# THE EFFECT OF GINGER ON SERUM CHOLESTEROL AND BLOOD GLUCOSE LEVELS

BY

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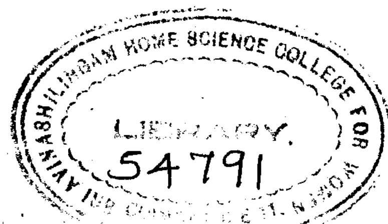

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## I. INTRODUCTION

Atherosclerosis resulting in Ischaemic heart disease, is the greatest epidemic that man-kind has ever faced. Coronary heart disease and the complications arising as its consequence are major problems facing modern society unlike the advanced countries precise data of mortality and morbidity caused by heart disease, covering the entire country is not available. It is however, on the increase in societies moving towards civilization (WHO, 1974).

Coronary heart disease results in more deaths and disability than cancer, diabetes and cell infections. The basic cause of coronary heart disease is atherosclerosis. A result from a series of changes in the intima of arteries caused by local accumulation of fatty material and fibrous tissue, disrupting its normal architecture (Kagan, 1977).

In the light of several experiments epidemiological and clinical investigations, it is generally accepted that among the many factors involved in the etiopathogenesis of atherosclerosis, hyper-cholesterolemia is perhaps the most important. Thus it is well known that the marked elevation of serum cholesterol seen in patients with familial

hypercholesterolemia is associated with the early development of atherosclerosis. Hypercholesterolemia is also associated with diabetes mellitus in some patients. (Yudkin, 1977).

Serum cholesterol concentrations can be successfully reduced by dietary modifications alone. Several studies have widely emphasized the effects of different components of the daily diet on the levels of cholesterol in the body. The role of nutritional factors such as the type and concentration of dietary fat, the type of carbohydrate and dietary fibre, in altering plasma cholesterol have been widely published (Zeister, 1975; Kritchevsky et al., 1975). Many of these dietary constituents have been used to check hypercholesterolemia though not as liberally as drugs.

The association of hypercholesterolemia to the development of atherosclerotic coronary heart disease has led to the search for cholesterol lowering agents which may moderate the course of the disease or prevent it (Gujaral, 1978). But the drugs used to lower cholesterol might be harmful.

Diabetes, commonly known as diabetes mellitus is a chronic hereditary disease characterised by hyperglycaemia and glycosuria, depending on a deficiency of insulin,

resulting from either insufficient supply or diminished effectiveness. It results from a serious dislocation of the endocrine balance that normally regulates carbohydrate metabolism, (Bajaj, 1977).

Diabetes mellitus is often associated with an excessive accumulation of one or more major lipids transported in plasma. High plasma triglycerides appear to be more common than hypercholesterolemia in middle-aged diabetes with vascular disease (New et al., 1973).

Diabetes is prevalent all over the world among both the sexes. The incidence ranges from 2 to 5% in the western world and 2.53 to 2.7% in different regions of India (WHO, 1977).

The most common causes of death from diabetes are cardiovascular degeneration and disease of the nervous system (Bryfogle, 1966). Diabetic nephropathy is a cause of death in young patients with long term diabetes (Kutmachar et al., 1964).

The developing countries like India with their meagre financial resources cannot avail themselves of the services of modern medicine in view of the huge investment involved in establishing and maintaining modern clinics and hospitals (Dechamp, 1968).

Hence it could be useful and valuable if we are able to get insights into the traditional system of medicine which is often cheaper (WHO, 1977).

Experiments have already been done with curcumin (Srinivasan, 1975) and various fractions of the guggal (Kapoor, 1973) for cholestrol lowering property.

Experiments done with Sarkaraikolli (<u>Gymnema Sylvestre</u>) and bitter guard juice have shown that they have a hypo-glycemic effect (Giri and Nageswari, 1978; Giri and Samuel, 1979).

Since ginger (<u>Zingeber Officinale Roscoe</u>) is a commonly used spice and is considered a valuable medicine because of its action as a rubefactant, diuretic and stimulant to the gastrointestinal tract, it was felt that a systematic, scientific study of the effect of ginger on serum cholesterol, liver cholesterol and blood glucose level would be highly interesting and useful.

## II. REVIEW OF LITERATURE

The literature pertaining to the study on 'the effect of ginger on serum cholesterol and blood glucose levels in rats' is discussed under the following headings.

