HYPOLIPIDAEMIC EFFECT OF *Allium sativum* AND *Emblica officinalis* IN RABBITS

By

K. P. MINI

THESIS

Submitted in partial fulfilment of the
requirement for the degree

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Faculty of Veterinary and Animal Sciences
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Department of Pharmacology and Toxicology
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Mannuthy Thrissur

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DECLARATION

I hereby declare that this thesis entitled **Hypolipidaemic effect of *Allium sativum* and *Emblica officinalis* in rabbits** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship, fellowship, or other similar title of any other University or Society

Mannuthy

K. P. MINI

CERTIFICATE

Certified that this thesis, entitled **Hypolipidaemic effect of *Allium sativum* and *Emblica officinalis* in rabbits'** is a record of research work done independently by **Dr. K. P. Muni** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her

Mannuthy
20 2 13

Dr. N. GOPAKUMAR
(Chairman, Advisory Committee)
Associate Professor
Dept of Pharmacology and Toxicology
College of Veterinary and Animal Sciences, Mannuthy

Dedicated to
my beloved parents

CERTIFICATE

We, the undersigned members of the Advisory Committee of Dr, K.P. Mini, a candidate for the degree of Master of Veterinary Science in Pharmacology and Toxicology, agree that the thesis entitled **Hypolipidaemic effect of Allium sativum and Emblica officinalis in rabbits** may be submitted by Dr K P Mini in partial fulfilment of the requirement for the degree

20/2/93

Dr N Gopakumar
Chairman, Advisory Committee
Department of Pharmacology and Toxicology
College of Veterinary and Animal Sciences
Mannuthy

20/2/93

Dr M. K. Rajagopalan
Professor & Head
Department of Pharmacology
and Toxicology

Dr Jacob V Cheeran
Senior Scientist
Department of Pharmacology
and Toxicology

Dr K. P Surendranathan
Professor
Department of Physiology
and Biochemistry

External Examiner 20/2/93

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Introduction

INTRODUCTION

There has been an increasing recognition in recent years that diet plays a major role in causing disease particularly diseases of Western Civilization Nutrition is presently regarded as a major factor in the development of both cancer and cardiovascular disease This notion is well supported by the finding that there is a strong association between dietary intake of cholesterol fats development of hypercholesterolaemia and risk of coronary heart disease Cholesterol is found to be a pivotal element in the etiology of cardiovascular disease Risl of developing the coronary heart disease increases exponentially with the serum cholesterol concentration For every 1-mg/dl reduction in plasma cholesterol, one per cent risk of coronary heart disease is reduced Due to the putative linkage of serum cholesterol level to incidence of atherosclerosis, dietary manipulation of serum cholesterol has become a public health issue Cholesterol and its esters are among the many substances contributing to the plaque on the inner valves of arteries in atherosclerosis The cholesterol in the plaques has

been shown to originate from cholesterol carried in the blood stream by the low density lipoproteins (LDL) Clinical concern arises because an elevated concentration of such lipoproteins can accelerate the development of atherosclerosis with its dual sequelae of thrombosis and infarction

Usually only total plasma cholesterol is measured to assess the hypercholesterolaemic status, but it is often useful to determine its distribution in the different lipoprotein fractions Plasma cholesterol and triglycerides are transported in lipoproteins, which are large globular particles that contain an inner core of cholesteryl esters or triglycerides surrounded by a polar coat of phospholipids, free cholesterol and apoproteins Some lipoproteins have shown association with cardiovascular disease while others do not It is the LDL and HDL (High density lipoproteins) which are thought to be of particular importance in atherosclerosis and coronary heart disease. When there is a high concentration of LDL, with a deficiency in LDL receptors, cholesterol accumulation can occur The HDL fraction on the other hand, is a scavenger lipoprotein responsible for removal of excess cholesterol from tissues Thus the LDL/HDL ratio is a convenient method of assessing the

extent of atherogenicity of an individuals s plasma lipoproteins (Thomas, 1988)

A more potent dietary influence on blood cholesterol concentration is the relative proportion of polyunsaturated (PS) to saturated (S) fatty acids in the diet Experimentally it has been shown that saturated fatty acids are about twice potent in raising the blood cholesterol concentration as polyunsaturated fatty acids are in lowering it A ratio of PS/S fatty acids of about 0 5 stabilizes blood cholesterol

Drug therapy is indicated in patients who have failed to achieve lower serum levels of cholesterol in spite of diet and life style modifications, and here the choice is between a drug that lowers cholesterol and one that lowers triglycerides

One of the commonly used category of hypo cholesterolaemic drug is, bile acid sequestrants namely Cholestyramine and Colestipol

Another group of drug is the aryloxyisobutyric acid derivatives such as Clofibrate, Gemfibrozil Fenofibrate and Bizafibrate Clofibrate was widely used in the treatment of hypertriglyceridaemia Later its use

became increasingly circumscribed because it has been proven to increase death rates Gemfibrozil which is related to Clofibrate chemically and therapeutically is the currently used drug It effectively lowers plasma triglycerides and reduces very low density lipoproteins (VLDL) and apoprotein B production in the liver

Nicotinic acid is also used as a hypo-lipoproteinaemic agent which lowers VLDL and LDL concentrations It also reduces triglycerides (Thomas, 1991)

Since time immemorial herbs have been an integral part of the health care Research on medicinal plants is an important facet of biomedical research in India because the country has an estimated number of 20,0000 plant species of which 2,500 are of medicinal value (Vohora, 1989) Compared to synthetic drugs herbal drugs are less expensive and easily available In the case of synthetic drugs, their adverse side effects and occassionally toxicities overshadow their potency and thereby limit their usage in unhealthy conditions Hence it is particularly appropriate at the present moment when the pharmaceutical companies of the world are emitting an unceasing flow of new synthetic drugs, that attention should be turned to the possible remedies that may be found among indigenous herbs of this country

Review of Literature

REVIEW OF LITERATURE

2.1 Plants in general

Attempts were made to study the hypolipidaemic activity of several plants in general This included plants like Commiphora mukul (guggulu) Phaseolus mungo, Phaseolus vulgaris, Helenium amaram Vigna sinensis, Aloe barbedensi , Medicago sativa, Plumbago zeylanica and several others

Commiphora mukul (Gum guggulu) has been reported to possess hypocholesterolaemic and hypolipidaemic properties (Satyavathi, 1966) Gupta et al (1974) have found that ether extract of gum guggulu could prevent the hyperlipidaemia induced in chicks

Long term clinical studies on the hypolipidaemic effect of ether extract of gum guggulu in patients with hyperlipoproteinaemia showed that it was comparable to clofibrate in lowering serum cholesterol and triglyceride significantly (Malhotra et al , 1977)

Steroid fraction of gum guggulu decreased total cholesterol, triglyceride, phospholipids and non esterified fatty acids to a greater extent than clofibrate (Bhargava, 1984)

Satyavathi (1988) reported on the hypolipidaemic effect of gum-guggulu It was found that the drug had a profound effect in hyperlipidaemia due to disorder of lipid metabolism with special reference to atherosclerosis and obesity

The effect of gum-guggulu in hyperlipidaemic patients with special reference to High density lipoprotein cholesterol (HDL-C) was investigated by Verma and Bordia (1988) Gum-guggulu was found to decrease total cholesterol and triglyceride significantly Significant increase in HDL-C levels together with a distinct decrease in low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) was reported

Sharma (1979) evaluated the effect of the various isoflavones of some commonly used legumes (Cicer arietinum, Phaseolus aureus, Phaseolus mungo, Cajanus cajan and Cicer kabuliam on lipid levels in triton treated rats The isoflavones biochanin A, formononetin and pratensien showed hypolipidaemic activity

Hall et al (1980) noticed that Helenalin from Balduina angustifolia tenulin and aromaticin from Helenium amaram, deoxyelephantopin from Elephantopus

carolinians and Eupahyssopin from Eupatorium hyssopifolium reduced the serum cholesterol by 30 per cent and serum triglyceride by 25 per cent in mice

Dixit and Joshi (1983) studied the hypolipidaemic effect of aqueous extract of Aloe barbedensis and observed that aloe was efficient in reducing the triton induced hyperlipidaemia in Presbytis monkeys The percentage reduction in blood parameters like cholesterol triglyceride phospholipid and non esterified fatty acids was more significant with aloe when compared to clofibrate

Kedar and Chakrabarthi (1983) found that Jambolan seed when given to streptozotocin induced diabetic rabbits was capable of reducing blood sugar cholesterol free fatty acids and triglyceride level comparable to phenformin

In rats fed with Zingiberis officinale along with cholesterol added ginger restrained the increase of blood cholesterol and hepatic cholesterol levels significantly (Giri et al 1984)

Aqueous extract and residual Vigna sinensis seed meal were found to decrease serum cholesterol significantly in hypercholesterolaemic rats (Joshi et al ,1984)

Dixit and Joshi (1985) have reported the anti-atherosclerotic effects of alfalfa meal ingestion in chicks fed high cholesterol diet to be similar to that obtained with clofibrate

Administration of Khellin and Khelloside isolated from Ammi visnaga decreased low density lipoprotein cholesterol and increased high density lipoprotein cholesterol in female cynomologus monkeys Total cholesterol also reduced Very low density lipoprotein and triglyceride did not change (Stevens et al , 1985)

The effect of Phaseolus vulgaris (locust bean) gum on total cholesterol in rats has been studied by Nandy et al (1987) Hepatic and serum cholesterol in rat was reduced by 30 and 13 per cent respectively

Goswami (1988) found that ingestion of Isapgul decreased the total serum cholesterol by 9 62 per cent and triglyceride by 8 6 per cent

Hypolipidaemic effect of guar gum obtained from the beans of Cyamopsis tetragonoloba guar seeds and splits were evaluated for their hypolipidaemic activity in rats and rabbits by Singh and Nityanand (1988) Guar gum produced significant reduction in serum cholesterol and

triglycerides and liver lipids whereas guar seeds and splits were devoid of this activity

Sharma et al (1989) evaluated raw Trichosanthus dioica whole fruit and pulp in normal and mild diabetic human volunteers in relation to lipid profile and has been found to exert significant hypocholesterolaemic, hypotriglyceridaemic and hyperphospholipidaemic effects in both the subjects High density lipoprotein-cholesterol was increased whereas low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol were reduced

Hypolipidaemic effects of Medicago sativa seed extract was studied by Dixit and Jain (1990) in rabbits They have found out that the low density lipoprotein-cholesterol decrease was maximum when the seed extract was fed without cholesterol in hypercholesterolaemic rabbits

Srivastava and Joshi (1990) observed that whole seed diet of Phaseolus mungo (black gram) produced a significant reduction in blood glucose serum total lipids, triglyceride and esterified fraction of cholesterol in alloxan induced diabetic guinea pigs Total cholesterol to phospholipid ratio decreased in both the normal and diabetic groups which indicated the antiatherogenic nature of black gram

Rao et al (1992) conducted a study on the anti hypercholesterolaemic activity of a mannogalactan obtained from the seeds of Strychnos potatorum in diet induced hypercholesterolaemic rats and found that its effect was 1/10th of clofibrate in terms of triglyceride reduction

Plumbagin (2 methyl-5-hydroxy 1 4 naphthoquinone) isolated from the roots of Plumbago zeylanica when administered to hyperlipidaemic rabbits reduced serum cholesterol and low density lipoprotein cholesterol Treatment with plumbagin prevented the accumulation of cholesterol and triglycerides in liver and aorta and regressed atheromatous plaques of thoracic and abdominal aorta (Sharma et al 1991)

Gupta et al (1966) have reported that addition of Allium cepa (Onion) to fatty diet could protect against changes like rise in serum cholesterol and lowering of fibrinolytic activity in individuals who reacted adversely to fatty diet but had little effect in normal individuals

Lipid lowering effect of allylpropyl disulphide isolated from A cepa on long term feeding to normal rats was reported by Augusti (1974) A significant decrease in serum and liver lipids was noticed

Onion juice and essential oil of onion was found to have protective action against fat induced increase in serum cholesterol and decrease in coagulation time as well as fibrinolytic activity in ten healthy subjects (Bordia et al 1974a)

Singh and Chaturvedi (1974) carried out a study on anticoagulant and fibrinolytic effects of three drugs namely Allium cepa Allium sativum and Allium ascalonicum in male albino rabbits It was found that all the three drugs increased the whole blood coagulation time in the rabbits and was most significant with A cepa group A significant reduction in serum cholesterol level was also produced by these three drugs

