetin isolated from it at the rate of 15mg/100g body weight p.o were studied for their hepatoprotective effect against country made liquor and Paracetamol challenge in albino rats and mice respectively. Both produced significant hepatoprotection (Gulati *et al.*, 1995).

The effect of *Emblica officinalis* on necrotizing pancreatitis induced by injecting a mixture of trypsin, bile and blood into duodenal opening of pancreatic duct in dogs was studied by Thorat *et al.* (1995). Pretreatment with *Emblica officinalis* at the rate of 28 mg/kg/day for 15 days prior to induction of pancreatitis gave significant protection.

Administration of *Emblica officinalis* fresh juice at the rate of 5 mg/kg significantly reduced the elevated serum lipid parameters in response to atherogenic diet and cholesterol feeding. The most pronounced effect was on serum LDL levels (Mathur *et al.*, 1996).

The protection offered by crude aqueous extract of *Emblica officinalis* at the rate of 685 mg/kg bwt against cytotoxic effects of Arsenic was studied by Biswas *et al.* (1999) in mice. Sodium arsenite at the rate of 2.5mg/kg bodyweight was given after a seven and 14 days of pretreatment with the fruit extract. The crude extract reduced Arsenic induced damage, bringing the cells to almost normal level.

Anila and Vijayalakshmi (2000) compared the effects of flavonoids from *Sesamum indicum, Emblica officinalis* and *Momordica charantia* and showed that flavonoids isolated from *Emblica officinalis* has highest hypolipidaemic and hypoglycaemic activity. These flavonoids were effective in raising the haemoglobin level in rats.

Pretreatment with butanol extract of water fraction of *Phyllanthus emblica* fruits at the rate of 100mg/kg for 10 days enhanced gastric mucous and hexosamine in indomethacin induced gastric ulcer in rats. No change in the levels of

malondialdehyde and superoxide dismutase was noticed in pretreated animals compared to those given indomethacin alone (Bandopadhyay *et al.*, 2000).

Bhattacharya *et al.* (2000) demonstrated that *Emblica officinalis* tannins at the rate of 10, 20 and 50 mg/kg body weight p.o for 10 days inhibited hepatic lipid peroxidation and increase in serum levels of alanine amino transferase, aspartate amino transferase and lactate dehydrogenase in rats having iron overload hepatic toxicity. A similar effect was produced by Silymarin at the rate of 20 mg/kg p.o.

Bhattacharya *et al.* (2000) studied the effect of chronic unpredictable foot shock induced stress induced perturbations in oxidative free radical scavenging enzymes in rat brain frontal, cortex and striatum over a period of 21 days. Administration of *Emblica officinalis* tannoids at the rate of 10 mg and 20 mg p.o concomitant with stress showed a tendency towards normalization of the activity of superoxide dismutase, catalase and glutathione peroxidase and decreased the lipid peroxidation.

The effect of *Emblica officinalis* tannins in tardive dyskinesia induced by Haloperidol at the rate of 1.5 mg/kg i/p for 28 days was studied by Bhattacharya *et al.* (2000). Involuntary orofacial movements like chewing movements, buccal tremors and tongue protrusion was taken as the parameters. *Emblica officinalis* tannins which was similar to Vitamin E at the rate of 400 mg/kg p.o. induced a dose related inhibition of all the three parameters.

Jose and Kuttan (2000) studied the hepatoprotective activity of *Emblica officinalis* and Chyavanaprash extracts in Carbontetrachloride induced liver injury in rats. Both the extracts significantly decreased the serum and liver lipid peroxides, glutamate pyruvate transaminase, alkaline phosphatase, collagen- hydroxy proline which were all increased in animals with liver injury.

Aqueous extracts of *Cassia occidentalis* and *Emblica officinalis* were screened for their effectiveness in inhibiting mutagenicity of aflatoxin B_1 and

benzo(a)pyrene (Sharma *et al.*, 2000). Both plants inhibited mutagenicity, but *Emblica officinalis* had more pronounced effect.

Sharma *et al.* (2000) investigated the effect of *Emblica officinalis* extract at the rate of 50, 250 and 500mg/kg on the in vivo genotoxicity of Benzo(a)pyrene and Cyclophosphamide in mice. Higher doses inhibited genotoxicity while 50mg/kg had no effect and Vitamin C at the rate of 9 mg/kg inhibited chromosomal aberrations and micronuclei induction in a non significant manner. The plant extract and Vitamin C significantly induced the levels of glutathione, glutathione peroxidase, glutathione reductase and glutathione –S-transferase.

The effects of Garlic, Amla and Onion on the hyperlipidaemia induced by feeding butterfat and beef fat at the rate of 21 percent by weight to albino rats was compared by Augusti *et al.* (2001). Incorporation of five percent Garlic, Amla or Onion separately in the fatty diet ameliorated the deleterious effects of animal fat and the effect of Amla was intermediate to Garlic and Onion.

Haque *et al.* (2001) showed the protective effects of aqueous extract of *Emblica officinalis* at the rate of 100 mg/kg orally on immunological parameters and antioxidants in kidney and liver in mice treated with Cyclophosphamide (50mg/kg). The extract reduced the suppression of humoral immunity, increased the reduced glutathione levels and restored the antioxidant enzymes.

The antitumor activity of aqueous extract of *Emblica officinalis* (1.25g/kg) and Chyavanaprash (2.5g/kg) in mice bearing ascites and solid tumors was studied by Jose *et al.* (2001). Both extracts could increase the life span of tumor bearing animals ; *Emblica officinalis* by 20 percent and Chyavanaprash by 60.9 percent and reduced the solid tumors. The results also suggested that the antitumor activity of *Emblica officinalis* extract may partially be due to its interaction with cell cycle regulation.

Manjunatha *et al.* (2001) compared the effects of Vitamin C (500mg/day) from *Emblica officinalis* with Chyavanaprash (15g/day) on glucose tolerance and

lipoprotein profile in healthy adult volunteers. Chyavanaprash reduced post prandial glycaemia in the oral glucose tolerance test and blood cholesterol level to a significantly greater extent than vitamin C.

Al-Rehaily *et al.* (2002) examined the ethanolic extract of *Emblica officinalis* for its antisecretory and antiulcer activities by pylorus ligation shay rats, Indomethacin, hypothermic restraint stress induced gastric ulcer and necrotizing agents like 80 percent ethanol, 0.2M Sodium hydroxide and 25 percent Sodium chloride. Oral administration of the extract at the rate of 250 mg and 500 mg/kg significantly inhibited the development of gastric lesions in all the test models. It also offered protection against ethanol induced depletion of stomach wall mucous and reduction in non protein sulfhydryl concentration.

The hypolipidaemic action of *Emblica officinalis* was by the concerted action of inhibition of synthesis and enhancement of degradation of cholesterol, as evidenced by inhibition of HMG CoA reductase and elevated levels of LCAT (Anila and Vijayalakshmi, 2002).

The efficacy of *Emblica officinalis* fruit juice at the rate of 500mg/kg in modifying the acute cytotoxicity of cadmium was evaluated by Khandelwal *et al.* (2002). Oral administration of fruit juice at the rate of 500mg/kg body weight for eight days followed by a single toxic dose of cadmium reduced the elevated mortality and levels of SGOT, SGPT, GGT, lipid peroxidation, metallothionein and total sulfhydryl content of liver and kidney. It also prevented to some extent histopathological lesions in kidney, liver and testes.

Immunomodulatory activity of an Ayurvedic poly herbal formulation, Immu-21 containing extracts of *Ocimum sanctum, Withania somnifera, Emblica officinalis,* and *Tinospora cordifolia* on proliferative response of splenic leukocytes to T-cell mitogens, Concavalin (Con)-A and phytohaemagglutinin (PHA) and B-cell mitogen, lipopoly saccharide (LPS) in vitro by [3H]-thymidine uptake assay was studied in mice. Treatment of Immu-21 (30mg/kg i/p) once daily for 14 and 21 days did not

cause any change in body weight and spleen weight, but splenocytes or spleen count was increased. Immu-21 (30mg/kg i/p) for 14 days and 1 mg/kg for 21 days significantly increased LPS induced leukocyte proliferation; NK cell activity was increased in pretreatment with 10 and 30 mg/kg i/p of Immu-21 for seven days (Nemmani *et al.*, 2002).

Sairam *et al.* (2002) studied the ulcer protective potential of methanolic extracts of *Emblica officinalis* at the rate of 10-50 mg/kg p.o. Twice daily administration of the extract showed a dose dependent ulcer protection (36-98.3 percent) in ulcers induced by aspirin, ethanol, cold restraint stress, pyloric ligation and acetic acid. It also decreased acid secretion by 65.9 percent, increased mucin secretion by 95 percent , cellular mucous by 53.4 percent and life span of mucosal cells by 42.1 percent . It also showed significant antioxidant effect.

The cytoprotective and immunomodulating properties of Amla on lymphocytes using Chromium VI as immunosuppressive agent was studied by Sairam *et al.* (2002). The enhanced cytotoxicity, oxidative stress, inhibition of lymphocyte proliferation, IL-2 and γ-IFN production, increased apoptosis and DNA fragmentation due to Chromium treatment were effectively countered by Amla.

The effect of *Emblica officinalis* on hepatic function in broiler chicken exposed to hepatotoxins like feed additives, antibiotics in excess, mycotoxins like aflatoxin was studied (Sajitha, 2002). The fruit pulp powder gave better feed conversion rate, reduced the toxicopathological changes at two percent rate, as evidenced by an increase in haemoglobin, packed cell volume, total and differential leukocyte count, total serum protein and albumin content and also a decrease in ESR and serum enzyme levels. Degenerative and necrotic lesions and fibrous tissue proliferation were repaired.

Methanolic root extracts of *Vitex Negundo* and *Emblica officinalis* significantly antagonized the *Vipera russelli* and *Naja kaouthia* venom induced lethal activity in vitro and in vivo. *Vipera russelli* venom induced hemorrhage,

coagulant, defibrinogenating and inflammatory activity was significantly neutralized (Alam and Gomes, 2003).