### A. Heart Distases:

1. Prevalence of heart disease in the world and India
2. Types of heart diseases
    - a) Coronary heart disease
    - b) Hypertensive heart disease
    - c) Rheumatic heart disease
    - d) Congenital heart disease
    - e) Myocardial infarction
3. Etiological factors promoting heart diseases
    - a) Nutritional factors
        - (i) Type and concentration of fat
        - (ii) Type of carbohydrate
        - (iii) Source of dietary protein
        - (iv) Vitamins
        - (v) Minerals
        - (vi) Heavy meals

b) Non-nutritional factors

4. Cholesterol and cardiovascular diseases

5. Lipoproteins and heart diseases

6. Factors lowering cholesteral level

   a) Fats

   b) Carbohydrates

   c) Proteins

   d) Vitamins

   e) Minerals

   f) Fibre

   g) Foods lowering cholesterol level

      (i) Onion

      (ii) Turmeric

      (iii) Guggal

      (iv) Katha

      (v) Wakame

      (vi) Garlic

      (vii) Yeast

7. Dietary control of hypercholesterolemia

B. <u>Diabetes Mellitus:</u>

1. Incidence of diabetes

2. Factors promoting diabetes

3. Complications of diabetes
    a) Acute complications
    b) Chronic complications
4. Methods of treatment of diabetes
5. Need for an inexpensive, indigenous and harmless cure for diabetes
6. Traditional Medicine
7. Ginger in the treatment of diabetes

1. <u>Prevalence of heart disease in the world and India</u>:

In economically advanced countries the leading causes of death among adults are degenerative diseases of the heart and blood vessels (Jones, 1970). The United States is reported to have the highest death rate from cardiovascular diseases in the world. Coronary diseases take the first place, with stroke in second place. The pattern of heart disease in India resembles that of United States. In India more persons are affected by strokes than by heart attacks. The basic cause for a stroke or heart disease is atherosclerosis or hardening and narrowing of the arteries through which blood flows. The incidence of infective endocarditis varies from 1.4 to 2.5% in various studies (Chopra <u>et al</u>., 1980).

## 2. Types of heart diseases:

### a) Coronary heart disease:

Atherosclerosis is a condition in which plaque like deposits of fatty material are found along the inner walls of arteries. It is accompanied by high blood cholesterol (Cooper, 197[illegible]).

### b) Hypertensive heart disease:

Hypertension or high blood pressure is an important factor in the development of heart disease (Kar, 1977).

### c) Rheumatic heart disease:

Rheumatic heart disease is one of the most common and serious forms of heart disease. It occurs mostly in the middle age group. It is generally agreed that, hemolytic streptococcus is the causative agent(White, 1975).

### d) Congenital heart disease:

### e) Myocardial infarction:

Myocardial infarction is associated with and possibly due to coronary artery spasm. (Hellstrom, 1979).

## 3. Etiological factors promoting heart disease:

### a) Nutritional factors:

Voskresenskii *et al.*, (1979) pointed out that atherosclerosis is a result of overeating, a deficiency of antioxidants in natural products and inactivity which causes a derangement of the physiological antioxidant system, leading to free radical oxidation, breakdown of β-lipoproteins, lipid infiltration of the intima, destruction of elasticity, fibrosis and calcinosis.

Cholesterol rich foods have long been cited by doctors as the primary cause of coronary artery disease.

The role of nutritional factors such as the type and concentration of dietary fat, the type of carbohydrate and proteins have been associated with raised cholesterol levels.

### i) Type and concentration of fat:

Both quantity and quality of dietary fat have an important bearing on cholesterol levels (Granda, 1975). Populations which consume a diet high in saturated fats have a higher incidence of hyperlipidemia and coronary heart disease (Reid *et al.*, 1977).

Jostet and Girardet (1979) revealed that butter, lard, palm oil, soyabean oil, hydrogenated groundnut oil, coconut oil and mustard oil are highly atherogenic.

Padmavati (1977) reported that diets that include a combination of fats from animal origin such as dairy products (butter, cream, cheese etc), egg yolk and saturated cooking fats such as ghee, vanaspati etc. have a marked cholesterol raising effect.

A high saturated fat diet results in the formation of metabolised products like oleic and palmitic acids that ultimately give rise to esters of these fatty acids with cholesterol which are highly atherogenic (Gopalakrishnan, 1979).

Lauric, myristic and palmitic acids are more atherogenic than stearic acid (Gopalakrishnan, 1979). Elaidic acid in the presence of cholesterol increases serum cholesterol more than oleic acid (Vergroesen, 1972).

(ii) Type of carbohydrates:

The major sources of carbohydrates in our diets are cereals, pulses, sugar, roots and tubers (Granda, 1975).