Long term effect of onion on experimentally induced hypercholesterolaemia and consequently decreased fibrinolytic activity was studied in rabbits by Sharma et al (1975) It was found that onion reduced serum cholesterol and brought back the decreased fibrinolytic activity to its normal

Sharma et al (1975) found that both raw and boiled onion given along with butter fat meal significantly reduced serum cholesterol in man

2. Allium sativum (Garlic)

Thiersch (1937) reported that garlic oil inhibited the development of arteriosclerosis in rabbits fed cholesterol

The effects of the essential oil and juice of Allium sativum (garlic) on serum cholesterol plasma fibrinogen whole blood coagulation time and fibrinolytic activity in five healthy subjects was studied by Bordia and Bansal (1973) It was found that garlic had a very significant protective action against hyperlipaemia and blood coagulation changes

Crystals of s allyl cysteine sulfoxide and s methyl cysteine sulfoxide isolated from garlic depressed the increase in plasma and liver cholesterol levels of rats fed a high cholesterol diet (Itokawa et al 1973)

The effect of A sativum juice and essential oil on alimentary hyperlipaemia and blood coagulation changes induced on feeding butter was studied in ten healthy subjects Both forms of garlic were found to have significant protective action against fat induced increase in serum cholesterol and plasma fibrinogen and decrease in fibrinolytic activity as well as coagulation time (Bordia et al 1974b)

The effect of onion and garlic in experimental cholesterol induced atherosclerosis in rabbits was studied by Jain (1976) Supplementation of garlic resulted in significant lower levels of total free ester cholesterol and phospholipids but onion was not found to produce this effect

Sharma et al (1976) evaluated the effect of raw and boiled A sativum on blood cholesterol in butter fat induced lipaemia in ten healthy individuals It was found that both the forms of garlic reduced the serum cholesterol level significantly

Garlic oil was found to be very effective in experimentally induced atherosclerosis in rabbits and it also reduced serum cholesterol and triglycerides (Bordia et al 1977)

Extracts from raw or boiled garlic reduced the rise in serum cholesterol and triglycerides in rabbits fed cholesterol (Sharma et al 1977)

The effect of raw A sativum on normal blood cholesterol level in adult males was studied by Bhushan et al (1979) Blood cholesterol level decreased significantly in all the subjects of the experimental group

Sainani et al (1979) studied the effect of dietary garlic and onion on serum lipid profile in Jain community People who totally abstained from onion and garlic had significantly higher levels of serum cholesterol, triglyceride and phospholipids whereas people who took small amounts and liberal amounts of onion and garlic maintained their serum lipid at low levels

Chang and Johnson (1980) studied the effect of A sativum on lipid synthesis and observed that incorporation of ^{14}C acetate into lipids or triglycerides and free fatty acids in liver and to total lipids in serum was lower with garlic diet than with the control atherogenic diet

Ten healthy individuals were given raw A sativum and its effect on blood lipids and blood glucose was investigated by Mahanta et al (1980) At the end of one month reduction in mean values of glucose total cholesterol phospholipids triglycerides free fatty acids and total fatty acids were noticed

Sainani et al (1980) observed that garlic and onion significantly inhibited the rise in serum cholesterol and triglycerides in hypercholesterolaemic rabbits

The effect of long term use of A sativum in healthy controls and patients with ischaemic heart disease was investigated by Arora et al (1981) Garlic therapy did not cause appreciable changes in serum cholesterol triglyceride lipoprotein plasma fibrinogen and coagulation time

Baldwa et al (1981) treated several cases of hyperlipidaemia with A sativum pearls guggul and clofibrate Fifteen days of treatment with the above drugs produced a fall in serum cholesterol level but the decrease was significant with garlic and guggul only after one month

The effect of giving garlic oil to normal as well as patients with Coronary Heart Disease (CHD) was studied by Bordia (1981) Serum cholesterol and triglycerides were found to decrease in both normal as well as CHD patients

Bordia et al (1982) reported that administration of A sativum oil checked the rise in serum cholesterol and serum triglycerides produced by fatty diet in man Fibrinolytic activity also increased in the drug treated group

Sainami et al (1982) reported the effectiveness of A sativum pearls in the treatment of hyperlipidaemia in man Serum cholesterol level was brought down to normal range in most of the patients

Hypocholesterolaemic activity of A sativum have been reviewed by Kamanna and Chandrasekhara (1983) A sativum powder was found to counteract the rise in serum cholesterol and low density lipoprotein in rats fed a cholesterol containing diet

Effect of A sativum on the lipid composition and lipid biosynthesis in the tissues of rabbits during experimental atherosclerosis was studied by Mirhadi et al (1983) It was observed that the atherogenic diet containing garlic decreased the biosynthesis of fatty acids

Bakhsh and Chughtai (1985) observed that inclusion of onion and A sativum in the diet of rats decreased the weight gain serum choesterol and liver cholesterol

Hypolipidaemic effect of a fraction derived from garlic has been reported by Dixit and Sinha (1985) in albino rats house rats and gerbils Significant depletion of total cholesterol phospholipids triglycerides and non esterified fatty acids occurred in garlic fed animals

Farva et al (1986) found that daily administration of garlic oil to diabetic rats decreased the raised blood sugar cholesterol triglycerides in serum , liver and total lipid and proteins in liver very significantly

The mechanism of hypolipidaemic effect of garlic oil extract in rats fed high sucrose and alcohol diets was studied by Adoga (1987)

Arora et al (1987) showed that Lipotab a poly pharmaceutical herbal drug (a combination of three plants viz Nepeta hindostana Allium sativum and Curcuma longa) could produce a mean reduction in cholesterol and triglyceride levels in hyperlipidaemic patients

Hypolipidaemic effect of garlic extract mixed with three per cent ethanol was reported in rats fed sucrose high fat diet by Ikpeazu et al (1987)

The protective effect of garlic in preventing aortic atherosclerosis in sheep was shown by Mirhadi and Singh (1987)

Paul and Prasad (1990) have given some evidence on the hypocholesterolaemic and anti atherosclerotic effects of garlic in goats They found that the increase in the concentration of serum lipid and total cholesterol was less significant in those goats which received garlic

along with high cholesterol diet Atherosclerotic lesions were also minimal in the garlic treated group

Mirhadi *et al* (1991) have found that supplementation of *A sativum* to cholesterol rich diet reduced the severity and extent of atherosclerosis in rabbits *A sativum* also decreased the plasma cholesterol considerably

2 *Emblica officinalis* (Indian gooseberry , Amla)

Quadry *et al* (1962) have reported that fruit pulp of amla is a rich source of ascorbic acid and that it is the most stable form among naturally occurring sources of ascorbic acid

A relationship between vitamin C and blood lipids was corroborated by Bates *et al* (1977) It was found that vitamin C increased plasma HDL cholesterol which has a protective role against hyperlipidaemia

Vijayakumar and Vasudevan (1980) found out from their study that administration of ascorbic acid resulted in a significant reduction of serum cholesterol levels both in normal and diabetic patients

The effect of Chyavanprash the principal constituent of which is amla in the reduction of serum

triglyceride significantly was reported by Bordia et al (1981) Serum cholesterol was also reduced

The effect of amla fruit and vitamin C on cholesterol induced hypercholesterolaemia and atherosclerosis was studied by Thakur and Mandal (1984) Both reduced serum cholesterol levels significantly Aortic sudanophilia was minimal in the group fed with cholesterol and amla fruit

Bordia et al (1985) have reported that amla juice amla pulp and vitamin C could prevent the increase in serum cholesterol serum triglyceride and experimental atheroma in cholesterol fed rabbits Amla juice and pulp were found to be significantly superior than Vitamin C

Shebib et al (1986) found that reversal and regression of experimental atherosclerosis and hypercholesterolaemia in rabbits was possible by administration of Vitamin C Vitamin C induced a significant reduction in the total serum cholesterol level

The development of experimental atherosclerosis with high cholesterol diet was superimposed by chronic ascorbic acid deficiency in guinea pigs Chronic ascorbic acid deficiency resulted in a significant increase in serum and hepatic lipids (Satinder et al 1987)

The effect of administration of vitamin C to guinea pigs fed on a high cholesterol diet was studied by Sharma et al (1988) It was observed that vitamin C exerted a significant hypolipidaemic effect by reducing all the elevated lipid fractions in the hyper cholesterolaemic guinea pigs

2 Gemfibrozil

Brown (1987) reported that since 1962 a series of aryloxyisobutyric acids were found to be effective in reducing plasma concentrations of triglyceride and cholesterol The first compound used in this group was clofibrate Subsequently its use became increasingly circumscribed because it has not been proven to be effective for the prevention of atherosclerosis Since the mid 1970 s Gemfibrozil which is chemically and therapeutically related to clofibrate has been used extensively in the United States and Europe It proved to be less toxic and more effective for the treatment of hypertriglyceridaemia and hypercholesterolaemia than clofibrate

The mechanism of action of fibric acids remains controversial Their primary effect is to increase the

activity of lipoprotein lipase which in turn promotes the catabolism of the triglyceride rich lipoproteins VLDL and IDL (Intermediate density lipoprotein) Gemfibrozil is currently the drug of choice for patients with hyper triglyceridaemia with or without hypercholesterolaemia

Present Investigation

PRESENT INVESTIGATION

Eversince hyperlipidaemia was identified as an important risk factor in the development of atherosclerosis leading to Ischaemic Heart Disease (IHD) and other complications there has been a continuous search for agents which can lower the serum lipids

Scrutiny of the literature would reveal that several chemical agents having hypolipidaemic effect have been identified and are extensively used clinically But information available on the efficacy of herbal agents to bring down the level of lipids including cholesterol is far from complete Bulk of the findings on these seem to be exploratory in nature more so with Allium sativum and Emblica officinalis As such further studies in this area are definite to yield rich dividents Therefore it was thought worthwhile to probe into the effects of these chosen herbal agents namely A sativum and E officinalis in rabbits and assess their efficacy in comparison with Gemfibrozil, an established chemical drug in this field Feasible way to demonstrate the hypolipidaemic effect is to try these agents in animals with experimentally induced hyperlipidaemia

The present study is done in rabbits with a view to gather details on the development of hypercholesterolaemia with dietary supplementation of cholesterol and fat Hypolipidaemic effect of the preparations tested were aqueous extract of A sativum and aqueous extract as well as pulverised form of E officinalis

The main objectives of study are

1 To detect an economic and potent indigenous hypo lipidaemic agent

2 To compare the efficacy of the indigenous agents with a known hypolipidaemic drug namely Gemfibrozil

Materials and Methods

MATERIALS AND METHODS

4 1 Pattern of the Experiment

The experiment was carried out in rabbits in two stages The first stage was to induce hyper cholesterolaemia in all the rabbits The second part of the study was to assess the hypolipidaemic effect of aqueous extract of Allium sativum and fruit pulp as well as aqueous extract of Emblica officinalis in hyper cholesterolaemic rabbits

Stage I

Thirty Newzealand White adult male rabbits were procured from Small An r al Breeding Station College of Veterinary and Animal Sciences Mannuthy The average body weight of the animals were 1 5 kg They were housed in cages designed for the purpose

The rabbits were given feed with the following composition

Bengal gram	50 per cent
Groundnut cake	15
Wheat	10
Rice polish	23
*Supplevite (M)	1 5
Salt	0 5

* Supplevite M Sarabhai Chemicals BARODA

Each rabbit received 200 g mixed feed per day To render them hypercholesterolaemic the ration was supplemented daily with 100mg *Cholesterol and 8 g Vanaspathi *(DALDA) per rabbit They were maintained on this ration under ideal conditions for 60 days when they developed hypercholesterolaemia as evidenced by blood analysis

Stage II

The rabbits rendered hypercholesterolaemic were subjected to experimentation to assess the hypolipidaemic effect of herbal agents (Allium sativum and Emblica officinalis) in comparison with Gemfibrozil For this purpose periodical blood analysis for cholesterol and triglycerides were conducted at regular intervals and at the end of the period histopathological studies were undertaken to demonstrate structural changes in tissues

4 2 Preparation of herbal agents

A sativum bulbs and fruits of E officinalis were procured from the local market

Allium sativum

The outer scaly leaves of garlic were removed and dried for a short time Two grams of this dried garlic

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* DALDA - Manufactured by Lipton India Ltd CALCUTTA