2.2.2 Curcumin

Turmeric (*Curcuma longa*) has been ascribed with several medicinal properties. The yellow colour of turmeric is due to a phenolic chemical called curcumin (diferuloyl methane). On an average turmeric contain 2-4 percent curcumin; however, turmeric from Kerala is having the maximum content (8-11 percent) of curcumin. Curcumin is present in turmeric in three forms curcumin I, II and III and curcumin I is the main curcuminoid making around 60 percent, followed by curcumin II (30 percent) and curcumin III (10 percent).

Shalini and Srinivas (1987) demonstrated that the aqueous extract of turmeric extended protection to DNA against peroxidative injury

Curcumin significantly reduced the elevated hydroxy proline, serum glutamate pyruvate transaminase and histopathological lesion in Carbontetrachloride induced liver damage. Curcumin partially reversed the lipid peroxidation and histopathological changes in lung damage caused by Paraquat (Sony and Kuttan, 1993).

Turmeric oil and curcumin were studied against fifteen isolates of dermatophytes, four isolates of pathogenic molds and six isolates of yeast for their antifungal activity (Apisariyakul *et al.*, 1995). All the dermatophytes were inhibited by turmeric oil at 1:40-1:320 dilutions, pathogenic fungi were inhibited at 1:40-1:80 dilutions. These were not inhibited by curcumin whereas both curcumin and turmeric oil inhibited the growth of yeasts.

Ruby *et al.* (1995) compared the activity of natural curcuminoids, curcumin I, II and III for their cytotoxic, tumor reducing and antioxidant properties. Curcumin III was found to be more active as a cytotoxic agent, antioxidant and inhibitor of Ehrlich ascites tumor in mice.

The turmeric antioxidant protein isolated from aqueous extract of turmeric was found to be a heat stable protein and its activity was abolished by trypsin (Selvam *et al.*, 1995). A 50 percent inhibitory activity of lipid peroxidation was observed at protein concentrations of 50µg/ml.

Curcumin effectively inhibited endothelial cell proliferation and basic fibroblast growth factor induced corneal neovascularisation. But it had no effect on phorbol ester stimulated vascular endothelial growth factor showing that curcumin has direct antiangiogenic activity in vitro and in vivo. (Arbiser *et al.*, 1998).

Shoskes and Daniel (1998) showed that pretreatment with quercetin and curcumin resulted in preservation of histological integrity with decrease in tubular damage, interstitial inflammation and serum creatinine caused by renal ischemia reperfusion. The flavonoids also attenuated the expression of allograft inflammatory factor, monocyte chemoattractant protein, showing a promise as agents that can reduce immune and non immune renal injury.

Administration of 0.2 percent curcumin during both initiation and post initiation periods significantly inhibited colon tumorigenesis. (Kawamori *et al.*, 1999). Synthetic curcumin (0.2 percent and 0.6 percent)in diet during progression or promotion stage significantly suppressed the incidence and multiplicity of non invasive adenocarcinomas and invasive carcinomas of colon.

Deters *et al.* (2000) observed that 250 and 500 mg/kg injections of curcumin increased dose dependent basal bile flow up to 200 percent, biliary bilirubin excretion up to 150 percent and biliary cholesterol excretion up to 113 percent in rats given Cyclosporine.

The antiallergic activity of n-hexane, benzene, ethyl acetate, n-butanol and water extracts from the rhizome of *Curcuma longa* was studied by Yano *et al.* (2000). The extracts moderately to markedly inhibited histamine release induced by compound 48/80 from rat peritoneal mast cells and maximum potency was shown by ethyl acetate extract.

Bhagath and Purohit (2001) showed that 50 percent alcoholic and aqueous extracts of *Curcuma longa* at the rate of 500 mg/kg caused degenerative changes in the spermatogonic germinal elements in testes. The cholesterol and glycogen content in testes and ascorbic acid in adrenals were significantly decreased.

Literat *et al.* (2001) demonstrated that curcumin produced significant inhibition of IL-1β and IL-8, but minimal inhibition of TNF-α expression by preterm lung inflammatory cells. Adult peripheral blood mononuclear cell expression of IL-8 was also significantly inhibited confirming the property of inhibition of proinflammatory cytokine production.

Administration of turmeric or curcumin to diabetic rats reduced the blood sugar, haemoglobin, glycosylated haemoglobin and the activity of sorbitol dehydrogenase. Curcumin was more effective in attenuating the diabetes related changes than turmeric (Arun and Nalini, 2002).

Efficacy of curcuminoids in inhibiting the hepatic microvascular inflammatory response to lipopolysaccharides was studied by Atmadja *et al.* (2002). Treatment with curcuminoids at the rate of 40 and 80 mg/kg body weight reduced the phagocytic activity of centrilobular kupffer cells and number of adhering leukocytes and swollen endothelial cells.

Das and Das (2002) studied the efficacy of curcumin as a free radical scavenger and showed that 2.75μM concentration caused 50 percent inhibition of singlet oxygen dependent 2,2,6,6-tetra methyl piperidine –N-oxyl formation. But it was not an efficient superoxide or hydroxyl radical scavenger.

The activity of synthetic curcuminoids on *Leishmania amazonensis* promastigotes was studied by Gomas-Dde *et al.* (2002). They found that compound 5 (1,7 bis (2-hydroxy-4-methoxy phenyl)-1,6-heptadiene-3,5-dione) was most effective.

Mahady *et al.* (2002) tested the methanolic extract of dried powdered turmeric rhizome and curcumin against 19 strains of *Helicobacter pylori*, a group I

carcinogen. Both of them inhibited the growth of all strains of *Helicobacter pylori* in vitro.

Rasyid *et al.* (2002) showed that a dose of 40 mg of curcumin was capable of a 50 percent contraction of gall bladder. But there existed no linear relationship between doubling curcumin dosage and doubling of gall bladder contraction.

Dietary curcumin inhibited chemotherapy induced apoptosis through inhibition of reactive oxygen species (ROS) and blockade of C-Jun NH (2)- terminal kinase (JNK) pathway which is needed for many chemotherapeutic drugs inducing apoptosis (Somasundaram *et al* ., 2002).

Sugimoto *et al.* (2002) observed that treatment of mice with curcumin at the rate of 0.5, two and five percent in diet prevented wasting and histopathologic signs of trinitro benzene sulphonic acid (TNBS) induced colitis. It was also found to suppress the proinflammatory cytokine m RNA expression in colonic mucosa.

Caughey *et al.* (2003) demonstrated that curcumin potently inhibits protease resistant prion protein in Scrapie agent infected neuroblastoma cells.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Fifty adult wistar rats, of 150-250g body weight, procured from Small Animal Breeding Station, Mannuthy were used for the study. They were maintained under identical managemental conditions. They were divided into five groups of 10 animals each.

3.2 GROUP DESIGN

3.2.1 Treatment Schedule

Group	Treatment
I	Control. Given high fat diet.
II	Control. Given one percent cholesterol diet and 0.2ml five percent gum acacia
III	One percent cholesterol diet and aqueous extract of *Emblica officinalis* at the rate of 50 mg/kg orally.
IV	One percent cholesterol diet and aqueous extract of *Emblica officinalis* at the rate of 100mg/kg orally.
V	One percent cholesterol diet and curcumin at the rate of 250 mg/kg in five percent gum acacia orally.

3.2.2 Composition of High Fat Diet

Wheat flour	64%
Casein	16%
Coconut oil	15%
Mineral mixture	4%
Vitamin	1%

3.2.3 Composition of One Percent Cholesterol Diet

Wheat flour	63%
Casein	16%
Coconut oil	15%
Cholesterol	1%
Mineral mixture	4%
Vitamin	1%

3.3 PREPARATION OF HERBAL AGENTS

3.3.1 *Emblica officinalis*

Emblica officinalis was purchased from local market, deseeded and dried under shade (Fig 1). The dried fruit pulp was pulverized and five gram of the material was mixed with 10 ml distilled water in a mortar with pestle. It was then sieved through a fine muslin cloth, centrifuged and the supernatant was collected. The residue was dried and weighed to assess the percentage of extract and was found to be 10 percent. It was fed orally to rats of group III and IV at the rate of 50 and 100mg/kg ie, 0.2ml and 0.4ml respectively.

3.3.2 Curcumin

Two gram of curcumin was mixed with 10ml of five percent gum acacia and fed orally at the rate of 0.25ml(250 mg/kg) to rats of group V(Fig 2).

The drugs were administered once daily simultaneously with one percent cholesterol diet for 60 days.

3.4 PATTERN OF THE EXPERIMENT

The total duration of the experiment was for 60 days. Initial body weight was taken on day zero of the experiment and final body weight on the day of slaughter of

Fig 1. *Emblica officinalis* fruit

Fig 2. Turmeric (*Curcuma longa*) rhizome

the animals. Fifty percent of the animals were sacrificed on the 30th day and rest on 60th day for the estimation of serum cholesterol, liver lipid peroxide, superoxide dismutase and catalase levels and haematological parameters like haemoglobin, total and differential leukocyte counts and total erythrocyte count.

3.5 COLLECTION OF BIOLOGICAL SAMPLES

3.5.1 Blood

Blood was collected from retro orbital plexus by puncturing with heparinised capillary tubes, into sterile vials containing disodium salt of ethylene diamine tetra acetic acid (EDTA Sodium) at the rate of 1mg/ml for estimation of haematological parameters. Blood was collected in sterile centrifuge tubes without anticoagulant for serum for the estimation of cholesterol.

3.5.2 Liver

The animals were euthanized and dissected upon and the liver wascollected. It was washed in running tap water to remove blood clots and kept in chilled 0.9% sodium chloride.

3.6 ESTIMATION OF VARIOUS PARAMETERS

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

Reagents

Sodium chloride 0.9%

Potassium chloride 150mM

Sodium dodecyl sulphate 8.1 percent (SDS)

Acetic acid 20%; pH adjusted to 3.5

Aqueous solution of Thiobarbituric acid 0.8% (TBA)

n-butanol :pyridine mixture (15:1)

Procedure

1. One gm of wet tissue was mixed with 9 ml of 150mM potassium chloride and homogenized in a tissue homogenizer
2. Tissue homogenates (0.2ml) were taken in test tubes , added 0.2ml of 8.1 percent SDS, 1.5ml of 20% acetic acid and 1.5ml of TBA. Blank contained 0.2ml of potassium chloride instead of tissue homogenate.
3. Made up the volume to 4ml with distilled water and heated on a water bath at 95° C for 60 minutes. Cooled under tap water.
4. Added 1ml of distilled water and 5ml of n-butanol : pyridine mixture. It was shaken well and centrifuged at 400rpm for 10 minutes.
5. The absorbance of colour of the organic layer was measured at 532nm.
6. The lipid peroxide level was calculated by using extinction coefficient of $1.56x10^5$ and the values were expressed in nmol of malondialdehyde (MDA)/g of wet tissue.