Ahrens (1974) revealed that sugar (sucrose) is an important factor in the development of coronary heart disease. Semipurified diets containing cellulose are more atherogenic than those with wheat straw (Kritchevsky, 1978). Kritchesky et al., (1973) reported that sucrose, starch, fructose, glucose and lactose are atherogenic in the declining order. Hahn and Koldovsky (1976) reported that early weaning to a high carbohydrate diet make adult rats more prone to high cholesterol levels.

(iii) Source of Dietary Protein:

Animal protein has been associated with high and vegetable protein with low plasma cholesterol levels; this differential effect is thought to be related to amino acid composition (Carroll et al., 1975 and 1978, and Leelamma et al., 1978).

(iv) Vitamins:

MCcully (1979) and Gruberg et al., (1979) implicate the deficiency of pyridoxine (Vitamin B6) as a factor leading to atherosclerosis. According to MCcully methionine is broken down by the body into homocystein a chemical that promotes atherosclerosis in laboratory animals. In the presence of vitamin B6, however, homocystein is converted into cystathionine, an innocuous by product.

Wilson *et al.*, (1978) reported that diet deficient in vitamin E may predispose one to atherosclerosis and hypercholesterolemia. Supplementation with butylated hydroxy anisol and butylated hydroxy toluene (commonly used as antioxidants in fats) in rats caused atherosclerosis. Erdman *et al.*, (1974) have theorised diet induced hyper-cholesterolemia in rats on a vitamin A free diet. However, the response is affected by the salt mixture used.

The deficiency of vitamin like substance known as co-enzyme-Q diminishes the heart's pumping ability to what has been termed as a deficiency of bioenergetics.

(v) Minerals:

A number of minerals have been associated with coronary heart disease. Masironi (1975) has reported that cadmium, cobalt and lead have detrimental effects on the cardiocirculatory system. According to Allen *et al.*, (1978) a deficiency of copper intensifies the risk of coronary heart disease by raising cholesterol levels. Turlaraty and Altura (1976) found that lower than normal levels of magnesium increased artery tone, made the arteries more responsive to body chemicals and could produce progressive vasoconstriction resulting in cardioarterial spasm leading to sudden death.

Klevay (1978) has reported that an imbalance in copper metabolism characterised by a high ratio of Zinc to copper (Zn/Cu) contributes to the risk of coronary heart disease.

(vi) <u>Heavy Meals:</u>

Koen (1978) postulates that a light rather than a heavy evening meal would reduce the risk of atherosclerosis. A heavy evening meal which is rich in saturated fats when digested, is dumped into the slow moving circulation during sleep. As this moves slowly through the arteries, the situation is ideal for clot formation.

b) <u>Non-nutritional factors:</u>

Analysis of the studies on coronary heart disease has led to the formulation of certain 'risk factors' some major and some minor, some reversible and some irreversible for coronary heart disease. Some of the major non-nutritional factors are hypertension, hypercholesterolemia, cigarette smoking, obesity, sex, sedentary living, oral contraceptives, infection and caffeine in coffee (Grande, 1975; Gordon *et al.*, 1978 and Mishkel *et al.*, 1977).

Hardness of drinking water and trace element imbalance in the environment and in the body also cause the development of coronary heart disease (Allen and Klevay, 1978).

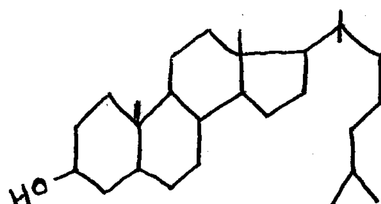
Srikantia (1971) lists stress and strain of modern living, anxiety, heredity and rich diet as major etiological factors of atherosclerosis.

Lyon (1971) reported that xanthine oxidase present in homogenised cow's milk is absorbed into the blood stream and causes the initial damage to the artery wall. After which cholesterol may deposit itself at the site of injury.

4. Cholesterol and cardiovascular diseases:

Cholesterol is a major component of all mammalian plasma membranes and is vital to cell growth and its survival. However, its excess is quite harmful to the body because cholesterol deposits harden arterial walls causing atherosclerosis.

Cholesterol belongs to the class of steriods with alcohol as one of the functional groups. Its molecular formula is $C_{27}H_{46}O$ and structural formula is



Cholesterol is obtained through diet and is also synthesised in various body tissues. It is absorbed from the intestines where about 70 per cent of it gets esterified with the fatty acids ( Gary and Sharma, 1979).