* Cholesterol crystalline powder Sisco Research Laboratories Pvt Ltd BOMBAY

Group III	Fruit pulp of *E officinalis* 1 g/kg body weight orally
Group IV	Aqueous extract of *E officinalis* 1 g fruit pulp in 10 ml of water/kg body weight orally
Group V	Gemfibrozil (LOPID) 120 mg/kg orally

The drugs were administered continuously for 75 days All the drug preparations except Gemfibrozil were administered using stomach tube once daily The diet supplemented with cholesterol and vanaspathi was continued throughout the period of study

4 4 Collection of Plasma

Blood was collected from the central ear vein of each rabbit with addition of anticoagulant ethylene diamine tetra acetic acid (EDTA) at the rate of 1 mg/ml The blood was centrifuged at an RCF of 2260 G for 20 minutes and plasma was separated The plasma was stored in the refrigerator at 4°C

---

* Gemfibrozil (LOPID) Parke-Davis HYDERABAD

Each capsule contains Gemfibrozil 300 mg

Plasma cholesterol and triglycerides were estimated initially and then fortnightly to determine whether the animals became hypercholesterolaemic or not The estimations were carried out during the course of administration of drugs in order to detect the changes produced by the drugs on the above parameters

Cholesterol was estimated according to the method described by Zak (1957)

Triglyceride level in plasma was estimated by Trigazyme TM - enzymatic kit (GPO PAP method) (Werner et al 1981)

Principle

Triglycerides are hydrolysed by lipase and liberated glycerol is phosphorylated with the help of glycerol kinase in presence of ATP to glycerol 3 phosphate Glycerol 3 phosphate is then oxidized in presence of glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate and hydrogen peroxide Phenol and 4 amino antipyrine then combines with hydrogen peroxide by oxidative condensation in presence of peroxidase to produce red coloured quinoneimine which shows

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* Kit obtained from Ortho Diagnostic Systems

BOMBAY

a maximum absorbance at 500 nm (500-530 nm) filter The intensity of the colour thus produced is directly proportional to triglyceride concentration

Reaction

Triglycerides $\xrightarrow{\text{lipase}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3 Phosphate+ADP

Glycerol 3 phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxy acetone phosphate + H_2O_2

H_2O_2 + 4 Aminoantipyrine + Phenol $\xrightarrow{\text{peroxidase}}$ Quinoneimine + H_2O

Readings were taken in Spectrophotometer at wave length 530 nm

The data obtained were analysed statistically using Students t test (Das and Giri, 1979)

4.5 Histopathological studies

In order to conclude the findings of the study at the end of the period two animals from each group were selected at random and sacrificed by cervical dislocation

Selective samples of aorta and liver were collected and preserved in neutral buffered formalin

Tıssue collected was processed by routıne paraffın embeddıng technique The sectıons were staıned usıng Haematoxylın and Eosın (Luna, 1968) and examıned under lıght mıcroscope Changes notıced were recorded on photomıcrographs

Results

RESULTS

The values obtained for cholesterol and triglyceride in plasma of rabbits in various groups during the course of the experiment are presented in Tables 1 10 Details of statistical analysis are given in Tables 11 20 and Figures 1 and 2 Observations made in the histopathological studies have been taken on photomicrographs and are presented in Plates 1 to 8

Table 1 Plasma Cholesterol level (mg/100 ml) of rabbits in Group I*

	Pre Treatment Period			Treatment Period				
S No	0-day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	70 00	98 00	125 00	142 85	145 16	150 16	163 26	170 91
2	55 50	132 14	150 00	238 77	245 16	251 61	280 12	280 00
3	52 50	64 10	138 88	178 58	184 54	190 32	193 46	210 40
4	45 00	50 00	100 00	103 57	163 46	190 01	230 46	242 15
5	50 00	74 35	85 71	138 88	164 52	213 40	242 00	263 00
Mean ±	54 60±	83 71±	119 92±	160 52±	180 57±	199 10±	221 86±	233 29±
S E	0 45	14 45	11 96	22 8	17 30	16 60	20 14	19 44

*Group I - Control group

Table 2 Plasma Triglyceride level (mg/100 ml) of rabbits in Group I *

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	50 00	90 00	170 00	210 00	243 75	300 00	343 21	360 10
2	107 00	100 00	229 99	260 00	276 47	293 04	310 10	327 04
3	46 87	80 00	90 00	232 40	235 29	254 54	270 00	279 50
4	46 87	53 00	55 00	175 50	222 72	226 10	264 00	293 00
5	50 00	65 62	74 35	200 00	232 63	279 00	304 00	354 00
Mean±	60 14±	77 72±	123 86±	215 58±	242 17±	270 53±	298 26±	322 72±
S E	11 73	8 42	32 96	14 30	9 20	13 55	14 43	16 04

Group 1 Control group

Table 3 Plasma Cholesterol level (mg/100 ml) of rabbits in Group II*

S No	Pre Treatment Period			Treatment Period				
	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	75 00	96 00	138 80	106 80	89 28	63 47	40.01	37 14
2	30 00	60 01	119 44	78 84	53 24	40 01	31 51	22 91
3	48 75	48 00	147 36	100 44	78 71	61 49	44 23	22 50
4	56 25	70 00	228 70	169 90	114 00	84 00	80 01	56 66
5	76 25	76 00	283 87	192 30	142 85	80 95	62 00	52 01
6	72 00	70 00	178 66	78 84	68 41	--	-	-
Mean ±	59 70±	70 00±	182 81±	124 52±	91 07±	65 98±	51 55±	38 24±
S E	7 46	6 57	25 52	21 50	13 30	7 92	8 70	7 13

* Group II Treated with aqueous extract of Allium sativum

Table 4 Plasma triglyceride level (mg/100 ml) of rabbits in Group II *

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	157 14	260 00	302 80	238 80	203 76	152 14	105 22	93 74
2	80 00	90 00	170 00	150 00	98 76	72 36	57 36	52 94
3	160 00	160 71	200 00	153 08	119 00	102 27	80 54	44 11
4	142 86	185 70	249 90	191 10	162 00	126 23	93 73	70 58
5	142 86	196 00	499 99	290 07	158 82	127 00	90 00	70 02
6	85 71	117 10	218 18	108 66	87 14			
Mean ±	128 09±	168 25±	273 47±	188 61±	137 24±	116 00±	85 37±	66 27±
S E	14 61	24 53	48 96	27 06	18 09	13 49	8 06	8 55

* Group II Treated with aqueous extract of Allium sativum

Table 5 Plasma Cholesterol level (mg/100 ml) of rabbits in Group III *

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	58 92	77 12	161 29	140 23	90 00	32 00	29 01	27 00
2	46 15	48 75	85 55	61 32	45 32	37 09	35 00	32 10
3	44 40	74 00	161 29	142 85	138 28	102 04	67 04	61 19
4	32 20	40 00	139 47	98 00	73 00	64 28	37 00	33 03
5	33 30	50 00	86 11	61 11	48 07	45 01	40 07	31 05
6	72 91	74 00	79 94	63 00	60 00	49 03	33 04	31 56
Mean±	47 98±	60 64±	118 94±	94 41±	75 77±	54 92±	40 19±	35 98±
S E	6 38	6 60	16 05	15 97	14 21	10 45	5 58	5 11

*Group III Treated with fruit pulp of Emblica officinalis

Table 6 Plasma Triglyceride level (mg/100 ml) of rabbits in Group III *

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	96 00	103 12	360 00	230 00	130 00	44 11	36 01	32 00
2	125 71	125 00	160 00	142 70	109 09	66 04	57 48	46 07
3	142 90	142 00	399 99	218 18	152 94	150 00	114 60	99 03
4	100 00	107 00	231 81	200 00	123 40	114 68	65 62	54 54
5	50 00	100 00	250 00	195 45	90 10	64 10	60 02	47 91
6	126 00	126 00	210 00	200 00	153 22	99 23	77 84	53 71
Mean±	106 76±	117 18±	268 63±	197 72±	126 45±	89 69±	68 62±	58 54±
S E	13 44	6 71	37 66	12 25	10 10	5 16	10 17	9 30

*Group III - Treated with fruit pulp of Emblica officinalis

Table 7 Plasma Cholesterol level (mg/100 ml) of rabbits in Group IV*

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	56 25	60 00	83 92	65 27	40 74	36 53	28 57	25 07
2	32 25	130 00	159 29	153 22	135 71	73 6	32 50	32 05
3	70 00	89 50	134 21	116 12	96 42	70 00	43 29	23 69
4	52 50	62 00	127 77	101 00	80 00	46 42	37 34	30 01
5	40 00	50 00	131 94	96 42	93 54	60 01	57 50	31 07
6	64 00	89 74	189 74	80 00	60 00	35 80	--	-
Mean±	52 50±	82 20±	137 81±	102 00±	84 40±	53 70±	39 84±	28 38±
S E	5 83	11 98	14 39	12 50	13 39	6 74	5 06	1 68

* Group IV - Treated with aqueous extract of Emblica officinalis

Table 8 Plasma Triglyceride level (mg/100 ml) of rabbits in Group IV*

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	114 28	163 00	179 00	139 99	109 09	60 03	44 11	38 04
2	99 29	180 00	390 00	207 14	163 63	101 38	44 11	36 41
3	80 00	100 00	189 99	153 81	113 81	87 39	60 09	40 00
4	71 43	80 00	150 00	100 00	79 41	45 71	39 46	20 00
5	80 00	80 00	349 90	230 00	154 54	139 9	129 86	82 09
6	117 10	110 00	300 00	115 90	85 62	60 82		
Mean±	93 80±	118 83±	251 77±	157 80±	117 68±	82 53±	63 52±	43 30±
S E	7 92	17 45	40 89	20 88	14 2	14 14	16 99	10 35

Group IV - Treated with aqueous extract of Emblica officinalis

Table 9 Plasma Cholesterol level (mg/100 ml) of rabbits in Group V*

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	70 00	70 00	83 87	63 7	51 71	40 01	38 93	29 04
2	70 00	79 00	228 00	165 22	131 22	72 12	43 12	32 12
3	56 25	148 00	178 00	148 04	107 04	61 53	40 81	33 09
4	62 50	66 00	83 33	74 31	66 21	50 09	44 25	29 50
5	75 00	70 00	195 55					
6	51 25	70 00	161 77	--	-	--		-
Mean±	64 16±	83 83±	143 46±	112 82±	89 04±	55 93±	41 77±	30 93±
S E	3 73	12 95	24 34	25 63	18 29	6 95	1 18	0 98

* Group V Treated with Gemfibrozil

Table 10 Plasma triglyceride level (mg/100 ml) of rabbits in Group V *

	Pre Treatment Period			Treatment Period				
S No	0 day	30th day	60th day	15th day	30th day	45th day	60th day	75th day
1	200 00	214 27	220 00	177 50	139 43	123 32	83 41	61 76
2	114 30	200 00	531 00	390 00	276 41	153 21	104 25	47 54
3	53 00	183 00	219 99	160 00	134 00	122 72	74 21	52 94
4	85 71	160 00	190 00	163 54	109 09	86 09	63 04	52 04
5	257 14	300 00	369 99	--	--		--	-
6	171 43	140 0	219 77	--	--	--	--	-
Mean ±	130 26±	199 54±	290 24±	222 76±	164 73±	121 33±	81 22±	53 57±
S E	43 55	22 87	54 52	55 52	37 80	13 73	8 73	2 97

* Group V-Treated with Gemfibrozil

Table No 11 CH 15 days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	15712 44	14275 63	529 37
Error	22	51888 19	40984 47	37193 25
Total	26	67600 63	55260 10	37722 63

** F 7 29 F4 21 1° = 4 37 CH = Cholesterol

Table No 12 CH 30 days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	15712 44	38229 72	7344 09
Error	22	51888 19	26762 00	27689 56
Total	26	67600 63	64991 72	20345 47

** F 20 54

Table No 13 CH 45 days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	14271 25	81582 56	11799 69
Error	21	51867 53	11991 91	10745 44
Total	25	66138 78	93574 47	1054 25

**F 42 90

Table No 14 CH 60 days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	14902 63	128057 20	13484 66
Error	20	48631 69	11083 91	5968 72
Total	24	63534 32	139141 10	7515 94

**F 58 69

Table No 15 CH 75 days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	14902 63	159749 50	16746 16
Error	20	48631 69	9422 88	4983 72
Total	24	63534 32	169172 30	11762 44