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

Reagents

Tris cacodylic acid buffer (50mM, pH 8.2)

Tris cacodylic acid 50 mM

Diethylene triamine penta acetic acid 1mM

Nitroblue tetrazolium 0.1mM

Triton X 100 0.001 percent.

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

Pyrogallol 0.2mM.

Procedure

1. Freshly excised liver was homogenized with 10 volumes of 0.9% sodium chloride followed by centrifugation at 400rpm for 10 minutes at 4°C to harvest the supernatant.
2. The assay mixture in a total volume of 3ml consisted of 1.4ml of 50mM tris cacodylic acid buffer, 1.4ml of 0.2mM pyrogallol and 0.2ml of enzyme preparation.
3. Blank contained distilled water instead of enzyme preparation.
4. The absorbance due to autooxidation of pyrogallol was read at 420 nm using spectrophotometer.
5. One unit of SOD activity was the amount of enzyme which inhibited pyrogallol autooxidation by 50% under experimental conditions.
6. The values were expressed in units/mg of protein after quantifying the protein content of supernatant by method of Lowry *et al.* (1951).

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

Reagents

Phosphate buffer-Hydrogen peroxide solution (10mM)

Phosphate buffer (0.05M pH 7.0)

0.2M sodium dihydrogen phosphate	39ml
0.2M disodium hydrogen phosphate	61ml
Distilled water	300ml

Immediately before use 0.12 ml of hydrogen peroxide was added to 100ml buffer.

Procedure

1. Three ml of the phosphate buffer-hydrogen peroxide solutions were taken in test tubes.

2. Blank contained distilled water instead of hydrogen peroxide solution.
3. Samples prepared in sodium chloride (as described in case of superoxide dismutase) were added to both and the absorbance was read at 240nm at the 20th second of addition of sample.
4. The time required for the initial absorbance to decrease by 0.05 units was noted.
5. The catalase activity in units/assay mixture was calculated by using the formula $\log E_1 / E_2 \times 2300 / 6.93 \times 1 / \Delta t$.

   E_1- Initial absorbance

   E_2- Absorbance after decrease by 0.05 units

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

3.6.4 Measurement of Cholesterol (CHOD-PAP Method; Allain *et al.*, 1974).

(used kit from Agappe Diagnostics)*

Reagents

Pipes buffer pH 6.7	50mmol/L
Phenol	24mmol/L
Sodium cholate	0.5mmol/L
4-amino antipyrene	0.5mmol/L
Cholesterol esterase	$\geq$ 180u/L
Cholesterol oxidase	$\geq$ 200u/L
Peroxide	$\geq$1000u/L
Cholesterol Standard	200mg/dl

*Agappe Diagnostics, Thane, Maharashtra.

Procedure

1. One ml of the working reagent was taken in test tubes marked standard, blank and sample.
2. Ten micro litres of serum and standard were taken in respective test tubes.
3. Mixed them well, incubated at 37°C for five minutes and took the optical density at 505nm.
4. The cholesterol concentration in mg% was calculated by the formula
   Cholesterol concentration= O.D of sample/ O.D. of standard x 200.

3.6.5 Estimation of Haematological Parameters

3.6.5.1 *Total Leukocyte Count*

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

3.6.5.2 *Differential Leukocyte Count*

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

3.6.5.3 *Total Erythrocyte count*

The erythrocytes were counted using standard dilution technique using Hayme's fluid diluent. Counting of erythrocytes was done under high power of the microscope in the zone for erythrocyte in the haemocytometer (Benjamin, 1985).

3.6.5.4 *Haemoglobin concentration*

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

3.7. STATISTICAL ANALYSIS OF DATA

Results were analysed by using student *t* test for comparison between control groups, analysis of variance for comparison between groups II, III, IV and V for parameters except body weight and body weight was analysed using Analysis of Covariance as described by Snedecor and Cochran (1985). Results are expressed as mean $\pm$ standard deviation.

Results

4. RESULTS

4.1 BODY WEIGHT

The results obtained are presented in Tables 1 and 2 and Fig. 3 and 4. The mean body weight gain were 10g, 24g, 06g, 20g and 14g after 30 days and 34g, 32g, 26g, 28g and 40g after 60 days for groups I to V respectively. There was no significant difference between the treatment and control groups in body weight gain.

4.2 BIOCHEMICAL PARAMETERS

4.2.1 Lipid peroxide level

The results are presented in Tables 3 and 4 and Fig. 5. The peak lipid peroxide level found was 248.672± 9.702 and 386.076± 16.05 nmol of MDA/g wet tissue in animals fed one percent cholesterol diet (group II) after 30 and 60 days respectively. Group II differed significantly from group I which had values 202.454± 9.747 and 330.752± 16.072 nmol of MDA /g wet tissue. The treatment groups (group III, IV and V) showed significant reduction in lipid peroxide compared to the animals fed one percent cholesterol diet (group II, $P<0.01$). The lipid peroxide level of animals given Emblica at the rate of 100mg/kg (group IV) and curcumin at the rate of 250 mg/kg (group V) after 30 days were 88.128± 45.90 and 103.664± 4.73 nmol of MDA/g wet tissue respectively. However after 60 days of treatment, animals fed curcumin (group V) had greater reduction in levels viz, 59.720± 14.55 where as for group IV it was 79.568±14.79 nmol of MDA/g wet tissue. The lipid peroxide level of group III (Emblica at the rate of 50mg/kg) was intermediate to the other treatment groups and control which were 148.368± 14.27 and 100.016± 6.57 after 30 and 60 days respectively. There was significant difference between the groups after both 30 and 60 days in lipid peroxide level ($P<0.01$).

Table 1. Initial and final body weights after 30 days of treatment, g

Animal No.	Group I		Group II		Group III		Group IV		Group V	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	220	220	200	220	200	220	160	200	200	230
2	220	210	160	200	210	210	220	200	160	180
3	220	220	220	230	230	220	180	180	200	200
4	180	220	160	180	210	210	180	220	240	260
5	220	240	160	190	160	180	300	340	240	240
Mean	212	222	180	204	202	208	208	228	208	222

Table 2. Initial and final body weights after 60 days of treatment, g

Animal No.	Group I		Group II		Group III		Group IV		Group V	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	160	190	180	200	200	240	200	220	260	290
2	180	200	220	270	180	230	220	260	180	220
3	140	200	180	200	260	270	240	270	200	260
4	200	240	200	210	220	230	180	200	200	240
5	180	200	140	200	220	240	220	250	220	250
Mean	172	206	184	216	216	242	212	240	212	252

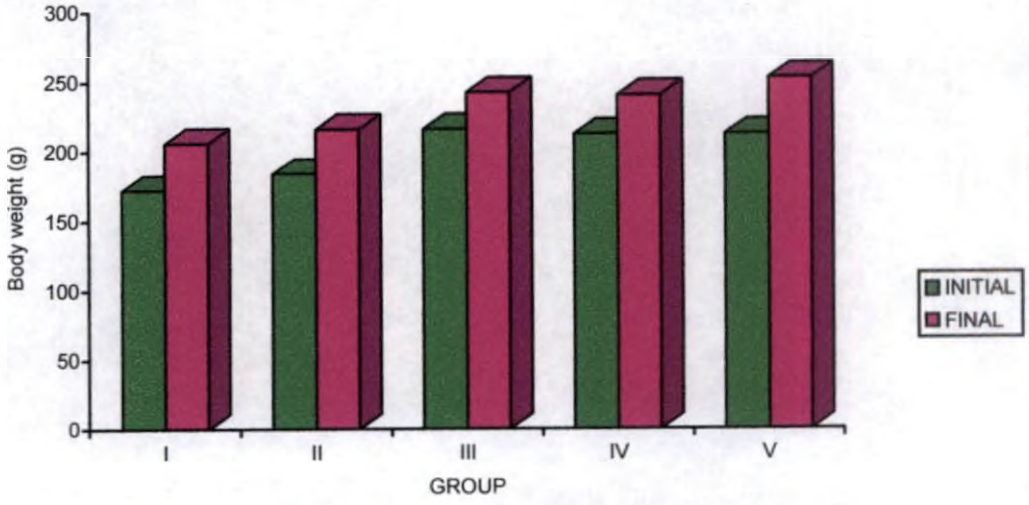

Fig. 3. Effect of treatments on body weight after 30 days

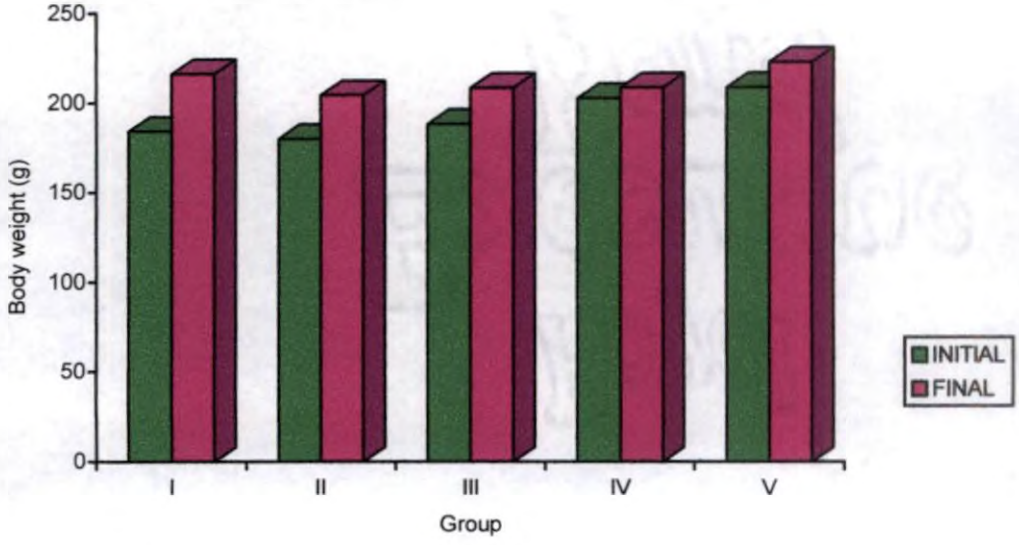

Fig. 4. Effect of treatments on body weight after 60 days

Table 3. Lipid peroxide level after 30 days of treatment, n mol of malondialdehyde (MDA)/g wet tissue