Gopalakrishnan (1979) declared that elevated levels of cholesterol in the body is a major factor leading to coronary heart disease and the risk is increased as the levels go above 220-250 mg/100 ml of blood.

Kudchodhar _et al._, (1973) have indicated that the amount of cholesterol absorbed has an excellent correlation with the dietary intake of cholesterol.

5. Lipoproteins and heart diseases:

The levels of lipoprotein in the plasma has been shown to be indivative of impending heart disease. As early as 1967 Fredrickson _et al._, identified four groups of lipoproteins important in clinical diagnosis. They were enumerated as high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), and chylomicrons.

The low density lipoproteins pick up cholesterol that originated from the diet or was manufactured in the liver and deposit it in cells for processing. If for dietary or other reasons, there is more cholesterol than is needed for daily metabolism, some of the LDL's may deposit their fatty cargoes on the interior linings of coronary arteries. Eventually this build up of fatty plaques sets up conditions for a heart attack.

High density lipoproteins floating around in the blood stream pick up excess cholesterol and appear to carry it back to the liver for excretion from the body (Engelbordt, 1978).

Barr (1973) had predicted that the level of HDL's is a powerful indicator of heart attack risk. Vegetarians have extremely, high HDL to LDL ratios. Dietary cholesterol besides altering the plasma cholesterol does both increase in LDL and decrease in HDL level.

According to Levy (1978) the higher the level of HDL the lower the risk and higher the level of LDL the greater the risk of coronary heart disease. Albers _et al_., (1978) reported that HDL levels were low in myocardial infarction survivors.

6. Factors lowering cholesterol levels:

There are a number of nutritional factors lowering cholerstarol levels and many of these from regular components of our daily diet.

a) Fat:

Poly unsaturated fats have a marked cholesterol lowering effect (Durrington et al., 1978).

Rao et al. (1977) reported that sardine oil and safflower oil decreased serum cholesterol levels within 22-44 days of feeding in adult males. The decrease was greater in the cases with higher initial values. Van lesseneny et al., (1978) reported increase in high density lipoprotein and decrease in serum triglycerides with a diet supplemented with linoleic acid. Premakumari, and Sundara-Valli (1976) pointed out that serum cholesterol decreases were more with sunflower oil than with groundnut oil. Calendra et al., (1978) remarked that an olive oil diet increases the plasma very low density lipoprotein and low density lipoprotein fractions and that an addition of cholesterol to this diet further increased these fractions and simultaneously decreased the high density - lipoprotein (HDL - 2) fractions.

4) improves the physiological function of the heart.

5) normalises bio-chemical abnormalities in obesity and maturity onset diabetes.

b) Carbohydrates:

Mac Donald (1973) revealed that fructose and starch are more atherogenic than glucose. According to Granda (1975) starch in general is not as atherogenic as sugar, while Berg et al., (1975) stated that fructose decreases plasma cholesterol and noted that sorbitol has no effect on serum triglycerides but causes a fall in cholesterol levels and that the effect of xylitol is negligible.

Pere et al., (1976) declared that feeding of ragi reduces serum cholesterol levels significantly, Judd et al., (1978) reported that rolled oats have a hypocholesterolemic effect. Garri-a flour prepared from Cassava Manihot Utilissima a staple food in urban communities of Nigeria, decreases plasma cholesterol concentrations but after the saturation point is reached garri has no further lowering effect on plasma cholesterol.

Erdman *et al.*, (1979) have pointed out that a 2% gelled agar diet decreased liver cholesterol in albino rats fed 1 per cent cholesterol diet, possibly by reducing absorption.

Tsuji *et al.*, (1979) reported that several polysaccharide derivative, synthetic polymers (like hydroxy ethil cellulose, carboxymethyl cellulose, propylene, gycol alginate) and lignin suppressed liver cholesterol.

Durringston *et al.*, (1976) and kelly *et al.*, (1978) are convinced that pectins decrease total serum cholesterol. Delbarre *et al.*, (1977) and Kay *et al.*, (1977) emphasized that apple pectin reduces blood cholesterol more effectively than lemon and citrus pectins. It is suggested that pectins decrease endogenous absorption of cholesterol and increases cholesterol turnover.

e. Proteins:

Carroll (1978) and Carroll *et al.*, (1978) have stated that replacement of animal protein with plant protein reduces plasma cholesterol, as the catabolism of cholesterol is enhanced in the latter diet.

Von Lossonczy *et al.*, (1978) reported that a fish diet given to human volunteers slightly but significantly lowered cholesterol levels.