** F - 84 25

Table No 16 TR 15 days

ANOVA Tables

Source	DF	SSX	SSY	SPXY
Treats	4	91068 88	13753 13	11477 50
Error	22	264190 90	81144 50	127376 90
Total	26	355259 80	94897 62	115899 40

** F -9 94 TR = Triglyceride

Table No 17 TR 30 days
ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	91068 88	53481 00	61455 88
Error	22	264190 90	38426 75	74929 38
Total	26	355259 80	91907 75	13473 50

** F = 22 69

Table No 18 TR 45 days
ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	94038 00	123099 40	99397 62
Error	21	260521 40	23213 44	44331 75
Total	25	354559 40	146312 90	55065 88

** F = 38 96

Table No 19 TR 60 days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	92846 12	202646 70	131084 40
Error	20	258583 60	15584 38	30633 81
Total	24	351429 80	218231 10	100450 60

** F 70 55

Table No 20 TR 75 Days

ANOVA Table

Source	DF	SSX	SSY	SPXY
Treats	4	92846 12	288526 90	157763 00
Error	20	258583 60	11439 13	17564 44
Total	24	351429 80	299966 00	140199 10

** F 108 38

Table No 21 Plasma Cholesterol level mean values (mg/100 ml)

Duration	Control	Aq Ex A s	Pulv E o	Aq Ex E o	Gemfibrozil
0 day	119 92	182 81	118 94	137 81	143 46
15 days	160 53	124 52	94 41	102 00	112 82
30 days	180 57	91 07	75 77	84 40	89 04
45 days	199 10	65 98	54 92	53 70	55 93
60 days	221 86	51 55	40 19	39 84	41 77
75 days	233 29	38 24	35 98	28 38	30 93

Aq Ex A s — Aqueous extract of A satıvum

Pulv E o — Pulverised E officınalıs

Aq Ex E o — Aqueous extract of E officınalıs

Fig. No. 1

TOTAL PLASMA CHOLESTEROL (mg./100ml.)- COMPARATIVE EFFECT OF DIFF. TREATMENTS

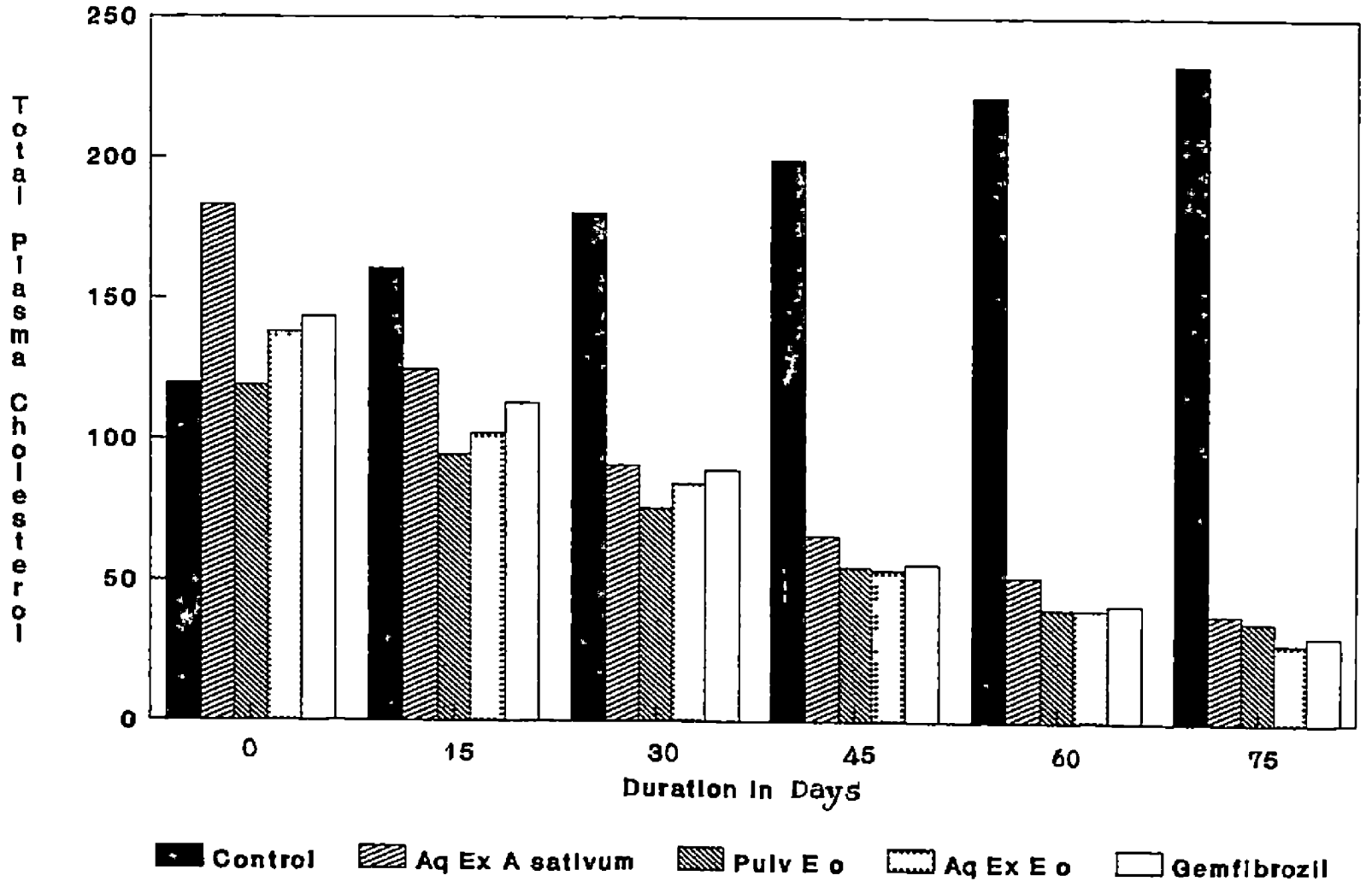


Table No 22 Triglyceride - mean values (mg/100 ml)

Duration	Control	Aq Ex A s	Pulv E o	Aq Ex E o	Gemfibrozil
0 day	123 86	273 47	268 63	251 77	290 24
15 days	215 58	188 61	197 72	157 80	222 76
30 days	242 17	137 24	126 45	117 68	164 73
45 days	270 53	116 00	89 69	82 53	121 53
60 days	298 26	85 37	68 62	63 52	81 22
75 days	322 72	66 27	55 54	43 30	53 57

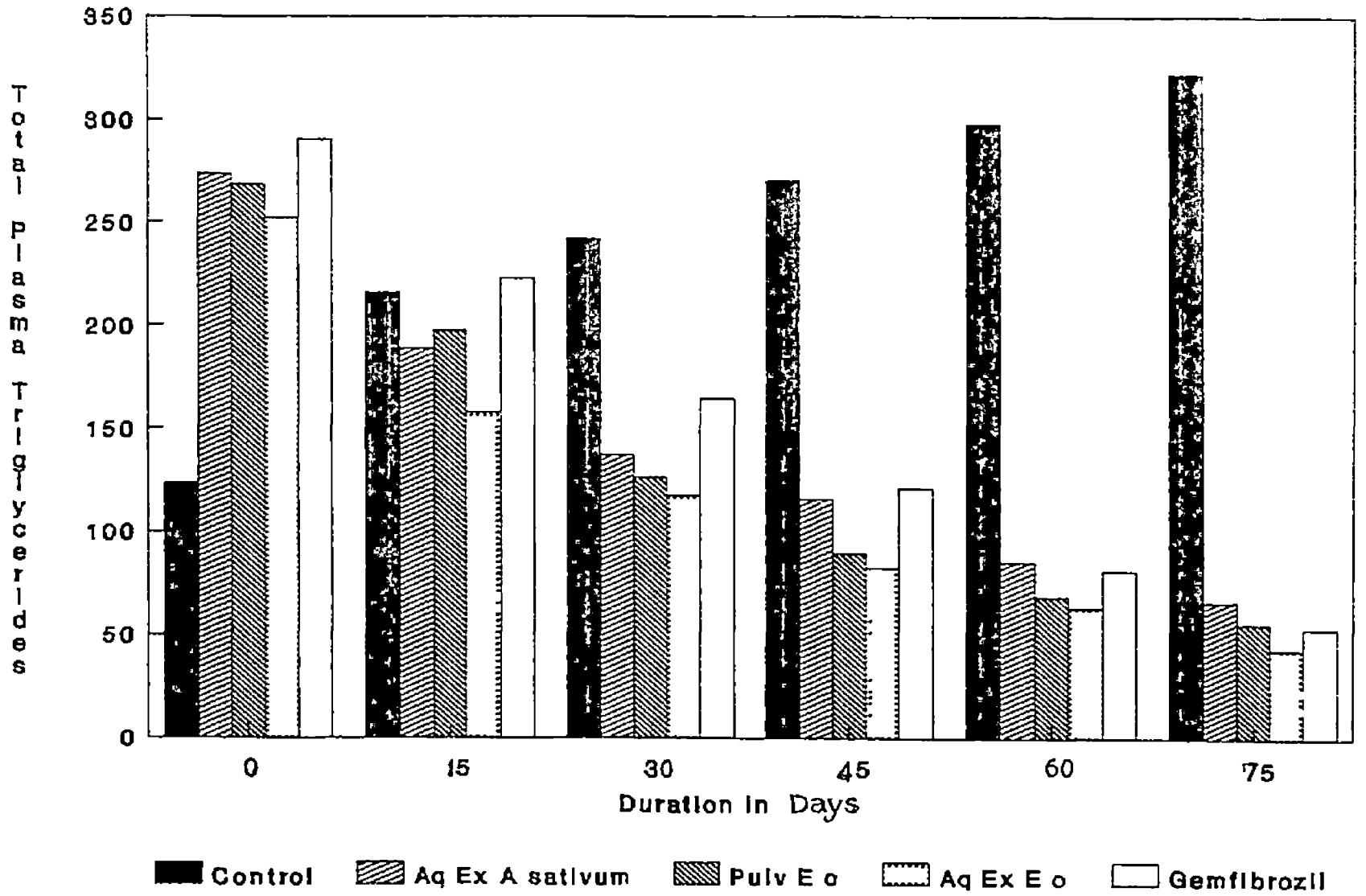
Aq Ex A s — Aqueous extract of A sativum