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	197.6	262.96	130.68	133.32	106.4
2	188.48	238.64	147.84	67.32	103.36
3	203.68	253.84	170.28	108.24	107.92
4	209.71	244.72	143.88	106.92	95.76
5	212.80	243.20	149.16	114.84	104.88
Mean ± SD	202.454 ± 9.747^{D}	248.672 ± 9.709^{A}	148.368 ± 14.27^{B}	88.128 ± 45.90^{C}	103.664 ± 4.73^{C}

Table 4. Lipid peroxide level after 60 days of treatment, n mol of malondialdehyde (MDA)/g wet tissue

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	338.96	364.80	95.76	65.36	76.00
2	334.40	404.32	98.80	74.48	54.72
3	326.80	399.76	92.72	104.48	47.12
4	348.08	380.00	103.36	79.04	74.48
5	305.50	381.52	109.44	74.48	46.28
Mean ± SD	330.752 ± 16.072^{E}	386.076 ± 16.05^{A}	100.016 ± 6.57^{B}	79.568 ± 14.79^{C}	59.720 ± 14.55^{D}

(Means bearing the same superscript do not differ significantly at $P< 0.01$).

4.2.2 Superoxide dismutase

The values of superoxide dismutase obtained are presented in Tables 5 and 6 and Fig.6. A significant increase in the superoxide dismutase level was noted in all the treatment groups compared to the controls ($P < 0.01$). Group III and IV had mean superoxide dismutase values of 11.518 $\pm$ 0.59 and 12.120 $\pm$ 0.53 units/mg of protein respectively after 30 days. But after 60 days, group IV had more pronounced levels with 14.865$\pm$ 2.02 units/mg of protein and group III had 12.036$\pm$ 0.720 units. Group V had significant difference ($P< 0.01$) with other treatments and had peak superoxide dismutase values of 12.758$\pm$ 0.570 and 22.090$\pm$ 2.290 units/mg of protein after 30 and 60 days respectively. The superoxide dismutase level in animals given high fat diet (group I) were 9.138$\pm$ 0.909 and 10.288$\pm$ 0.305 units after 30 and 60 days respectively. The superoxide dismutase level was lowest in group II ie, 8.730$\pm$ 1.22 and 10. 174 $\pm$ 0.53 units respectively after 30 and 60 days of treatment. There was no significant difference between the two control groups.

4.2.3. Catalase

The values of catalase are given in Tables 7 and 8 and Fig.7. There was significant increase in the mean catalase level of groups III, IV and V compared to group II ($P< 0.01$). The catalase level of group III after 30 days of treatment was intermediate to group IV and group V with values reading 18.316 $\pm$ 2.690, 20.326 $\pm$ 4.370 and 15.656$\pm$ 0.460 units/assay mixture respectively after 30 days. Group I had values 10.5$\pm$ 0.510 and group II 12.422 $\pm$ 1.29 units/assay mixture respectively. After 60 days, maximum catalase level was shown by group IV and V and the values were 21.788$\pm$ 2.880 and 21.40$\pm$ 2.840 units/assay mixture respectively. Group III was intermediate to group II and other treatments with 18.154$\pm$ 2.230 units whereas groups II and I did not differ significantly and the mean catalase level was 10.964$\pm$ 1.329 and 12.194$\pm$ 1.490 units/assay mixture respectively.

Table5. Superoxide dismutase level (units/mg of protein) after 30 days of treatment

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	9.470	7.750	10.886	11.550	12.120
2	9.532	9.392	10.848	12.980	12.570
3	9.402	9.992	12.000	12.100	12.790
4	9.756	9.392	11.848	11.870	13.670
5	7.530	7.126	12.000	12.100	12.640
Mean ± SD	9.138 ± 0.909^{C}	8.730 ± 1.220^{C}	11.518 ± 0.590^{B}	12.120 ± 0.530^{B}	12.758 ± 0.570^{A}

Table6. Superoxide dismutase level (units/mg of protein) after 60 days of treatment

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	10.100	9.440	11.340	12.136	20.756
2	10.530	10.760	11.260	13.730	25.130
3	10.040	10.010	12.160	16.950	20.310
4	10.070	10.060	12.910	14.850	23.960
5	10.700	10.600	12.510	16.660	20.300
Mean ± SD	10.288 ± 0.305^{C}	10.174 ± 0.530^{C}	12.036 ± 0.720^{C}	14.865 ± 2.020^{B}	22.090 ± 2.290^{A}

(Means bearing the same superscript do not differ significantly at $P< 0.01$).

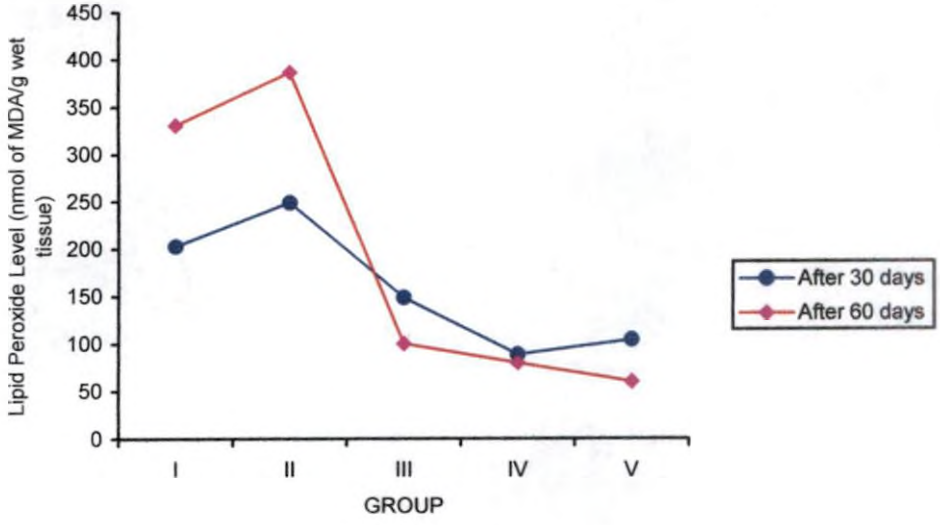

Fig. 5. Effect of treatments on lipid peroxide level

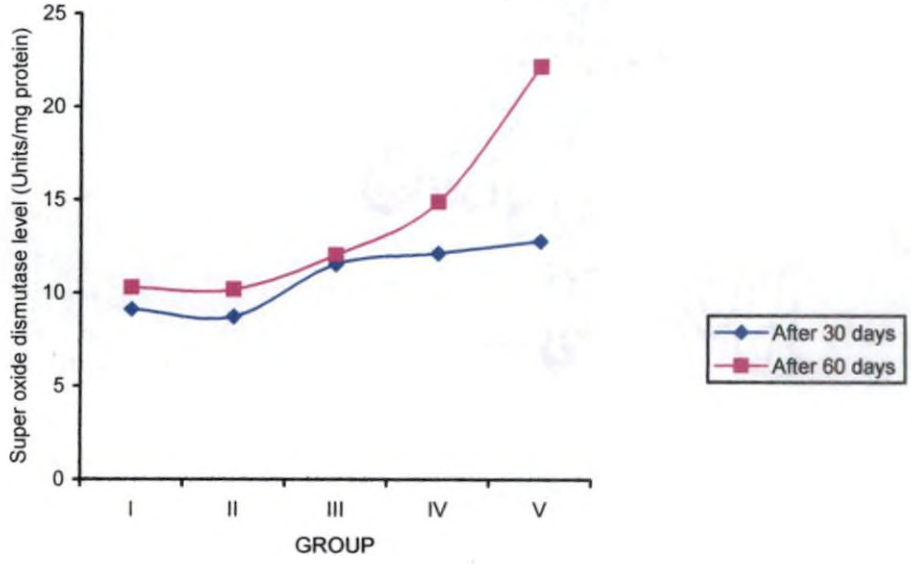

Fig. 6. Effect of treatments on superoxide dismutase level

Table 7. Catalase level after 30 days of treatment, units/assay mixture

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	10.2	10.8	16.45	16.5	15.10
2	10.4	14.13	17.81	22.95	15.9
3	11.4	11.89	22.95	19.67	16.23
4	10.2	13.29	17.92	26.39	15.7
5	10.3	12.1	16.45	16.07	15.3
Mean ± SD	10.5 $\pm 0.510^{D}$	12.442 $\pm 1.29^{C}$	18.316 $\pm 2.69^{AB}$	20.326 $\pm 4.37^{A}$	15.656 $\pm 0.46^{BC}$

Table 8. Catalase level after 60 days of treatment, units/assay mixture

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	9.95	11.06	15.49	22.74	19.91
2	10.7	10.6	18.05	18.05	25.5
3	13.27	13.29	16.50	20.06	22.74
4	10.7	14.13	20.06	25.5	18.05
5	10.2	11.89	20.67	20.67	22.74
Mean ± SD	10.964 $\pm 1.329^{C}$	12.194 $\pm 1.49^{C}$	18.154 $\pm 2.23^{B}$	21.40 $\pm 2.84^{A}$	21.788 $\pm 2.88^{A}$

(Means bearing the same superscript do not differ significantly at $P< 0.01$).