Nair *et al.*, (1971) and Mann (1977) revealed that milk has cholesterol lowering properties and that the active factor in milk is β-hydroxy β-methyl glutaric acid. Hermus (1976) said that casein of milk has marked hypocholesterolemic effects. He further affirms as a result of elaborate experiments that the amino acid glycine possesses the most cholesterol lowering capacity. Leelamma *et al.*, (1978) reported that soya beans and black gram are hypocholesterolemic.

Singh *et al.*, (1977) demonstrated that the protein of *Acacia milanoxylon* and *Bauhina retusa* (wild leguminous seeds) were both hypoglycemic and hypocholesterolemic. Mathur (1971) and Reddy (1979) have pointed out that bengal gram is positively a lipodistretic.

Recently, protein from algal has been found to help in lowering the cholesterol level in the body.

d. Vitamins:

Vitamin C:

Garg and Sharma (1979) have pointed out that adequate tissue ascorbic acid is necessary for the catabolism of

cholesterol to bile salts. It has therefore been concluded that vitamin C lowers cholesterol levels.

<u>Nicotinic Acid</u>:

Charmann *et al.*, (1973) reported that large doses of nicotinic acid reduces both free and esterified cholesterol levels.

<u>Folic acid</u>:

Oster as quoted by Lyon (1977) who substantiated the theory that cow's milk xanthine oxidase causes the initial damage to the artery wall, treated his patients with folic acid and got phenomenal results. He therefore calls folic acid the penicillin of atherosclerosis.

<u>Lipoic acid</u>:

Lipoic acid decreases total cholesterol (Ivanov, 1975).

<u>Vitamin A</u>:

Erdman *et al.*, (1974) reported that pharmacological levels of vitamin A and recently β-Carotene have been shown to retard the accumulation of cholesterol in the serum and liver and also reduce elevated serum cholesterol levels. But lycopene, although structurally similar to β-carotene does not depress diet induced hyper-cholesterolemia.

**Vitamin D:**

Recently, activated 7 - dehydrocholesterol has been shown to decrease serum cholesterol level considerably (Fabry, 1978).

**Vitamin E:**

Wilson *et al*., (1978) showed that vitamin E inhibits atherosclerosis by preventing hyper-cholesterolemia.

**e. Minerals:**

Nagyvary *et al*., (1978) informed that $Al^{3+}$ complexes reduce serum cholesterol in rats. Klevay (1977) indicated that sodium phytate supplements in the diet of rats, significantly reduced cholesterol levels in the plasma. Diets rich in chromium protect from coronary proneness.

**f. Fibre:**

Dietary fibre, the non-digestible component of our diet has been implicated in recent years as causing a reduction in serum and body cholesterol and it has been referred to as a natural hypocholesterolemic agent (Tsai *et al*., 1976 and Domingo *et al*., 1978).

Vijayagopal et al., (1973) Heaton et al., (1974) and Mathe et al., (1978)said that wheat bran is a hypocholesterolemic agent. This property is attributed to the presence of uronic acid. Kritchevsky (1978) has indicated that pectin, gaurgum and lignin reduce serum and liver cholesterol in rats.

Alfa Alfa meal, rolled oats, are other fibres reducing cholesterol level (Malinow et al., 1978).

Trowell (1972 and 1978) attributed the fibre in cerdals (2g/100g) leguminous seeds (3-5g/100g), whole fruits and vegetables for their lipid lowering effect.

The lipid lowering effect of fibres is advocated to be due to the increase in the excretion of bile salts and faecal sterols (Trowell, 1972 and 1974, Stary et al.,1978).

g) Foods lowering cholesterol level:

i) Onion:

Jain et al., (1978) and Saindni et al., (1979) reported that onion as such and essential oil of onion reduce cholesterol levels significantly. Basarkar et al.,(1975) have revealed that quercetin obtained from onion reduces liver cholesterol but not serum cholesterol in rats.

ii) Turmeric:

Curcumin obtained from turmeric is an active principle in reducing cholesterol level (Rao et al., 1970).

iii) Guggal:

Various fractions of the guggal resin from Commiphora mukul show serum cholesterol lowering activity in rats (Nityanand, 1973).

iv) Katha

Katha containing epicatechin reduces both serum and liver cholesterol in rats (Basarkar et al., 1975)

v) Wakame:

Wakame is a sea weed (Undaria pinnatifida) which stimulates the excretion of cholesterol in the faeces (Iritani et al., 1972).

vi) Garlic:

Garlic and garlic oil possess marked cholesterol lowering properties due to an active principle, suplhide of allyl (Bordia et al., 1977). Elaborating on the beneficial effects of garlic, Bordia et al., (1977) and Jain et al., (1978) revealed that garlic prevents the fall in the α lipoprotein fraction, enhances fibrinolytic activity, lowers serum cholesterol and triglyceride and decrease aortic atheroma.

vii) Yeast:

Dietary yeast and yeast fractions have cholesterol lowering effects (Seeley, 1978).