Pulv E o — Pulverised E officinalis

A Ex E o — Aqueous extract of E officinalis

Fig. No. 2

TOTAL PLASMA TRIGLYCERIDES (mg /100ml.)- COMPARATIVE EFFECT OF DIFF TREATMENTS



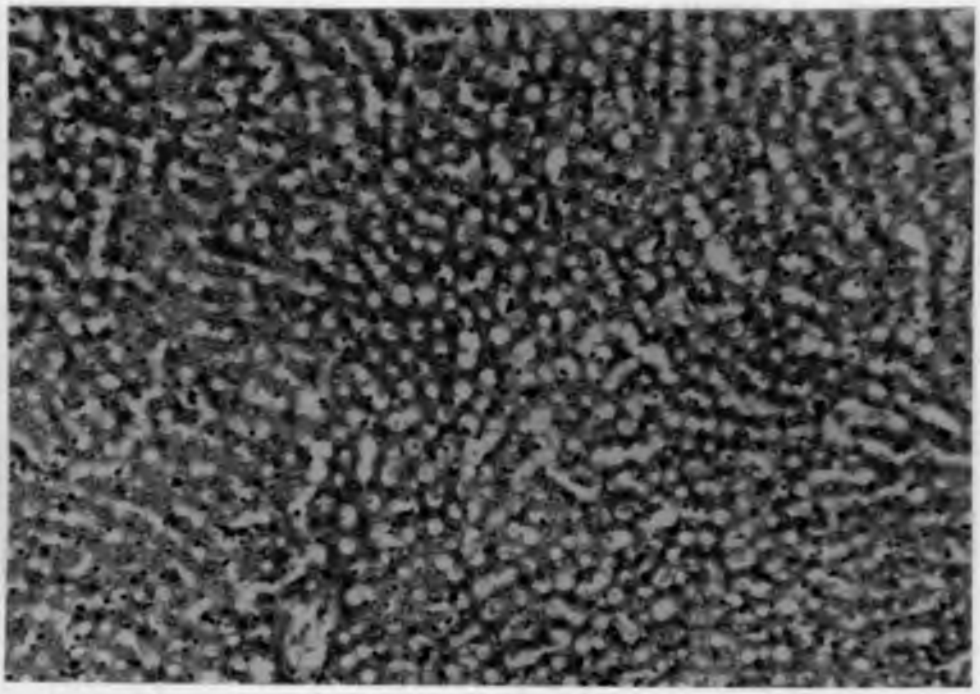


Plate No. 1

Group I - Rabbit liver - Diffused fatty changes
H & E x 250

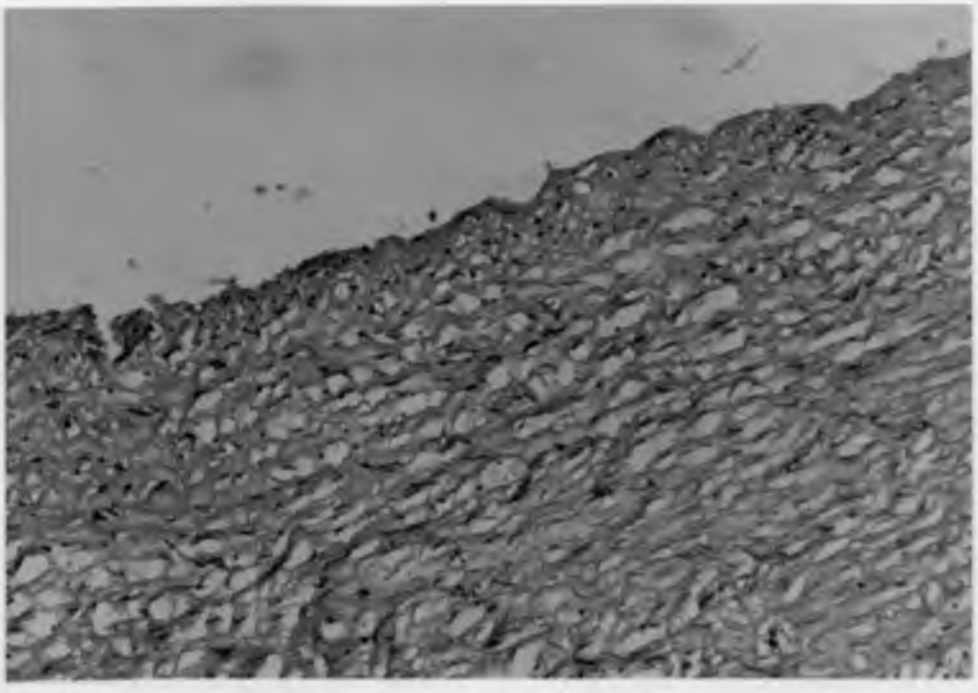


Plate No.2

Group I - Rabbit aorta - Diffused fatty changes
H & E x 250

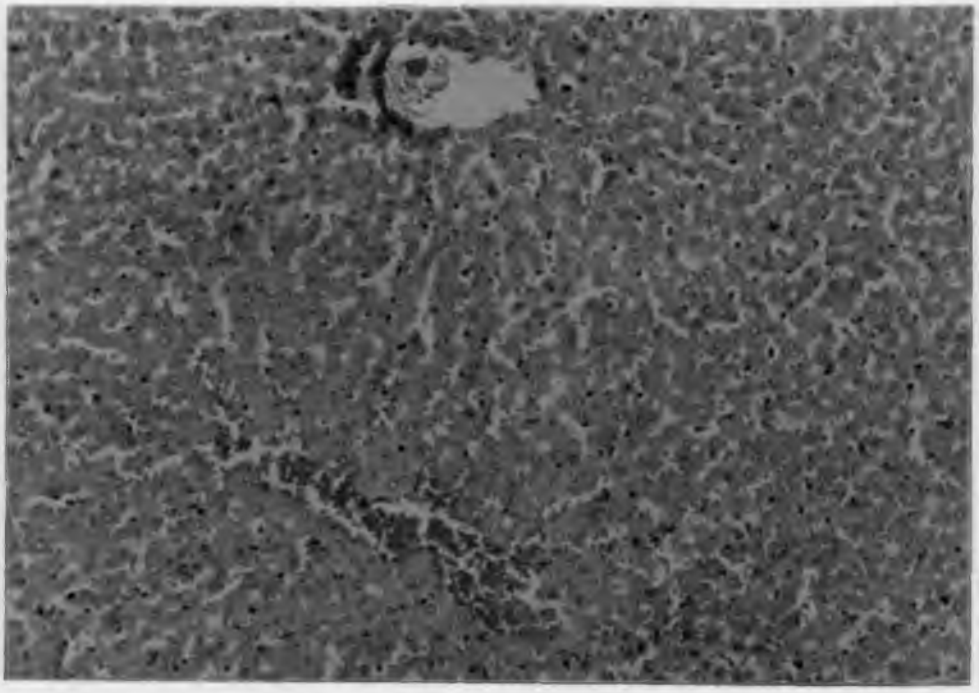


Plate No. 3
Group II - Rabbit liver - Mild degree of fatty change
H & E x 250

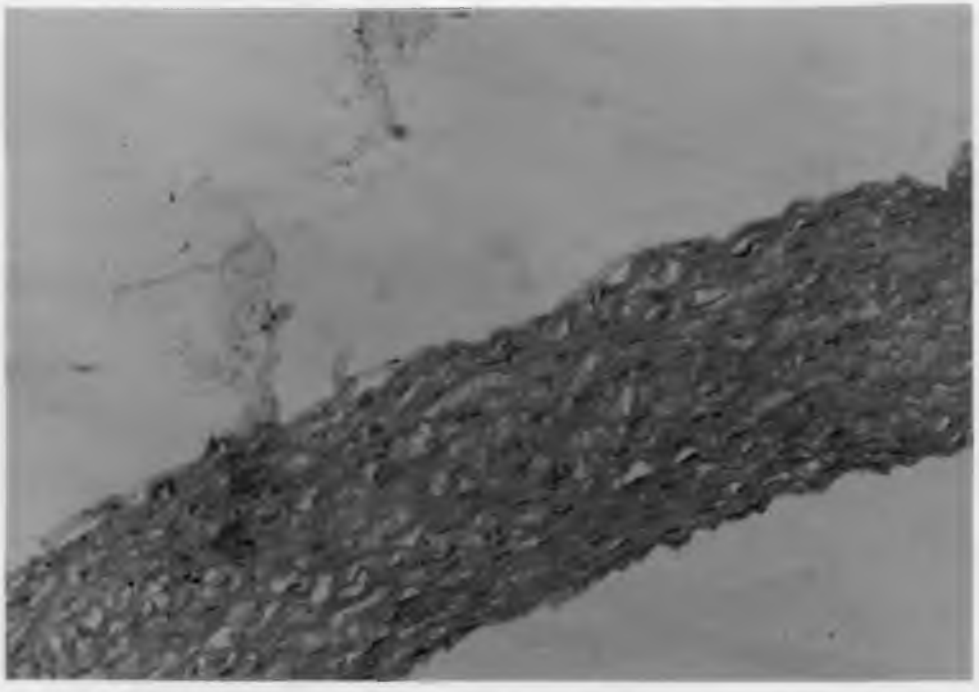


Plate No. 4
Group II - Rabbit aorta - Mild degree of fatty change

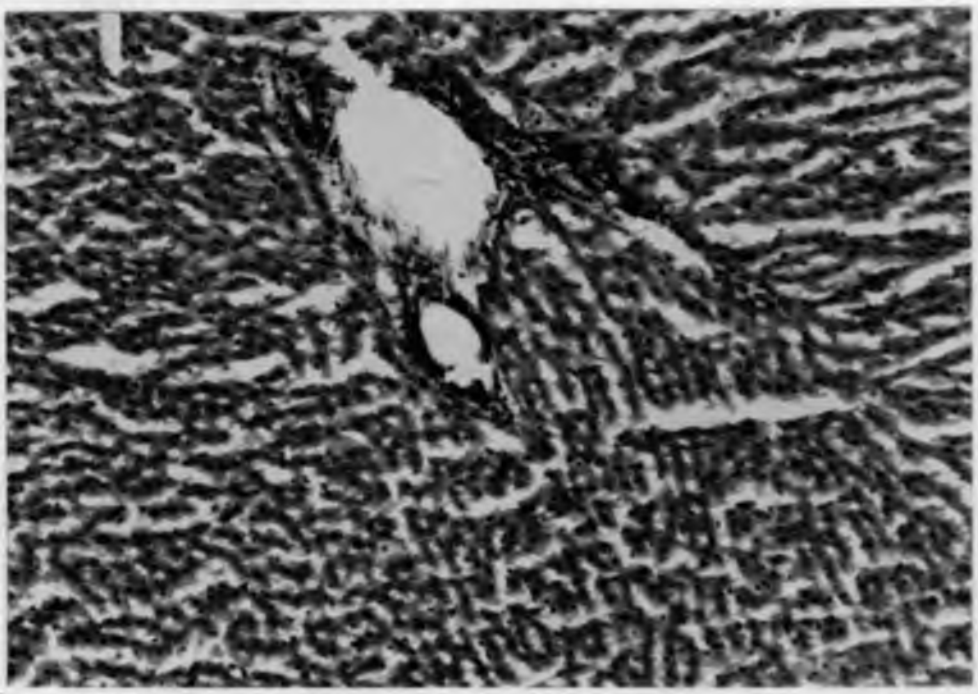


Plate No.5
Group III - Rabbit liver - Moderate degree of fatty change
H & E x 250

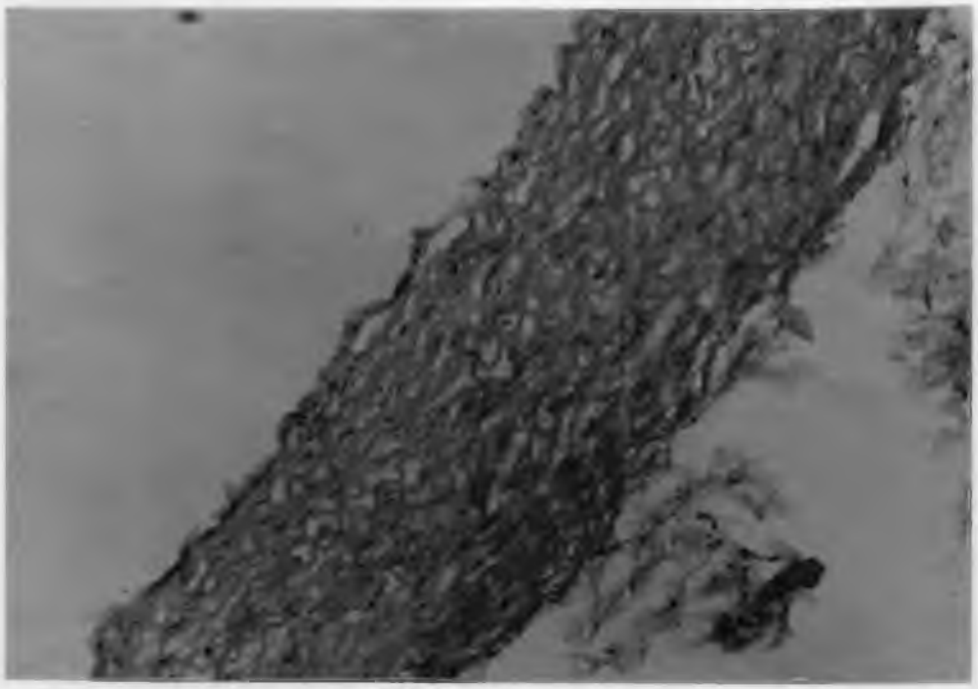


Plate No. 6
Group III- Rabbit aorta - Moderate degree of fatty change
H & E x 250