4.2.4 Serum Cholesterol

The results obtained are presented in Tables 9 and 10 and Fig. 7. The lowest level of mean serum cholesterol after 30 days of treatment was observed in group IV ie, 89.814± 7.38 mg/dl; group III was intermediate to other treatment groups with 92.45± 5.573 and group V had 99. 796± 11.55 mg/dl. Group I and II did not differ significantly with respect to serum cholesterol levels and the mean values were 102.940±3.859 and 104.344± 6.98 respectively after 30 days. The serum cholesterol levels after 60 days were similar in groups III and IV which were 69.214± 0.916 and 64.554± 1.16 mg/dl ($P< 0.05$). The value of group V was intermediate to the other treatments and controls and the mean level was 97.098± 7.54 mg/dl. The mean serum cholesterol level was maximum in group II, 112.088± 5.24mg/dl where as group I had 105.656± 2.821 mg/dl. There was significant decrease in serum cholesterol level ($P< 0.05$) when compared to the control groups in groups III, IV and V after 30 and 60 days of treatment.

4.2 HAEMATOLOGICAL PARAMETERS.

4.3.1 Total Leukocyte Count

The results are given in Tables 11 and 12 and Fig.8. The values of total leukocyte count (TLC) were in normal range in all groups throughout the experiment. The highest count was obtained in group IV and V which was 7800± 632.46 and 7960± 260.77/µl of blood after 30 days and did not differ significantly between them. The values of group III (7380± 469.84/ µl) was intermediate to group II (6790± 674.91µl of blood) and other treatment groups. However, group I had a TLC of 6090± 207.36 cells /µl of blood after 30 days. After the completion of experiment, there was no significant difference between groups. The total leukocyte counts of groups III, IV and V were 7230± 148.32, 7160± 221.92 and 7180± 168.08

Table 9. Serum cholesterol level after 30 days of treatment, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	108.69	97.82	89.09	102.27	116.81
2	101.63	101.63	101.81	89.54	92.80
3	104.34	114.67	93.18	88.63	88.49
4	101.63	99.45	86.81	83.63	106.19
5	98.36	108.15	91.36	85.0	94.69
Mean ± SD	102.940 ±3.859^{C}	104.344 ± 6.98^{C}	92.45 ±5.753^{A}	89.814 ± 7.38^{A}	99.796 ±11.55^{B}

Table 10. Serum cholesterol level after 60 days of treatment, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	109.84	111.84	66.39	63.22	109.00
2	102.84	109.84	68.73	64.37	96.08
3	106.12	102.40	71.82	63.81	96.46
4	103.24	106.12	69.32	65.23	95.95
5	106.24	112.24	69.82	66.14	88.00
Mean ± SD	105.656 ± 2.821^{D}	112.088 ± 5.24^{A}	69.214 ± 0.196^{C}	64.554 ± 1.16^{C}	97.098 ± 7.54^{B}

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

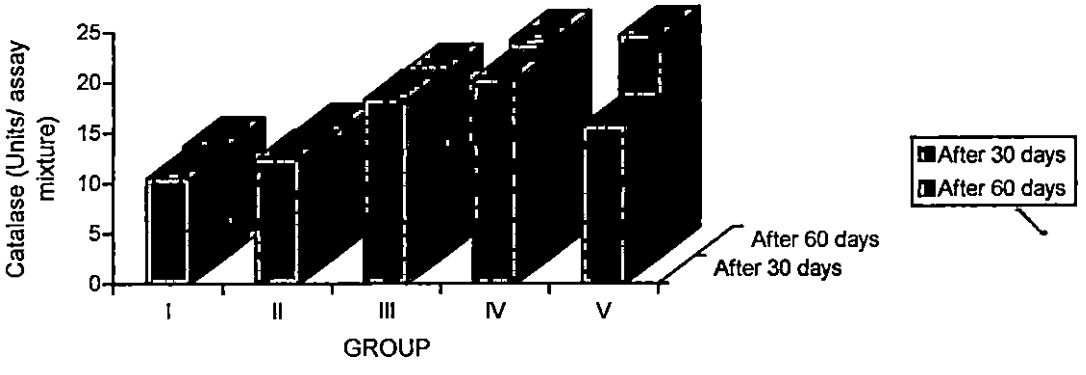

Fig. 7. Effect of treatments on catalase level

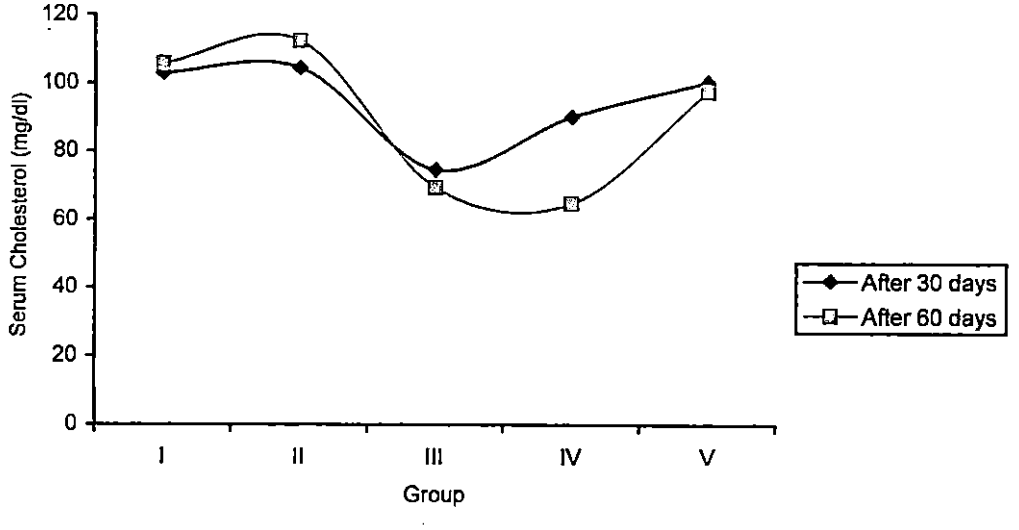

Fig. 8. Effect of various treatments on cholesterol level

WBC's /µl of blood respectively. Group I had a count of 6280± 347.49 and group II 6730± 494.47 WBC's /µl of blood.

4.3.4 Differential Leukocyte Count.

4.3.2.1 *Neutrophils*

The data is presented in Tables 13 and 14 and Fig.9 and 10. The neutrophil count was highest in group IV after 30 days with 15.8± 3.11%, The counts were 12.0± 1.58% in group V, 11.00± 2.24% in group III 14.4± 3.05% in group I and 9.6± 3.05% in group II. Group II and III were similar, group V was intermediate to the other treatments and group II ($P< 0.01$). There was no significant difference between the groups after 60 days. The values however after 30 and 60 days were within the normal range of 9.0- 34%.

4.3.2.2 Lymphocytes

The results obtained are presented in Tables 13 and 14 and Fig.9 and 10. The lymphocyte counts in groups II and III were similar ($P< 0.01$) with values of 89.00 ± 2.24% and 90.4± 3.05 % respectively after 30 days. The counts were 84.60 ± 3.44%, 83.6± 3.21% and 87.6± 1.67% for groups I, IV and V respectively. Group V was found intermediate to other treatment groups and group II. After 60 days of treatment there was no significant difference between groups. All the values were within the normal range of 65-85%.

4.2.4.1 Eosinophils

The data is presented in Tables 13 and 14 and Fig. 9 and 10. The eosinophil counts of different groups did not differ significantly after 30 days. After 60 days of treatment groups I and II had counts of 2.0% and 1.6 %respectively. But the values were within normal range of 0-5%.

Table 11. Total leukocyte count after 30 days of treatment, per microlitres of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	6,000	6,000	7,800	7,600	7,800
2	5,900	7,200	7,600	8,200	7,600
3	6,200	7,600	6,800	8,000	8,200
4	6,400	6,950	6,950	6,800	8,000
5	5,950	6,200	7,750	8,400	8,200
Mean ± SD	6090 ± 207.36^{C}	6790 ± 674.91^{B}	7380 ± 469.84^{AB}	7800 ± 632.46^{A}	7960 ± 260.77^{A}

(Means bearing the same superscript do not differ significantly at $P < 0.01$).

Table 12. Total leukocyte count after 60 days of treatment, per microlitres of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	6,800	6,250	7,000	7,200	7,200
2	6,400	7,100	7,200	7,250	7,250
3	5,900	7,300	7,400	6,800	7,400
4	6,050	6,800	7,300	7,150	6,950
5	6250	6,200	7,250	7,400	7,100
Mean ± SD	6280 ± 347.49	6730 ± 494.47	7230 ± 148.32	7160 ± 221.92	7180 ± 168.08

Table 13. Differential leukocyte count after 30 days of treatment, %

Animal No.	Group I			Group II			Group III			Group IV			Group V		
	N	L	E	N	L	E	N	L	E	N	L	E	N	L	E
1	17	80	3	17	81	2	14	86	0	11	89	0	11	88	1
2	11	88	1	13	86	1	12	88	0	8	92	0	13	86	1
3	14	86	0	12	88	0	11	89	0	6	94	0	12	88	0
4	12	87	1	19	81	0	8	92	0	14	86	0	14	86	0
5	18	82	0	18	82	0	10	90	0	9	91	0	10	90	0
Mean ± SD	14.4 ± 3.05 C	84.6 ± 3.44 C	1± 1.23	9.6± 3.05 A	90.4 ± 3.05 A	0.6± 0.89	11± 2.24 A	89.0 ± 2.2 A	0	15.8 ± 3.11 A	83.6 ± 3.21 B	0	12.01.58 AB	87.6± 1.67 AB	0.4 ± 0.5

(Means bearing the same superscript within a column does not differ significantly (P< 0.01)