7. Dietary control of hypercholesterolemia:

It has been widely accepted that a wise selection, a judicious combination and a proper knowledge of the optimum quality and quantity of foods can have far reaching effects in preserving and maintaining desirable health standards.

The same is true for checking an elevation in serum cholesterol levels which has been implicated as a major factor promoting to coronary heart disease. Dietary modification can retard and check significantly the cholesterol levels in the body (Williams et al., 1976 and Marr et al., 1974).

According to Turner (1978) and Gopalakrishnan (1979) the dietary control which have to be undertaken to maintain serum cholesterol level are as follows.

a. Avoid cholesterol rich foods like deep fried foods, rich pastries, cakes and puddings.

b. Eat less and lesser meat, chicken and eggs.

c. Avoid sugar and salt as much as possible.

d. Avoid butter, ghee, cream, whole milk, Include skim milk.

e. Eat more whole grain bread, cereals and pulses.

f. Include large quantities of green leafy vegetables, root vegetables like carrots and other vegetables, vegetables like onion and garlic are beneficial. All fruits are allowed in moderation.

g. Avoid animal fats and saturated fats for cooking including margarine, vanaspathi and coconut oil. Use more vegetable oils like safflower oil, gingelly oil, groundnut oil and cotton seed oil.

Fat content of diet should not be more than 20-30% of the total calories. The daily intake of cholesterol in the diet should be less than 300 mg (one egg contains 300mg cholesterol).

h. Calorie intake must be adjusted to maintain ideal body weight.

i. Eat small and light meals. Eating should be done slowly and food should be thoroughly chewed.

WHO has promoted a multifactorial approach to the prevention of coronary heart disease (Marmot, 1979). Examples are health education, advice on diet, antismoking, physical activity, weight reduction, prevention of obesity which is associated with several risk factors such as high blood pressure, elevated serum lipids, diabetes and hyperglycaemia and information on hypertension.

B. Diabetes mellitus:

1. Incidence of Diabetes:

Diabetes mellitus is not a new disease, it has been known for over 2,500 years, but the real progress in the understanding of the disease has taken place only in the last seventy five years (Ajgaonkar, 1962). The incidence of known cases of diabetes in western countries is close to 2% of the population. It is highest in communities with a high incidence of obesity. Diabetes is more frequent among relatives of known diabetes than among relatives of non-diabetes (Joslin et al., 1959; Steinberg, 1959).

Prevalence rate of diabetes has varied between 2.53 to 2.7 per cent in different regions of India (Ahuja, 1976). Peak incidence occurred in age groups of 35 to 55 years and is male predominated. Juvenile diabetes is very rare (Hanumantarao, 1975).

2. Factors Promoting Diabetes:

The several contributing factors are hereditary, age, sex, obesity, infections and stress (Davidson et al., 1973).

## 3. Complications of Diabetes:

Diabetes is generally considered to be a disease of complications. In most of the cases it is severe from the start, and trends a narrow path between ketosis and hypoglycaemia, developing vascular and coronary complications within a few years and finally leads to death (Malins,1975). Exact records of the cause of death in diabetes are virtually impossible to compile. The most common cause of death are cardiovascular degeneration and disease of the nervous system (Bradely and Bryfogle, 1956). Diabetic nephropathy is a cause of death in young patients with long term diabetes (Entmacher *et al.*, 1964).

The complications of diabetes can be divided into

(a) Acute complications and

(b) Chronic complications.

### a) Acute complications:

Diabetic Ketosis, carbuncle and acute gangrene are considered to be the acute complications.

Diabetic Ketosis, acidosis, Ketoacidosis - Diabetic precoma and diabetic coma, are terms used to describe the grave metabolic illness which, if untreated, is the end of severe diabetes. Discription of gangrene and its treatment in the diabetic, show wide variations because the elements of neuropathy and infection are not always realised. Poorly controlled diabetes encourages the

development and spread of minor infections (Malins, 1975).

b) Chronic complications:

Diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy are considered to be the most important chronic complications (Ajgaonkar, 1962).

Diabetic neuropathy is considered to be the disturbances of sensation to heat, pain, cold, touch, vibration and other nerve functions (Ajgaonkar, 1962).