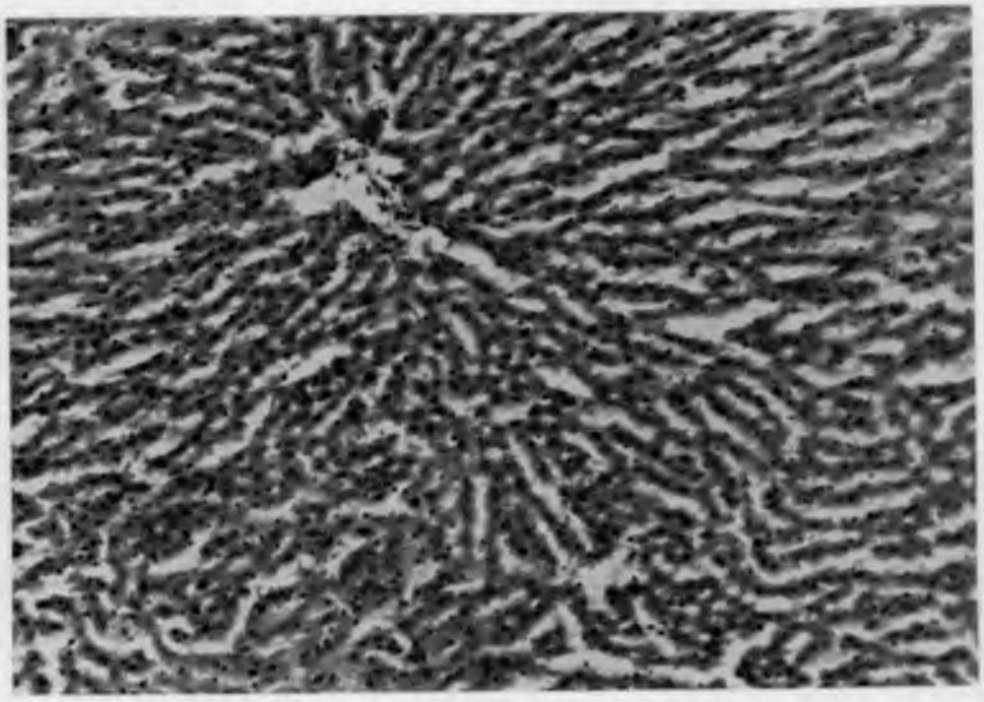


Plate No. 7

Group V - Rabbit Liver - Moderate degree of fatty change
H & E x 250

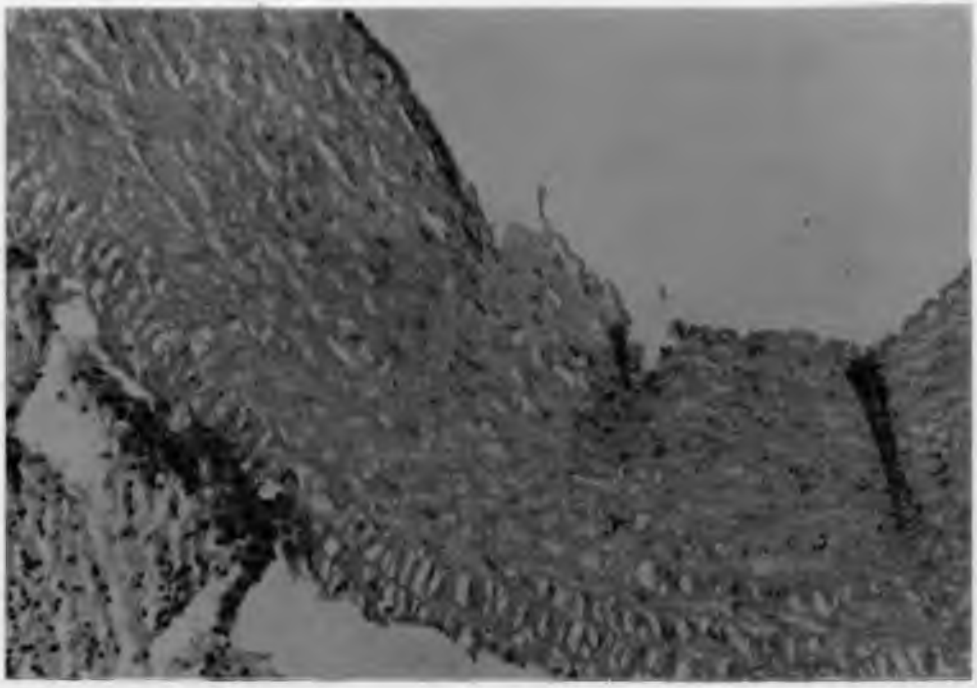


Plate No. 8

Group V - Rabbit aorta - Moderate degree of fatty change
H & E x 250

RESULTS

5 1 Group I Control

Tables 1 and 2 show the effect of atherogen c d et on plasma cholesterol and triglyceride levels (mg/1 0 ml of control group animals in the serial order

In the hypercholesterolaemic state th e a mals n the control group sho ved a mean l na c oles rol val e of 119 92 mg/100 ml and tr c c ride value of 23 86 mg/100 ml from the initial values of 54 6 a d 60 14 mg/100 ml respectively On continuatio of t e atherogenic diet throughout the per od of study t e plas a levels of above parameters continued to increase significantly As shov n in the tables the average maximum value of plasma cholesterol attained after 135 days was 233 29 mg/100 ml and that of triglycerides vas 322 72 mg/ 100 ml The increase in the plasma levels of the above parameters was significant (P<0 01) (Anova Tables 11 20)

Histopathological changes were observed in the liver and aorta of animals which belonged to the control group (Plate No 1 and 2) Moderate to diffused fatty change was noticed in the liver The hepatocytes ere filled with fat and the nucleus was pushed towards one

side The aorta on examination sho ed that the cont uit of elastic tissue vas lost due to accumulation of f t between elastic tissue The continuity of endot elial lining was lost due to fat deposition

5 2 Group II - Treated with aqueous extract of Allium sativum (Garlic)

Tables 3 and 4 show the effect of aqueo s e rac of A sativum on plasma cholesterol an tr glyceri e levels (mg/100 ml) in hypercholesterolaem ra bits

The animals of this group had on a ave age plasma cholesterol level of 182 81 m /100 triglyceride level of 273 47 ng/100 ml n t e pe cholesterolaemic state Fifteen d ys after t administration of aqueous extract of garl oral t dose rate of 2g/kg prepared in 10 ml of ater t e le e s of the above parameters were found to be 124 52 mg/100ml and 188 61 mg/100 ml respectively On f rther administration of the drug the plasma levels of cholesterol and triglyceride continued to decrease Afte 75 days of continued administration of the drug the plasm cholesterol level decreased to a minimum of 38 24 mg/100m and 66 27 mg/100 ml in the case of triglyceride T e decrease in the plasma level of above parameters were found to be significant (P<0 01) (Anova Tables 11 20)

The sections of liver and aorta taken from animals in the group which was given aqueous extract of A sativum did not show pathological changes (Plate No 3 and 4) The degree of fatty changes noticed in the liver of garlic treated animals were very mild compared to that of the control group Aorta did not reveal prominent changes due to fat accumulation Compared to the control group the lining of the wall of aorta was more intact and only very little fatty infiltration could be seen

5.3 Group III - Treated with fruit pulp of Emblica officinalis (Indian Gooseberry)

Tables 5 and 6 show the effect of fruit pulp of E officinalis on plasma cholesterol and triglyceride levels (mg/100 ml) in hypercholesterolaemic rabbits

Fifteen days of continued administration of the above drug at a dose rate of 1g/kg body weight orally to this group of animals brought down their plasma cholesterol level to 94 41 mg/100 ml from a value of 118 94 mg/100 ml which was attained in the hypercholesterolaemic state Similarly the triglyceride level was found to decrease from 268 63 to 197 72 mg/100 ml The decrease in the plasma values of the above parameters on continued administration of the drug at the same dose

for 75 days was to a minimum of 35 98 mg/100 ml of plasma cholesterol and 58 54 mg/100 ml of plasma triglyceride The decrease in the plasma values were found to be significant at one per cent level (P<0 01) (Anova Tables 11 to 20)

5.4 Group IV - Treated with aqueous extract of Emblica officinalis

The effect of administration of aqueous extract of E officinalis on plasma cholesterol and triglyceride levels (mg/100 ml) in hypercholesterolaemic rabbits is evident from Tables 7 and 8

The mean plasma cholesterol and triglyceride levels of the animals of this group in the hypercholesterolaemic state were 137 81 and 251 77 mg/100 ml respectively They reduced to 102 00 and 157 80 mg/100 ml respectively after 15 days of administration of the above drug orally at a dose rate of 1g/kg body weight prepared in 10 ml of water Seventy five days of administration of the above preparation at the same dose, further decreased the above values to a minimum of 28 38 mg/100 ml of cholesterol and that of triglyceride to 43 30 mg/100 ml The reduction brought about by the aqueous extract of E officinalis during the period of study was found to be significant at one per cent level (P<0 01) (Anova Tables 11 to 20)

The groups which were given fruit pulp and aqueous extract of E officinalis did not show much histopathological changes (Plate No 5 and 6) The sections of liver showed only mild to moderate degree of fatty change The degree of fat infiltration in between the lining of wall of aorta was also very mild when compared to that of the control group

5.5 Group V - Treated with Gemfibrozil

Tables 9 and 10 show the effect of administration of Gemfibrozil on plasma cholesterol and triglyceride level (mg/100 ml) in hypercholesterolaemic rabbits

Gemfibrozil when given at a dose rate of 120 mg/kg body weight orally for 15 days reduced the plasma cholesterol level to 112 82 from the initial hypercholesterolaemic value of 143 46 mg/100 ml and that of triglycerides from 290 24 to 222 76 mg/100 ml After 75 days of treatment with Gemfibrozil the cholesterol and triglyceride levels were reduced to 30 93 mg/100 ml and 53 57 mg/100 ml respectively The reductions brought about by the drug were found to be significant at one per cent level ($P<0.01$) (Anova Tables 11-20)

Histopathological examination of liver and aorta of animals in the group treated with Gemfibrozil revealed that fat accumulated in the liver and aorta only in a mild to moderate degree compared to the control group (Plate No 7 and 8)

The comparison between the effects produced by different treatments with that of the control group has been represented in the figures 1 and 2 The mean values represented in the figures have been given in Tables 21 and 22 Analysis of the results obtained in the present study revealed that all the treatments were effective in producing the hypolipidaemic effect, but there was no significant difference between the treatments, as evident from the figures 1 and 2

Discussion

DISCUSSION

Cholesterol is an important structural component of all cellular and intracellular membranes as well as of plasma lipoproteins The cholesterol present in plasma is only a small fraction of the total present in the body but is important in many respects Patients with hypercholesterolaemia have a greater risk of developing atherosclerosis of the coronary arteries Conversely reduction in plasma cholesterol may reduce the risk of death from myocardial infarction Elevation of plasma cholesterol is assumed to increase the rate of deposition of cholesterol in the arterial intima which is the site of atherosclerotic lesions Attempts to decrease plasma cholesterol by diet or drugs are based on the hope that reduction in the rate of deposition of cholesterol may retard the development of lesions and the risk of myocardial infarction (Sodhi 1975)

From the results obtained, it is obvious that the agents tried namely aqueous extract of Allium sativum fruit pulp as well as aqueous extract of Emblica officinalis have reduced the plasma cholesterol and triglycerides similar to that of the known hypocholesterolaemic drug used in the study namely Gemfibrozil,

whereas the control group which received no treatment remained hypercholesterolaemic and hypertriglyceridaemic throughout the period of study

6.1 Control Group

The control group of animals had their mean plasma cholesterol and triglyceride values 54 6 and 60 14 mg/ 100ml initially In the hyperlipidaemic state these values increased to 119 92 and 123 86 mg/100 ml respectively At the end of the period of study the control group animals accumulated plasma cholesterol and triglyceride to a maximum value of 233 29 and 322 72 mg/100 ml respectively The increase observed in these parameters were significant at one per cent (P<0 01) (Anova Tables 11-20)

Histopathological changes were observed in the liver and aorta of animals which belonged to the control group (Plate No 1 and 2) Moderate to diffused fatty change was noticed in the liver The hepatocytes were filled with fat and the nucleus was pushed towards one side The aorta on examination showed that the continuity of elastic tissue was lost due to accumulation of fat in between the elastic tissue The continuity of endothelial lining was lost due to fat deposition

6.2 Group treated with Gemfibrozil

On analysis of the results of the study it can be seen that Gemfibrozil the current drug of choice in the treatment of hyperlipidaemia in human beings when given

orally to the positive control group of animals at a dose rate of 120 mg/kg for 75 days reduced the plasma cholesterol from a mean value of 143 46 mg/100 ml to 30 93 mg/100 ml and triglyceride level from 290 2 mg/100 ml to 53 37 mg/100 ml The percentage reduction produced was 78 43 with respect to cholesterol and 81 54 in the case of triglyceride which were statistically significant ($P<0.01$) (Anova Tables 11-20) The percentage reduction obtained in the case of triglyceride and cholesterol is similar to that reported in human beings by Brown (1987) Clinical use in human beings has revealed that it effectively lowers plasma triglycerides and reduce very low density lipoprotein and apoprotein B production in the liver