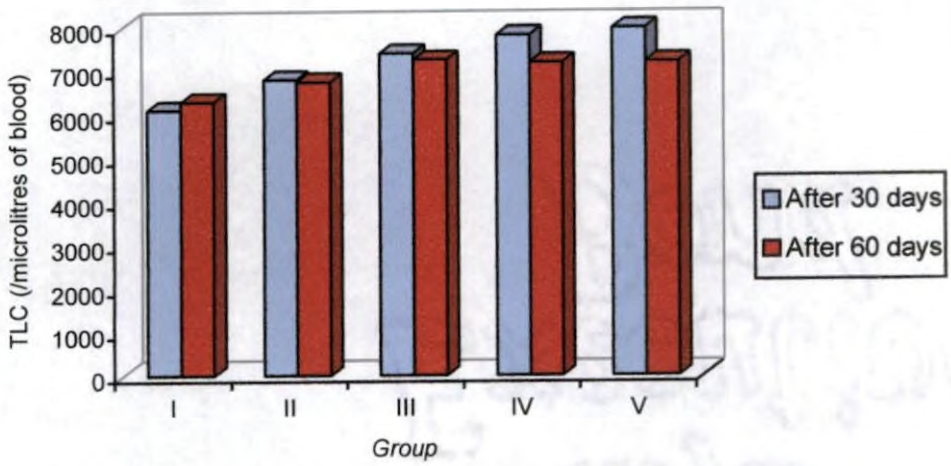

Fig. 9. Effect of treatments on total leukocyte count

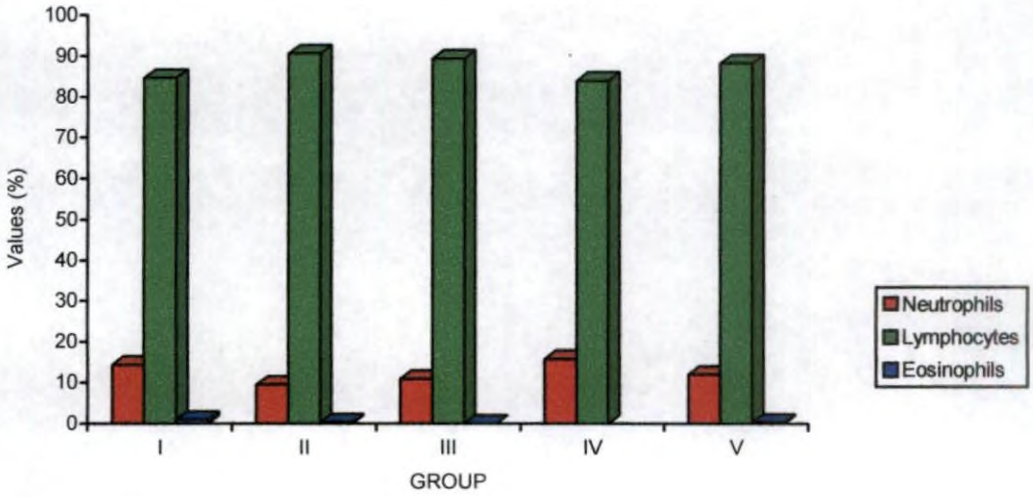

Fig.10. Differential leukocyte count after 30 days

Table 14. Differential leukocyte count after 60 days of treatment, %

Animal No.	Group I			GroupII			GroupIII			GroupIV			Group V		
	N	L	E	N	L	E	N	L	E	N	L	E	N	L	E
1	16	80	3	17	81	2	14	86	0	13	86	1	12	88	0
2	11	87	2	12	87	1	13	87	0	14	86	0	14	86	0
3	13	86	2	13	86	1	14	86	0	10	90	0	10	90	0
4	11	86	3	14	82	4	18	82	0	11	89	0	10	89	1
5	18	0	0	17	83	0	10	90	0	14	86	0	10	90	0
Mean ± SD	13.80 ± 3.11	84.2 ± 3.02	2.0± 1.27	14.6 ± 2.3	83.8 ± 2.59	1.6± 1.51	13.8 ± 2.86	86.2 ± 2.86	0	12.4 ± 1.82	87.4 ± 1.95	0.2± 0.45	11.2± 1.79	87.4± 1.95	0.2 ±0. 45

Table 15. Total erythrocyte count after 30 days of treatment, millions/mm^3 of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	8.00	7.82	7.91	8.11	8.10
2	6.27	8.62	7.97	7.99	8.13
3	7.83	7.61	7.82	8.23	7.99
4	8.21	8.11	7.99	8.10	7.63
5	7.93	8.32	8.11	7.63	7.81
Mean ± SD	7.65± 0.78	8.096± 0.4	7.960± 0.11	8.012± 0.23	7.932± 0.21

Table 16. Total erythrocyte count after 60 days of treatment, millions/mm^3 of blood

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	7.27	7.91	8.23	7.94	7.94
2	8.11	7.81	8.11	7.64	8.20
3	7.93	7.61	7.94	7.81	8.04
4	7.21	8.00	8.23	8.05	7.92
5	7.82	8.10	7.27	8.21	7.97
Mean ± SD	7.67±0.40	7.886± 0.19	8.356± 0.52	7.930± 0.22	8.014±0.11

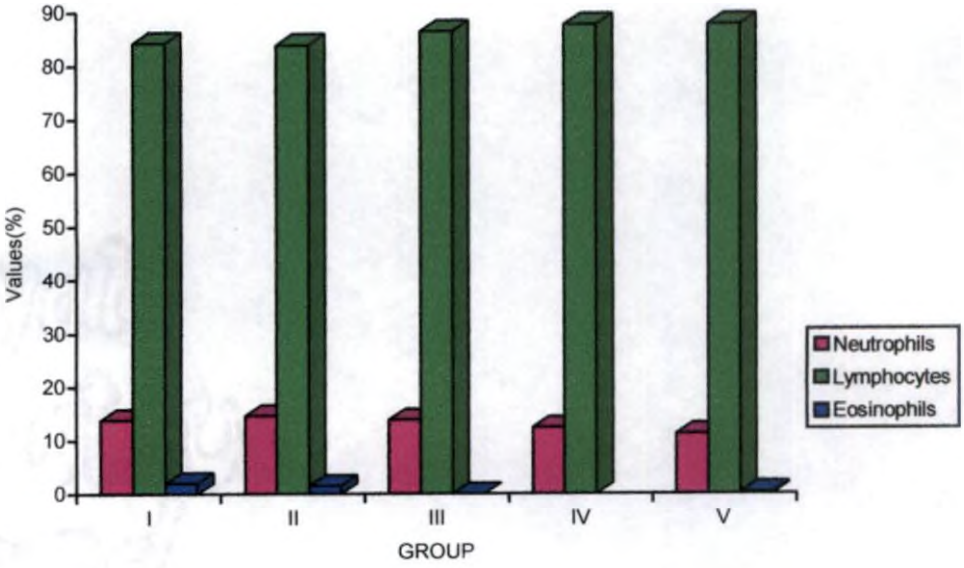

Fig. 11. Differential leukocyte count after 60 days

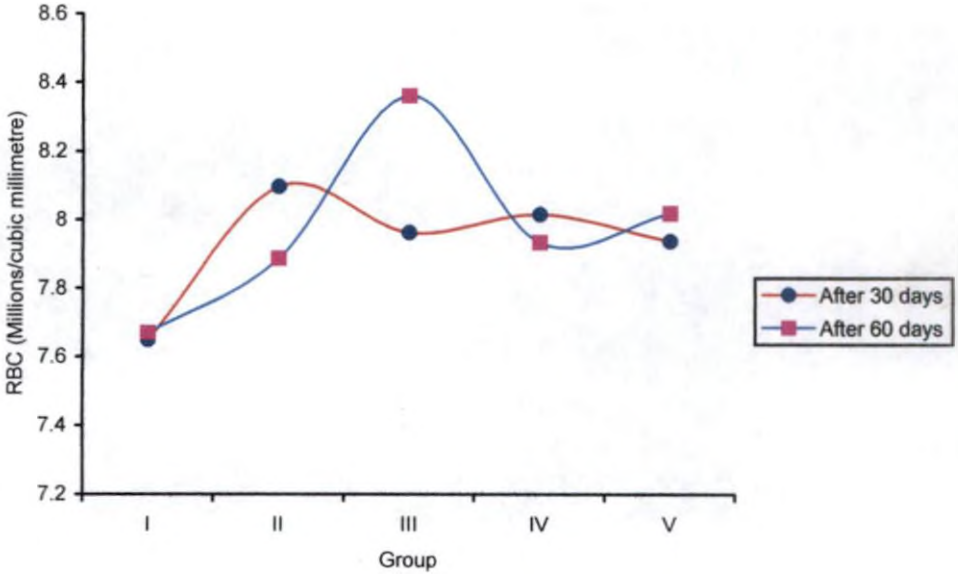

Fig. 12. Effect of treatments on total erythrocyte count

4.2.5 Total Erythrocyte Count

The results are presented in Tables 15 and 16 and Fig.11. The total erythrocyte count after 30 and 60 days in both the treatment and control groups were within the normal range of 7.0- $10x10^6$ cells/ µl of blood. There was no significant difference between the groups.

4.2.6 Haemoglobin concentration

The values are represented in Tables 17 and 18 and Fig.12. The haemoglobin concentration in animals of groups III, IV and V were similar, but differed significantly from I and II ($P< 0.01$) after 30 days of the experiment. The values were 13.92± 0.795, 14.220± 0.62, 15.360± 0.38, 15.160± 0.48 and 15.240± 0.30 g/dl for groups I to V respectively. However, no significant difference could be seen between groups after 60 days. The values were within the normal range on both occasions in all groups which is 10- 18 g/dl.

Table 17. Haemoglobin content after 30 days of treatment, g/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	15.0	14.6	14.8	15.2	15.2
2	13.8	15.1	15.2	14.6	14.8
3	12.8	14.0	15.6	14.8	15.4
4	13.8	13.8	15.4	15.4	15.2
5	14.2	13.6	15.8	15.8	15.6
Mean ± SD	13.92± 0.795^{B}	14.22± 0.62^{B}	15.36±0.38^{A}	15.16±0.48^{B}	15.24±0.30^{B}

(Means bearing the same superscript do not differ significantly at P< 0.01).