Diabetic nephropathy is a term which is confined to the glomerular changes, tubular lesions, renal artery, atherosclerosis and pyelonephritis. It also includes changes due to antheroma and glomeruler lesions (Marble, 1963).

Diabetic retinopathy becomes commoner with advancing age, but much more with the duration of diabetes.

4. Methods of treatment of Diabetes:

There are three methods of treatment they are;

a) Diet

b) Diet and oral hypoglycaemic drugs and

c) Diet and insulin

The most important and basic treatment of diabetes is through diet. Diet must be adjusted according to the needs of the body and not by hunger. The diabetic should use fewer fats containing saturated fatty acids, ghee, butter, animal fats and oils containing unsaturated fatty acids like safflower oil, groundnut oil and sunflower oil (Daughaday, 1968).

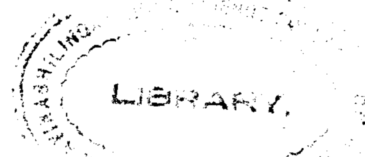
Besides diet, oral hypoglycaemic drugs such as sulphonylurea compounds and biguanides are very useful in controlling diabetes. Biguanides was widely used over long periods without serious toxic effects.

Apart from the maintenance of diet and oral treatment with hypoglycaemic drugs, insulin takes a key role in the treatment of diabetes. Insulin therapy is not curative but substitutive therapy. However, with one or more of the preparations of insulin available, it is usually possible to keep the blood glucose within reasonable limits throughout the day and night, without undue risk of hypoglycaemia. Insulin is essential in the following circumstances.

1. For new cases with severe dehydration or ketoacidosis.
2. For emergencies associated with ketosis, such as acute infection, gastro enteritis and some surgical operations and
3. For the treatment of nearly all young patients (Davidson, 1973).

5. Need for An Inexpensive, Indigenous and harmless Substitute:

In the case of oral hypoglycaemic drugs, the patients have to face many problems. Many side effects result. A fatal case due to massive overdosage of sulphonylurea drugs was recorded by Gilli et al., (1960). Severe hypoglycaemic attacks may be prolonged and refractory to treatment, so that fatalities with both tolbutamide and chlorpropamide have been reported (Cushman et al., 1963). Gastro intestinal upset is the commonest side effect and occurs in about 6 per cent of patients treated with chlorpropamide, perhaps a little more often with tolbutamide. Next, skin rashes (Toxic erythema) have been reported in about 4.5 per cent of chlorpropamide cases and 3 per cent of those taking tolbutamide. Also jaundice results within the first month of treatment. A complaint of transient giddiness or dizziness, in fact, a sensation of unsteadiness with weakness or lethargy, is not rare during sulphonylurea treatment, especially with chlorpropamide in full dosage. The side effect is thought to be due to a direct action of the drug on the central nervous system (Skinner et al., 1959) Pancytopenia is reported following tolbutamide treatment (Chapman and Cheung, 1963). A case of eosinophilic pulmonary infiltration due to chlorpropamide has been reported by Bell (1964). An interesting and common side effect of chlorpropamide is alcohol intolerance.



In the case of biguanides, the incidence of side effects has been high and has necessitated stopping the drug in about 10 per cent. The symptoms are nausea and vomiting, and occasionally diarrhoea. Also there is a tendency to ketonuria without hyperglycaemia and a rise in the blood lactic acid (Craig *et al.*, 1960).

Also the oral hypoglycaemic drugs may act for sometime and then fail to act, as sometimes happens with insulin (Ajgaonkar, 1963). Though insulin is very useful in the treatment of diabetes, there is a possibility of the development of lipoatrophy and lipohypertrophy. Lipoatrophy is a local disappearance of subcutaneous fat, usually but not always, at the site of repeated insulin infections.

Eventhough insulin has been a boon to the diabetic, it may cause him harm if not used intelligently. Insulin injections lower blood sugar. If blood sugar falls below the normal level, the diabetic may develop any one or a combination of symptoms termed as 'insulin reactions'. The symptoms of insulin reactions may appear gradually, or suddenly and unexpectedly when the blood sugar level comes down to 50 to 60 mg per cent or less in blood. The more common symptoms are fatigue, weakness, nervousness, dizziness,

hunger, restlessness, headache, cold, sweat etc. In some people there may be a mental lapse leading to loss of consiousness, if not treated promptly (Ajgaonkar, 1962).

So there is a dire need to find an effective, harmeless, substitute for diabetes which does not produce toxicity, side effects and disorders; also, it should be inexpensive.