Histopathological examination of liver and aorta of animals in the Gemfibrozil treated group (Plate No 7 and 8) revealed that fat accumulated in these tissues only in a mild to moderate degree compared to the control group which shows reparative process in the treated group

6. 3. Group treated with A. sativum

Aqueous extract of A sativum at a dose rate of 2g/kg given orally decreased the level of plasma cholesterol from 182 8/mg/100 ml to 38 24 mg/100 ml within a period of 75 days treatment The triglyceride value decreased from 273 47 mg/100 ml to 66 27 mg/100 ml The

percentage reduction produced by this agent was 79 08 per cent with cholesterol and 75 76 per cent with triglycerides Both the reductions were significant (P<0 01) as shown in the Anova Tables (11-20)

In the case of animals treated with aqueous extract of A sativum, the liver and aorta did not show much pathological changes (Plate No 3 and 4) The degree of fatty changes noticed was very mild compared to that of the control group The lining of the wall of aorta was more intact and only very little fatty infiltration could be seen

Though there was a significant difference between the control group and the group treated with aqueous extract of A sativum no significant difference was observed in between the group treated with A sativum and the positive control namely Gemfibrozil which is evident from Figures 1 and 2

Similar lipid lowering activity of garlic in rabbits has been reported by many workers previously (Jain 1976 Bordia et al 1977 Sharma et al 1977 Sainani et al 1980 Mirhadi et al 1983 and Mirhadi et al 1991) Most of these studies have also given due recognition to garlic as a powerful antiatherosclerotic element

Jain (1976) observed that supplementation of garlic to cholesterol fed rabbits caused a significant lowering of total free, ester cholesterol and phospholipids and the degree of atherosclerosis was also found to be less Augusti (1974) and Sharma et al (1975) noticed that addition of onion to fatty diet produced a significant reduction in serum cholesterol and triglyceride In these studies it was further stated that the hypolipidaemic activity of onion was due to the allylpropyldisulphide present in its volatile oil The observation made that onion acts as a choleretic agent also accounts for its hypolipidaemic activity

Jain (1976) suggested that the mechanism of action of garlic responsible for its lipid lowering effect to be the increased excretion of cholesterol end products in faeces which is well supported by a progressive increase in the excretion of total bile acids A diminished endogenous synthesis of cholesterol was also stated as a possibility

Bordia and Bansal (1973) concluded that the hypolipidaemic activity of garlic resides in the essential oil which chemically is a combination of sulphur containing compounds The above finding in human beings gained further support from the studies conducted by

Sharma et al , 1976 Sainani et al , 1979 Mahanta et al , 1980 and Bordia 1981

Certain studies conducted in rats also revealed that the sulphur containing compound present in the volatile oil were responsible for the hypolipidaemic effect of garlic (Itokawa et al , 1973 Farva et al ,1986 and Adoga, 1987)

Sharma et al (1976) also demonstrated that the essential oil was bound in garlic in such a form that garlic when boiled in water for 30 minutes does not extract it

Sainani et al (1979) observed from their study that people who totally abstained from garlic and onion had significantly higher levels of serum cholesterol, triglycerides, β -lipoproteins and phospholipids compared to those who consumed liberal amounts of garlic and onion in their diet

Bordia (1981) also suggested that administration of garlic oil not only caused a significant reduction in serum cholesterol and triglyceride levels, but also was accompanied by an increase in HDL in normal as well as patients with coronary heart disease and that withdrawal of the oil caused all the serum components to revert to the original levels The above mentioned studies have

also shown that garlic oil prevented the increase in β and pre β lipoproteins and the decrease in α-lipoproteins produced by cholesterol feeding

Itokawa et al (1973) isolated crystals of S allylcysteine sulphoxide (SACS) and S-methyl cysteine sulphoxide (SMCS) from garlic and found that administration of these agents decreased the elevated plasma cholesterol level of rats and also the liver total and free cholesterol The rationale proposed for the hypolipidaemic activity of SACS and SMCS was the increased faecal sterol and cholic acid excretion observed in rats under study

Farva et al (1986) and Adoga (1987) explained the reduction in serum lipids and liver lipids to be due to the unsaturated disulphide bonds of the garlic oil Adoga (1987) also remarked that the active principle diallyl disulphide in active enzymes and substrates containing thiol groups, increased hydrolysis of triacylglycerols in an exchange reaction An increased activity of the lipase enzyme by the oil was also suggested

Alterations produced in the fatty acid biosynthesis and enzyme activities in rabbits by adminis tration of garlic has been put forward as an explanation for the hypolipidaemic activity of garlic (Mirhadi et al

1983 and Mirhad et al 1991) It was evident from these studies that garlic supplementation to the atherogenic diet decreased the biosynthesis of fatty acids from 2 $^{-14}$C-acetate and of glycerolipids from 1^{-14} C palmitate The beneficial role of garlic in hyperlipidaemia was also attributed to the increased activity of phospholipase induced in liver and aorta

The antiatherosclerotic effect of garlic in rabbits has been reported by earlier workers (Thiersch, 1937 Jain, 1976 Sainani et al 1980 Mirhadi and Singh 1987 Kaul and Prasad 1990 and Mirhadi et al 1991) Thiersch was probably the first to report that garlic oil inhibited the development of atherosclerosis in rabbits fed cholesterol Jain (1976) also found that atherosclerosis was produced experimentally in rabbits by prolonged cholesterol feeding and that addition of garlic to cholesterol caused only a lesser degree of atherosclerosis, which was evident from visual grading itself Sainani et al (1980) also made the same remark Mirhadi and Singh (1987) studied the effect of garlic extract on in vitro uptake of calcium and orthophosphate ions by matrix of sheep aorta It was observed that addition of garlic to the system completely inhibited the uptake of calcium and orthophosphate ions and this effect increased proportionately with the quantity of garlic

extract used Hence it was suggested that ingestion of fresh garlic may reduce calcification of aorta which is an integral part of atherosclerosis

Kaul and Prasad (1990) also shared the above opinion that garlic proved to be antiatherosclerotic in goats The aortic intimal surface area involvement by fatty spots or streaks found to be minimum in kids fed cholesterol and garlic and maximum in kids fed cholesterol alone

Mirhadi et al (1991) associated the anti atherosclerotic effect of garlic to its action on certain enzymes, namely phospholipase and lecithin cholesterol acyl transferase An increase in the activities of these enzymes was reported

Most of the above mentioned studies were conducted with different forms of garlic such as raw, boiled, aqueous extract, essential oil and crushed paste and a major proportion of the results of these studies have attributed the hypolipidaemic activity of A sativum due to its allylpropyl disulphide content in the essential oil Hence these studies help to reach a conclusion that the essential oil is not destroyed in any of the above used preparations Since aqueous extract of A sativum was used in the present study and the results obtained

were almost similar to that of the previous studies it can be suggested that the hypolipidaemic activity of A sativum is probably due to the unsaturated allylpropyl disulphide content of the garlic oil

The above stated findings are well supported by the histopathological findings of the present study The histopathological changes produced by the high cholesterol diet in the control group and the changes noticed in the Gemfibrozil treated group as compared to the garlic treated group showed that garlic was effective in counteracting the fatty changes produced in the liver and aorta to a large extent The significant decrease in the values of cholesterol and triglycerides bears testimony for the reparative efficacy of garlic

6.4 Groups treated with E officinalis

The results also revealed that the fruit pulp & aqueous extract of E officinalis were effective in lowering the high plasma cholesterol and triglyceride levels in hypercholesterolaemic rabbits

Fruit pulp of E officinalis given at a dose rate of 1 g/kg body weight orally for a period of 75 days reduced the plasma cholesterol from the initial value of

118 94 mg/100 ml to 35 98 mg/100 ml and triglyceride from 268 63 mg/100 ml to 58 54 mg/100 ml The percentage reduction in cholesterol and triglyceride were 69 74 and 78 20 respectively

Aqueous extract of E officinalis when given at a dose rate of 1g/kg prepared in 10 ml of water also proved to be hypolipidaemic in the present study Treatment using this agent caused reduction in plasma cholesterol from 137 81 mg/100 ml to 28 38 mg/100 ml the percentage reduction being 79 40 per cent The triglyceride level decreased from a maximum value of 251 77 mg/100 ml to 43 30 mg/100 ml which accounted for a percentage reduction of 82 80 per cent The reductions produced by both the forms of E officinalis were significant ($P<0.01$) as evident from the above Tables (11 20)

The groups which were given fruit pulp and aqueous extract of E officinalis did not show much histopathological changes The sections of liver showed only mild to moderate degree of fatty change The degree of fat infiltration in between the lining of wall of aorta was also very mild when compared to that of the control group

On the basis of the present study it can be said that the hypolipidaemic activity of E officinalis was

highly significant and also comparable to that produced by the known drug, Gemfibrozil Another feature noticed was that, the difference in cholesterol and triglyceride levels produced by E officinalis was not statistically significant from that of the positive control drug but both the groups differed significantly from that of the control group, which remained hyperlipidaemic throughout the course of study It is evident from the figures 1 and 2 that there was no significant difference in the hypolipidaemic activity between the treatments

The hypolipidaemic activity of E officinalis observed in this study is in agreement with that reported by many previous workers (Thakur and Mandal 1984 Bordia et al , 1985) Thakur and Mandal (1984) observed that both Vitamin C and E officinalis reduced serum cholesterol levels significantly in rabbits fed high cholesterol diet Bordia et al (1985) also achieved the same results with juice as well as fruit pulp of E officinalis and Vitamin C in cholesterol fed rabbits E officinalis was found to be superior to Vitamin C All the above workers have attributed the hypolipidaemic activity of E officinalis to its high content of Vitamin C which inturn is strongly supported by studies conducted by several other workers (Vijayakumar and Vasudevan 1980

Bordia et al , 1981 Shebib et al , 1986 and Sharma et al , 1988)

Hypocholesterolaemic effect of ascorbic acid was reported in both normal and diabetic patients by Vijayakumar and Vasudevan (1980) The impairment in glucose metabolism in ascorbic acid deficiency is thought to cause an increase in serum cholesterol levels Bordia et al (1981) also thought that Chyavanprash , the principal constituent of which is E officinalis produced characteristic hypolipidaemic activity due to the high content of Vitamin C in it Administration of this ayurvedic medicine was found to increase serum ascorbic acid to a statistically significant level in healthy adults, which was accompanied by a distinct fall in serum triglyceride and cholesterol The hypocholesterolaemic activity of ascorbic acid has been further approved in the studies conducted by Shebib et al (1986) in rabbits and Sharma et al (1988) in guinea pigs Sharma et al (1988) observed that high cholesterol diet when fed to guinea pigs, which like human beings could not synthesise Vitamin C, resulted in a significant increase in all the lipid fractions Vitamin C was thought to exert its hypolipidaemic effect by increasing the mobilisation and transport of cholesterol to the liver which inturn was brought about by the HDL fraction This view is supported

by the finding that Vitamin C administration caused a relative increase in HDL cholesterol Vitamin C - was also found to have a beneficial role in enhancing the conversion of cholesterol to bile acids in the liver and their eventual excretion Bates et al (1977) corroborated such a Vitamin C blood lipid relationship and reported a positive correlation between Vitamin C and plasma HDL cholesterol which has a protective role against hyperlipidaemia

Several scientists have reported on the anti atherosclerotic effect of E officinalis and Vitamin C Thakur and Mandal (1984) studied the effect of E officinalis in cholesterol induced atherosclerosis in rabbits in comparison to Vitamin C It was observed that the degree of fatty changes as indicated by Sudanophilia was less in the group treated with E officinalis compared to that treated with Vitamin C Bordia et al (1985) reported that E officinalis juice E officinalis pulp and Vitamin C were effective in preventing experimental atheroma in cholesterol fed rabbits E officinalis juice and pulp were found to be significantly superior to Vitamin C and that pulp of E officinalis was even superior to the juice

An attempt on the reversal and regression of experimental atherosclerosis in rabbits by administration of Vitamin C was carried out by Shebib et al (1986) The study revealed that Vitamin C could improve and regress the experimental lesions produced