Table 18. Haemoglobin content after 60 days of treatment, g/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	14.2	14.8	16.2	15.2	15.2
2	13.8	15.2	14.8	15.6	14.6
3	13.6	14.4	14.4	15.0	15.2
4	13.8	14.2	18.0	13.8	15.4
5	14.8	14.0	15.2	14.4	15.4
Mean ± SD	14.04± 0.477	14.52± 0.480	15.72± 1.44	14.80± 0.71	15.16± 0.33

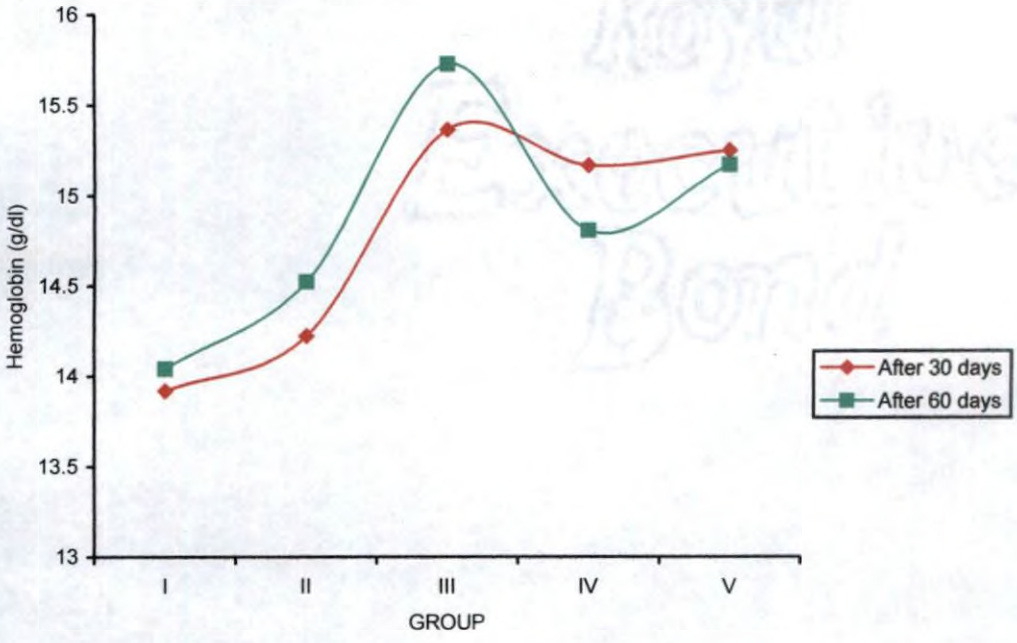

Fig.13. Effect of treatments on haemoglobin level

Discussion

5. DISCUSSION

The present study was undertaken to assess the antioxidant activity of *Emblica officinalis* and to compare it with that of curcumin. Aerobic cells are dependent on high concentration of poly unsaturated fatty acids for membrane elasticity, but paradoxically, this feature subjects them to peroxidative damage arising from the normal products of oxidative metabolism (Goyal, 1981). There is extensive evidence to implicate free radicals in the development of degenerative diseases and pathological changes associated with aging. Free radicals may also be a contributing factor in progressive decline in the function of immune system (Pike and Chandra, 1995). The consequences of oxidative stress are serious and in many cases are manifested by increased activities of enzymes involved in detoxification (Kim *et. al.*, 2003). Lipid peroxidation has gained more importance now a days because of its involvement in the pathogenesis of many diseases like atherosclerosis, cancer, diabetes mellitus, myocardial infarction and also aging (Cheeseman and Scater, 1993).

In the present study, a high fat diet was given to rats of group I. Animals of other four groups were given one percent cholesterol diet. Animals of group III and IV were given aqueous extract of *Emblica officinalis* simultaneously with one percent cholesterol diet at the rate of 50 and 100 mg/kg respectively. Animals of group V were given curcumin at the rate of 250mg/kg along with one percent cholesterol diet. The various physiological, biochemical and haematological parameters were studied which included body weight, liver lipid peroxide, superoxide dismutase and catalase levels, serum cholesterol, total and differential leukocyte counts, total erythrocyte count and haemoglobin level.

From the results, it is obvious that the herbal agents tried namely aqueous extract of *Emblica officinalis* at the rate of 50 and 100 mg/kg as well as curcumin at the rate of 250 mg/kg have reduced the lipid peroxidation and serum cholesterol

levels and increased the superoxide dismutase and catalase levels, where as the control groups remained in a state of oxidative stress and hypercholesterolaemia.

5.1 BODY WEIGHT

There was no significant difference in the mean body weight gain between any of the groups (Tables 1, 2 and Fig 3 and 4). There was no significant difference in the feed intake also during the entire course of experiment.

Gulati *et al.* (1995) have studied the effect of *Phyllanthus emblica* on body weight of rats having liver damage induced by feeding alcohol and found that there was increase in the body weight. This was substantiated by Sajitha (2002) in broiler chicken having hepatic damage, given fruit pulp powder of *Emblica officinalis*. In the present study, the effect of Amla and curcumin in rats without hepatic damage was studied. Compared to the other groups, the gain in body weight was lower in treatment groups. This may be due to the fact that *Emblica officinalis* and curcumin encouraged the excretion of cholesterol (Rao *et al.*, 1970; Mathur *et al.*, 1996).

5.2 LIPID PEROXIDE

The lipid peroxide levels were very high in control groups compared to the treatment groups, both after 30 and 60 days of experiment (Tables 3, 4 and Fig 5). *Emblica officinalis* at the rate of 100 mg/kg was more effective in reducing the lipid peroxide level than Emblica at the rate of 50 mg/kg. The activity of former was comparable to curcumin at the rate of 250mg/kg after 30 days of experiment, but after 60 days, curcumin showed more potency in reducing the lipid peroxide level.

High fat diet significantly increased the level of lipid peroxide in both serum and liver in Wistar rats (Sharma and Sharma, 2001). High oxidative stress of the high fat diet fed animals pointed towards a possibly high free radical generation, resulting in peroxidative damage and lowered activities of the antioxidant enzymes.

Pandey *et al.* (1994) states that free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies like

aging, atherosclerosis, delayed wound healing, oxygen toxicity, liver disorders, inflammation etc. Protection of the cell membrane from lipid peroxidation could prevent, cure or delay the afore said pathologies.

Daniel *et al.* (1998) observed a decrease in level of lipid peroxides in flavonoid treatment of hyperlipidaemic rats suggesting that these compounds can counteract the deleterious effects of a cholesterol diet.

Aqueous extracts of *Rhodiola sachaliensis* at the rate of 50, 100 and 200 mg/kg/day significantly decreased the malondialdehyde levels to 79 percent, 71 percent and 53 percent respectively to the levels with saline treatment during Carbontetrachloride intoxication in rats (Nan *et al.*, 2003).

Jose and Kuttan (1995) observed that *Emblica officinalis* fruit pulp at concentrations of 1000 µg/ml and 640 µg/ml caused a 50 percent inhibition of lipid peroxidation generated by Fe^{2+}/ascorbate and Fe^{3+}/ADP ascorbate in rat liver homogenate.

Curcumin also was found to decrease the lipid peroxide level in mice. (Soudamini *et al.*, 1992). Animals which were given curcumin for 14 days showed a significant decrease in the lipid peroxide level in tissue homogenates which were elevated by Carbontetrachloride and Paraquat injection. Selvam *et al.* (1995) also observed a concentration dependent inhibition of the promoter induced lipid peroxidation by turmeric antioxidant protein.

The results of the present study strongly agree with the previous studies and thus establishes the antioxidant property of *Emblica officinalis* and curcumin.

5.3 SUPEROXIDE DISMUTASE

Superoxide dismutase level was least in animals fed one percent cholesterol diet alone. But there existed no significant difference between them and those fed high fat diet. There was significant increase in the levels of superoxide dismutase in treatment groups (Tables 5, 6 and Fig 6). After 30 days of treatment, both dose levels

of Emblica were equal in action and curcumin had highest activity. After 60 days, the activity of *Emblica officinalis* at the rate of 100mg/kg was more compared to the same at the rate of 50mg/kg. But curcumin proved to be far more potent in its activity.

Daniel *et al.* (1998) observed that high fat diet decreased the activity of superoxide dismutase compared to animals fed a normal diet as observed during the present study. Similar observations were made by Sheela and Augusti (1995) and Dhuley (1999). Activity of superoxide dismutase was decreased by ethanol feeding to rats (Rajasree and Rajamohan, 1998).

Sheela and Augusti (1995) reported that administration of S-allyl cysteine sulphoxide and Gugulipid to rats fed cholesterol diet almost reversed the decreased level of superoxide dismutase. Dhuley (1999) reported that cinnamon bark and greater cardamom seeds also produced the same effect in hypercholesterolaemic rats.

Emblica officinalis tannins at the rate of 5 and 10 mg/kg intraperitoneally induced a dose related increase in superoxide dismutase activities in frontal cortex and striatum in rats (Bhattacharya *et al.*, 1999). In the present investigation, it was found that there was an increase in the level of superoxide dismutase in liver in rats fed with aqueous extract of *Emblica officinalis* at the rate of 50 and 100mg/kg. This highly indicates that *Emblica officinalis* has effectively countered stress due to high fat diet.

Curcumin however was more effective than Emblica as an antioxidant. Similar observations were made by Wang *et al.* (2000) in rats fed high fat diet and curcumin. The increase in activity of the enzymes may be due to the removal of toxic oxygen intermediates (Khan *et al.*, 1997).

5.4 CATALASE

As stated earlier in case of superoxide dismutase, the level of catalase was also lowest in the control groups (Tables 7, 8 and Fig 7). The activity was lowest in those animals fed high fat diet. Similar observation was made by Khan *et al.* (1997)

when rats were fed with high fat diet. Catalase levels were lower in animals fed two percent cholesterol diet than the animals fed normal diet (Daniel *et al.*, 1998).

There was an increase in the activity of catalase in the treatment groups. Emblica at the rate of 100 mg/kg exerted maximum activity after 30 days where as curcumin could produce an effect lesser than *Emblica officinalis* at the rate of 50 mg/kg. But after 60 days, both Emblica at the rate of 100mg/kg and curcumin exerted similar effects.

An increase in the level of catalase was observed by Sheela and Augusti (1995) when hyperlipidaemic rats were fed with S-allyl cysteine sulphoxide at the rate of 200mg/kg and Gugulipid at the rate of 50mg/kg. Similar results were obtained by Rajasree and Rajamohan (1998) with garlic protein which was fed to ethanol fed rats.

Emblica officinalis tannins at the rate of 5 and 10 mg/kg increased the level of catalase in rat frontal cortex and striatum by 72.9 percent, 61.5 percent and 42.5 percent and 69 percent respectively (Bhattacharya *et al.*, 1999).

5.5 SERUM CHOLESTEROL

An increase in the serum cholesterol level was observed in the control groups. Maximum serum cholesterol levels were noticed in animals fed one percent cholesterol diet (Tables 9,10 and Fig 8). Emblica at both dose rates reduced serum cholesterol better than curcumin.

Itokawa *et al.* (1973) showed that S-allyl cysteine sulphoxide isolated from garlic counteracted the effects of high cholesterol diet in rats viz, the increased serum and tissue cholesterol level, triglyceride, phospholipids and free fatty acids were reversed by a simultaneous administration of S-allyl cysteine sulphoxide with high cholesterol diet.

Daniel *et al.* (1998) observed that the flavonoids from the bark of *Ficus bengalensis* Linn. could reduce the elevated serum cholesterol, phospholipid, triglyceride and free fatty acids in rats fed two percent cholesterol diet.

Administration of *Emblica officinalis* fruit pulp decreased the elevated plasma cholesterol and triglyceride levels in rabbits given atherogenic diet by 69.74 percent and 78.20 percent respectively where as aqueous extract reduced them by 79.40 percent and 82.80 percent respectively. Similar observations were made by Mathur *et al.* (1996) who attribute the hypolipidaemic action to the increased excretion of cholesterol and phospholipids suggestive of ineffective fat absorption.

Oral administration of curcumin was also found to have a cholesterol lowering effect (Soudamini *et al.*, 1992; Wang *et al.*, 2000).

5.6 HAEMATOLOGICAL PARAMETERS

The total leukocyte count was highest in group IV and V, compared to the group II after 30 days (Tables 11, 12 and Fig 9). The values were within the normal range in all the groups (Hrapkiewics *et al.*, 1998). After 60 days of experiment, there existed no significant difference between the treatments. Praveenkumar *et al.* (1994) observed that Brahma Rasayana , a preparation made from *Emblica officinalis* stimulated bone marrow and proliferation of WBC in Cyclophosphamide treated animals.

Concomitant with the total leukocyte count, there was an increase in the neutrophil count and a decrease in lymphocyte count in animals treated with *Emblica officinalis* at the rate of 100mg/kg. No significant difference in total erythrocyte counts could be observed in all the groups (Tables 13 to 16 and Fig 10 to 12).

Haemoglobin level was more in treatment groups compared to controls after 30 days of experiment. Sajitha (2002) reported a significant increase in the levels of haemoglobin in broiler chicken fed Amla compared to those of control which had hepatic damage.

Sharma and Sharma (2001) observed that high fat diet significantly increased the level of thiobarbituric acid reacting substances in both serum and liver and decreased the red cell count, liver glutathione and hepatic antioxidant enzymes. Increased level of lipids due to high dietary fat seemed to be related to their more effective utilization in tissue lipid synthesis. Higher oxidative stress of the high fat diet fed animals points towards a possibly high free radical generation, resulting in a peroxidative damage, lowered activities of antioxidant enzymes. Free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies like aging, atherosclerosis, delayed wound healing, oxygen toxicity, liver disorders, inflammation etc. (Pandey *et al.*, 1994).

Potential antioxidant therapy include either free radical scavenging enzymes or agents that are capable of augmenting the activities of these enzymes which include superoxide dismutase, catalase, glutathione peroxidase etc. (Bhattacharya *et al.*, 1999). Regarding the relationship between the antioxidants and rasayana drugs, both have two common roles in health management ie, preventive and curative. They are responsible for maintaining the health of a healthy person and also for curing the metabolic diseases. It is also mentioned in Ayurvedic texts that rasayana drugs enhance the body defence capacity to fight against various causative factors and to improve the metabolism of different nutrients in the body (Acharya, 1978).

The results of the present study indicate a strong antioxidant activity of *Emblica officinalis* fruit. This property may be partially responsible for the biological properties manifested by the fruit. *Emblica officinalis* fruit contain tannins such as gallic acid, albumin, cellulose, nicotinic acid, amino acids like glutamic acid, proline, aspartic acid, alanine, lysine, minerals like chromium, zinc, iron, copper etc. (Khandelwal *et al.*, 2002). These components may be responsible for the protective effect. The results of the present study show that *Emblica officinalis* fruit induces endogenous antioxidant defence system and reduces lipid peroxidation in animals fed high fat diet, thus producing cytoprotective effects, especially in the event of oxidative stress induced injury by modifying the cell membrane integrity. Curcumin,

which is a proven antioxidant, was found to be a better antioxidant than Emblica by the present investigation.

The present investigation confirms the antioxidant and hypocholesterolaemic effect of *Emblica officinalis* and curcumin and their incorporation in diet help in prevention of pathologies due to oxidative stress.

Summary

6. SUMMARY

The present study was undertaken to assess the antioxidant activity of *Emblica officinalis* (Amla) on rats fed with one percent cholesterol diet and to compare it with the activity of curcumin. Fifty adult Wistar rats of 150-250 g body weight were divided into five groups of 10 each. High fat diet was fed to group I and the rest one percent cholesterol diet. Gum acacia, Emblica at the rate of 50 and 100 mg/ kg and curcumin at the rate of 250 mg/kg were fed simultaneously with one percent cholesterol diet to groups II, III, IV and V respectively for 60 days.

Different parameters like body weight gain, lipid peroxide, superoxide dismutase and catalase levels in liver, serum cholesterol, total and differential leukocyte count, total erythrocyte count and haemoglobin level were studied on 30^{th} and 60^{th} day of experiment.

There was no significant increase in body weight in any of the groups. Amla treated groups showed minimum change in body weight.

The animals of group II showed highest lipid peroxide level followed by those of group I. Maximum decrease in the levels was noticed in case of animals fed curcumin. All the treatments could significantly decrease the lipid peroxide level at both 30 and 60 days of experiment. Emblica at the rate of 50 mg/kg was having less effect than Emblica at the rate of 100 mg/kg and curcumin in lowering the lipid peroxide level.

Liver superoxide dismutase levels were lowest in group II. There was no significant difference between groups I and II. Emblica at the rate of 50 mg/kg could induce only a modest increase in the enzyme levels. Maximum response was produced by curcumin after 30 days, but after 60 days both curcumin and Emblica at the rate of 100 mg/kg produced high and comparable increase in the levels of superoxide dismutase.

Catalase levels were also lowest in the control groups. There was a significant increase in the levels in the treatment groups. Maximum levels were

recorded in animals fed with curcumin and Emblica at the rate of 100 mg/kg. This indicates that both show significant and comparable antioxidant property.

The high fat diet induced a hyperlipidaemic state in the animals of group I and II and maximum levels were recorded in animals of group II. Emblica was proved to be a better hypolipidaemic agent than curcumin at both dose levels. Curcumin however could significantly reduce the elevated serum cholesterol levels.

The haematological parameters were within the normal range on both occasions of analysis. The total leukocyte count was maximum with curcumin and Emblica at the rate of 100mg/kg after 30 days of experiment. Neutrophil counts were maximum in the group IV. All the three treatments could produce same increase in the haemoglobin levels. After 60 days of experiment, no significant difference existed between groups on any of the haematological parameters. Total erythrocyte counts showed no variation between groups on both occasions.

The results of the present study indicate that *Emblica officinalis* and curcumin could effectively reduce the oxidative stress induced by a high cholesterol diet in rats. Both of them are reported to have free radical scavenging activity which offers the protection. Also they could produce significant hypolipidaemic effect which is of great value in persons having the threat of atherosclerosis.

Significance of antioxidants, particularly 'herbal' antioxidants in modern veterinary practice can be visualized by the fact that the livestock and pet animals are increasingly exposed to prooxidants in the era of intensive production and industrialization. In addition to the management of stress and production linked diseases, herbal antioxidants also can be used as supportive therapy in mastitis, hepatitis, myocardial and neurological diseases of livestock and pet animals.

Considering the above facts and the findings of the present study, it could be concluded that *Emblica officinalis* and curcumin could be used as antioxidants. Evaluation and scientific validation of such agents provide a safe, cost effective and ecofriendly way of veterinary therapy in the future to come.

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COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY OF *Emblica officinalis* (AMLA) AND CURCUMIN IN RATS

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ABSTRACT OF THE THESIS

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ABSTRACT

The antioxidant activity of *Emblica officinalis* and Curcumin was investigated in rats fed with one percent cholesterol diet for a period of two months. They were divided into five groups of ten each. First group was fed a high fat diet; others one percent cholesterol diet. Group II did not receive any treatment. Animals of groups III and IV were given *Emblica officinalis* at the rate of 50 and 100 mg/kg. Group V was given curcumin at the rate of 250mg/kg. The liver samples of experimental animals were analysed for lipid peroxide level, superoxide dismutase and catalase activities. The blood samples were collected for serum cholesterol and routine haematological parameters. Body weight gain in them was also looked into.

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

Higher level of lipid peroxide and serum cholesterol was obtained in the control groups (groups I and II) compared to the treatments. Peak mean lipid peroxide and serum cholesterol levels were noticed in group II after 60 days of experiment viz, 386.076± 16.05 nmol of malondialdehyde (MDA)/ g wet tissue and 112.088± 5.24 mg/dl respectively. Curcumin reduced lipid peroxidation better than Emblica where as *Emblica officinalis* at both dose rates reduced serum cholesterol level than curcumin.

The activity of antioxidant enzymes like superoxide dismutase and catalase were lowest in group II. The peak superoxide dismutase level shown by group V was 22.090± 2.29 units/mg protein after 60 days of treatment. Emblica at 100 mg/kg and curcumin at 250 mg/kg were equally effective in inducing peak catalase levels. This showed that both Emblica and curcumin are having potent and comparable level of antioxidant property.

The haematological parameters from samples of all the groups were within the normal range. The maximum total leukocyte count was noticed in group V after 30 days of treatment. After 60 days of treatment, there was no significant difference

between the groups. Variability in differential leukocyte count was noticed only up to half way of experiment.

From the study it can be inferred that both *Emblica officinalis* and curcumin are potent antioxidants and that their antioxidant property is comparable.