The observation that Vitamin C influenced serum and aortic lipid profile was further substantiated by Sharma et al (1988) The histopathological changes recorded in the above study showed that cholesterol feeding significantly increased all the lipid fractions in the wall of the aorta Vitamin C supplementation did not affect phospholipids and it was suggested that plasma phospholipids stabilised the colloidal dispersion of cholesterol and thereby prevented its deposition in the arterial wall in the cholesterol fed guinea pigs

Since the finding of the present study that fruit pulp and aqueous extract of E officinalis are effective as hypolidaemic agents is well supported by the reports given above, it may be presumed that the hypolipidaemic effect of E officinalis is mainly due to its high content of Vitamin C E officinalis is a very rich natural source of Vitamin C containing around 600-800 mg/100 g of fresh amla fruit Hence the hypolipidaemic effect can very well be ascribed to this principal constituent The

histopathological observations of the present study also points out that *E officinalis* has been reasonably effective in counteracting the fatty changes produced by high cholesterol diet

Based on the above details it can be concluded that the indigenous preparations tested for their hypo lipidaemic efficacy in the present study namely *A sativum* and *E officinalis* were effective in lowering the plasma cholesterol and triglyceride levels signi ficantly The study also pointed out that the hypolipidaemic effect of the agents tried was comparable to that of Gemfibrozil in statistical significance as shown by the analysis of the results obtained Therefore these herbal agents can well be suggested for clinical trials in animals and human beings as hypocholesterolaemic and hypolipidaemic drugs

Summary

SUMMARY

In the present study an attempt was made to assess the hypolipidaemic efficacy of two indigenous agents namely aqueous extract of Allium sativum and fruit pulp as well as aqueous extract of Emblica officinalis in comparison with the established hypolipidaemic drug Gemfibrozil in rabbits

Thirty Newzealand White adult male rabbits were procured for the study from Small Animal Breeding Station College of Veterinary and Animal Sciences Mannuthy The average body weight of the animals were 1 5 kg They were housed in cages designed for the purpose Each rabbit received 200 g standard rabbit feed per day

The experiment was carried out in two stages The first stage was to induce hypercholesterolaemia in all the rabbits For this purpose the standard ration was supplemented daily with 100 mg cholesterol and 8 g Vanaspathi (DALDA) per rabbit They were maintained on this ration for 60 days when they developed hypercholesterolaemia as shown by blood analysis The parameters checked were plasma cholesterol and triglycerides

Plasma cholesterol and triglycerides were estimated initially and then fortnightly to assess whether the animals became hypercholesterolaemic or not

Cholesterol was estimated according to the method described by Zak (1957) Triglyceride level in plasma was estimated by Trigazyme T M - enzymatic Kit (GPO PAP method) (Werner et al 1981)

The second stage of the present study was to determine the hypolipidaemic effect of herbal agents (A sativum and E officinalis) in comparison with Gemfibrozil in the rabbits rendered hypercholesterolaemic during the first stage For this purpose the hypercholesterolaemic rabbits were divided into five groups of six each The first group was kept as control animals which did not receive treatment with any drug The second group was given aqueous extract of A sativum orally at a dose rate of 2g/kg body weight prepared in 10 ml of water The third group was treated with fruit pulp of E officinalis at a dose rate of 1g/kg body weight orally The fourth group was administered aqueous extract of E officinalis orally at a dose rate of 1g/kg body weight prepared in 10 ml of water The fifth group was taken as the positive control group which received the known hypolipidaemic drug namely, Gemfibrozil at a dose

rate of 120 mg/kg body weight orally All the agents were tried for a period of 75 days The diet supplemented with cholesterol and Vanaspathi was continued throughout the period of study Plasma cholesterol and triglycerides were estimated every fortnightly during the course of administration of drugs so as to determine the changes produced by the drugs on the above parameters At the end of the period of study histopathological studies of liver and aorta were conducted to demonstrate the structural changes in tissues produced by administration of the drugs

From the results of the study it was evident that the control group of animals which received no treatment with drugs continued to be hypercholesterolaemic throughout the period of study The animals of this group had a mean value of 54 6 and 60 14 mg/100 ml of cholesterol and triglyceride initially which increased upto 233 29 and 322 72 mg/100 ml respectively at the end of the period [illegible] produced in the liver and aorta of animals of this group was also in agreement with the above findings Moderate to diffused fatty changes were noticed in the liver The hepatocytes were filled with fat and nucleus was pushed to one side Due to fat accumulation in between the elastic tissue, the continuity of the wall of aorta was lost

In the case of Group II which received aqueous extract of A sativum the mean plasma values of cholesterol and triglyceride in the hyperlipidaemic state were 182 81 and 273 47 mg/100 ml respectively which were reduced to 38 24 and 66 27 mg/100 ml after 75 days of administration of the drug The reductions produced by the agent was statistically significant (P<0 01) (Anova Tables 11-20) Histopathological examination of liver and aorta of these animals revealed not much changes The fatty changes in the liver and aorta was only of a mild degree compared to that of the control group The lining of the wall of aorta was also more intact and continuous without much fat accumulation in between the elastic tissue

Group III animals which were treated with fruit pulp of E officinalis had a mean plasma cholesterol value of 118 94 mg/100 ml and 268 63 mg/100 ml of plasma triglycerides on attainment of hyperlipidaemia Seventy five days of administration of fruit pulp of E officinalis brought down the above values to a minimum of 35 98 mg/100 ml of cholesterol and 58 54 mg/100 ml of plasma triglyceride On statistical analysis it was found that , the decrease in plasma cholesterol and triglyceride produced by the above mentioned agent was highly significant (P<0 01) (Anova Tables 11 20)

The mean plasma cholesterol and triglyceride levels of animals of Group IV which were given aqueous extract of E officinalis were 137 81 and 251 77 mg/100 ml respectively in the hyperlipidaemic condition Administration of the drug for a period of 75 days significantly reduced the above values to a minimum of 28 38 mg/100 ml of cholesterol and 43 30 mg/100 ml of triglycerides (P<0 01) (Anova Tables 11 20)

The histopathological studies of the animals treated with fruit pulp and aqueous extract of E officinalis did not reveal pathological changes like that of the control group The fatty changes noticed in the liver and aorta was only of a mild to moderate degree The fat accumulation in the wall of aorta was only of a mild degree compared to that of the control

Gemfibrozil the drug used as the reference standard, when administered to animals of Group V reduced the plasma cholesterol level from the initial hypercholesterolaemic value of 143 46 mg/100 ml to 30 93 mg/100 ml and that of triglyceride from 290 24 mg/100 ml to 53 57 mg/100 ml within a period of 75 days The reductions produced by Gemfibrozil in both the parameters were highly significant (P<0 01) (Anova Tables 11-20)

Histopathology of liver and aorta of animals of this group was more or less similar to that of the group treated with E officinalis

Based on the above mentioned observations made from the present study it may be summarised that the different preparations of A sativum and E officinalis tested for their lipid lowering effects in hyperlipidaemic rabbits, proved to have a satisfactorily beneficial role in the treatment of hyperlipidaemia So also both A sativum and E officinalis were also effective in reducing the fatty changes induced in the liver and aorta by high cholesterol diet as evident from the histo pathological studies conducted Gemfibrozil produced an effect on the tissues more or less similar to that of E officinalis However garlic was found to be most effective in correcting the histopathological changes

Another observation made from the present study was that the control group differed significantly from the various treatment groups, but the different treatment groups did not differ significantly from one another Figures 1 and 2 depict the comparative efficacy of the different treatments in lowering plasma cholesterol & triglyceride respectively Hence it may be concluded that supplementation of high cholesterol diet causes hyper lipidaemia and that A sativum and E officinalis were as

effective as Gemfibrozil in reducing the increased lipid levels Compared to Gemfibrozil and E officinalis garlic was found to be more effective in counteracting the histopathological changes produced on feeding high cholesterol diet as revealed by the photomicrographs

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HYPOLIPIDAEMIC EFFECT OF *Allium sativum* AND *Emblica officinalis* IN RABBITS

By

K. P. MINI

ABSTRACT OF A THESIS

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Thrissur

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ABSTRACT

The present study was undertaken with the objective of determining the hypolipidaemic effect of Allium sativum and Emblica officinalis in hyperlipidaemic rabbits The different forms of the indigenous agents tried were aqueous extract of A sativum and fruit pulp as well as aqueous extract of E officinalis The effects produced by the above agents were compared with that of the known hypolipidaemic drug namely, Gemfibrozil which served as the positive control drug

Thirty Newzealand White adult male rabbits were used for the study The average body weight of the animals were 1 5 kg They were housed in cages designed for the purpose Each rabbit received 200 g standard rabbit s feed per day

The experiment was conducted in two stages The first step was to render all the rabbits hyper cholesterolaemic In order to achieve this goal the standard ration was supplemented daily with 100 mg cholesterol and 8 g Vanaspathi for each rabbit The rabbits were fed on this diet continuously for 60 days when they developed hypercholesterolaemia This was ascertained by the estimation carried out initially and

every fortnightly during this period The parameters estimated were plasma cholesterol and plasma triglyceride

The second part of the study was to evaluate the hypolipidaemic efficacy of the chosen indigenous preparations in comparison to that of Gemfibrozil Each agent was tried on a separate group by dividing the hypercholesterolaemic rabbits into five groups of six each Group I was kept as the control group which received no treatment Group II was administered aqueous extract of A sativum 10 ml (2g/kg b wt) orally The animals of Group III were treated with fruit pulp of E officinalis at a dose rate of lg/kg orally Group IV animals received aqueous extract of E officinalis orally at a dose rate of 1 g/kg prepared in 10 ml of water The Group V served as the positive control which received Gemfibrozil at a dose rate of 120 mg/kg orally All the drugs were administered for a period of 75 days The high cholesterol containing diet was continued throughout the period of study The difference brought about by the above agents on plasma cholesterol and triglyceride of hyperlipidaemic rabbits was determined by routine estimations of the above parameters carried out every fort nightly At the end of the period of study histo pathological studies of liver and aorta were also

performed in order to detect the structural changes in tissues caused by the different treatments

The control group of animals increased their plasma cholesterol and triglyceride by 76 59 and 81 36% respectively This increase was found to be statistically significant The liver and aorta of these animals also supported the above finding on histo pathological examination Diffused fatty changes was noticed throughout the section of liver and aorta The hepatocytes were filled with fat and the nucleus was displaced Lining of the wall of aorta also showed severe fatty infiltration in the control group

It was found that administration of aqueous extract of A sativum reduced plasma cholesterol by 79 08 per cent and plasma triglycerides by 75 76 per cent within a period of 75 days Both the reductions were highly significant Compared to the control group the histo pathological findings of this group showed that garlic was very effective in counteracting the fatty changes induced by high cholesterol diet in rabbits The fatty changes of liver was only of a mild degree and the fatty infiltration of aorta was also very mild

The percentage reduction obtained in the case of fruit pulp of E officinalis was 69 74 and 78 20 with

respect to cholesterol and triglycerides which was also statistically significant Aqueous extract of E officinalis administered to the fourth group of animals could produce a reduction in plasma cholesterol and triglyceride by 79 40 per cent and 82 80 per cent respectively

The histopathological studies conducted in the above two groups showed almost similar findings Compared to the control group the degree of fatty changes was only mild to moderate Infiltration of fat into the elastic tissues of aorta was also very mild Hence it can be suggested that E officinalis is capable of counteracting the fatty changes in liver and aorta partially

Gemfibrozil which served as the positive control brought about a percentage reduction of 78 43 with respect to cholesterol and 81 54 per cent in the case of triglyceride, both were found to be highly significant The above observation was well supported by the photomicrographs of liver and aorta taken from the animals of this group Mild to moderate degree of fatty changes was noticed in the liver and aorta Aorta did not reveal prominent changes like that of the control group due to fatty infiltration The histopathological observation made in the case of Gemfibrozil resemble that of E officinalis

From the results of the present study it can be inferred that aqueous extract of A sativum and fruit pulp as well as aqueous extract of E officinalis are effective as hypolipidaemic agents and this finding is further asserted by the simultaneous histopathological studies carried out Both the indigenous agents were capable of correcting the fatty changes produced by the fat containing diet to a considerable extent and garlic was found to be superior to E officinalis in this respect The efficacy of these agents in lowering plasma cholesterol and triglyceride was comparable to that of Gemfibrozil as shown by the statistical analysis of the results obtained Hence these agents prove to be of value as hypolipidaemic agents in the future clinical trials that can be carried out in animals and also in human beings