6. Traditional Medicine:

The traditional systems of medicine have played and will continue to play a very important part in providing curative services to very large numbers of people, particularly in the rural areas of almost all countries of the world. Because of local availability of herbs, leaves etc. the indigenious system of medicine is often cheaper (WHO, 1977).

The Indian Medicinal plants take a major role in the indigenous system of medicine. They represent a vast sources of drug materials. Throughout India, the root bark of Ficus religiosa is used as a remedy for diabetes and together with garlic and common onion (Allium caper) appears to have some blood sugar lowering effect (Brachmachari and Augusti, 1962). More striking is the hypoglycaemic action of the unripe fruit of Blighia sapida. This is due to

hypoglycins, biologically active polypeptides which cause a marked fall in the concentration of liver glycogen and a profound drop in blood sugar (Hassal *et al*., 1954) capsule vijayasar is prepared from *pterocarpus marsupium* (Beejak) and is given to the diabetic patients. It is proved to be antidiabetic and astringent. By this administration polyuria, polyphagia and polydypsia are reduced (Rajasekaharan and Tuli, 1976). During recent years the plant *Catharanthus roseus* has come into prominence in the medical field, as the plant yeilds an alkaloid vincaleukoblastine. ~~The leaf extract was reported to cure diabetic ulcaleukoblastine.~~ The leaf extract was reported to cure diabetic ulcers and as an effective oral hypoglycaemic agent (1977). The alcoholic extract of the whole plant - *Clerodendron phelmidis* (Arani) is proved to be a hypoglycaemic drug. Bitter gourd *Momordica charantia* is considered to have the hypoglycemic effect when it is administered . (Giri and Samuel, 1979). A volatile constituent of onion *(Allium cepalinn)* allyl propyl disulphide (an essential oil) has been found to produce hypoglycaemia (Sharma and Gupta, 1977). *Cephalandra indica* or Tendli has been proved to reduce glycosuria. A shrub called *Casearia essentialita* has been found to be useful in diabetes, where the liver

function is affected and it has marked efficiency in reducing glycosuria. A water soluble fraction of alcoholic extract of *Coccinia indica's* (Kunden-Ki-bal) roots gives a significant improvement in the diabetic state. This improvement persisted for roughly three weeks. Jambul seed which contains gallic acid, tannic acid and kino, with their astringent effect on the gut, and glycosides, help in reducing blood sugar level in some mild cases of diabetes. The extract of *Pterocarpus marsupium* containing kinotannic acid is proved to have astringent effect on gastrointestinal tract and retard the absorption of sugar (Tulli, 1976). Many herbal drugs, have been reported to possess antidiabetic properties. Vaman Ganesh Desai in his 'Aushadhi Sangrah has mentioned ten herbs while chopra in his indigenious drugs in India mentions eight (Patel and Talwalkar, 1966).

## 7. Ginger in the treatment of diabetes:

Ginger is a widely distributed plant, used in all parts of the world not only as spice in food but also popular remedy for various ailments. Its importance as a medical plant was recognised several thousand years ago (Aman, 1973).

Food value of ginger per 100g.

| | |
|---|---|
| Carbohydrate | - 11.2g |
| Protein | -2.3g |
| Fat | - 1.5g |
| Calcium | - 21.1mg |
| Phosphorus | - 61 mg |
| Iron | - 2.3 mg |
| Vitamin A | - 65 IU |
| Vitamin C | - 7 mg |
| Nicotinic acid | - 0.7 mg |
| Calories | - 75 |
| Digesting time | - 2 hrs. |

Regarding the oleoresin content of Riode Janeiro variety which is the principle factor present in ginger is as follows (Lewis, 1973).

| Constituent | Amount per cent |
|---|---|
| Moisture | 7 |
| Volatile oil | 2.5 |
| Acetone extract | 10.3 |
| Protein | 11.1 |
| Fibre | 9.1 |
| Starch | 43.4 |
| Ash | 9.4 |

Ginger is taken raw or cooked, separately or with other vegetables. Many workers used ginger to study its hypoglycaemic effect. Sharma (1977) had found out the hypoglycaemic effect of ginger.

## III. EXPERIMENTAL PROCEDURE

In the present investiggtion three experiments were conducted. They are described in the following sequence:

### A. Experiment I:

This experiment was conducted in two phases. The grouping of animals in this experiment was given in chart 1.

#### 1. Phase I:

In this phase of the experiment, the effect of ginger on serum cholesterol in rats fed ginger for 24 days was studied.

a. Formulation of diets

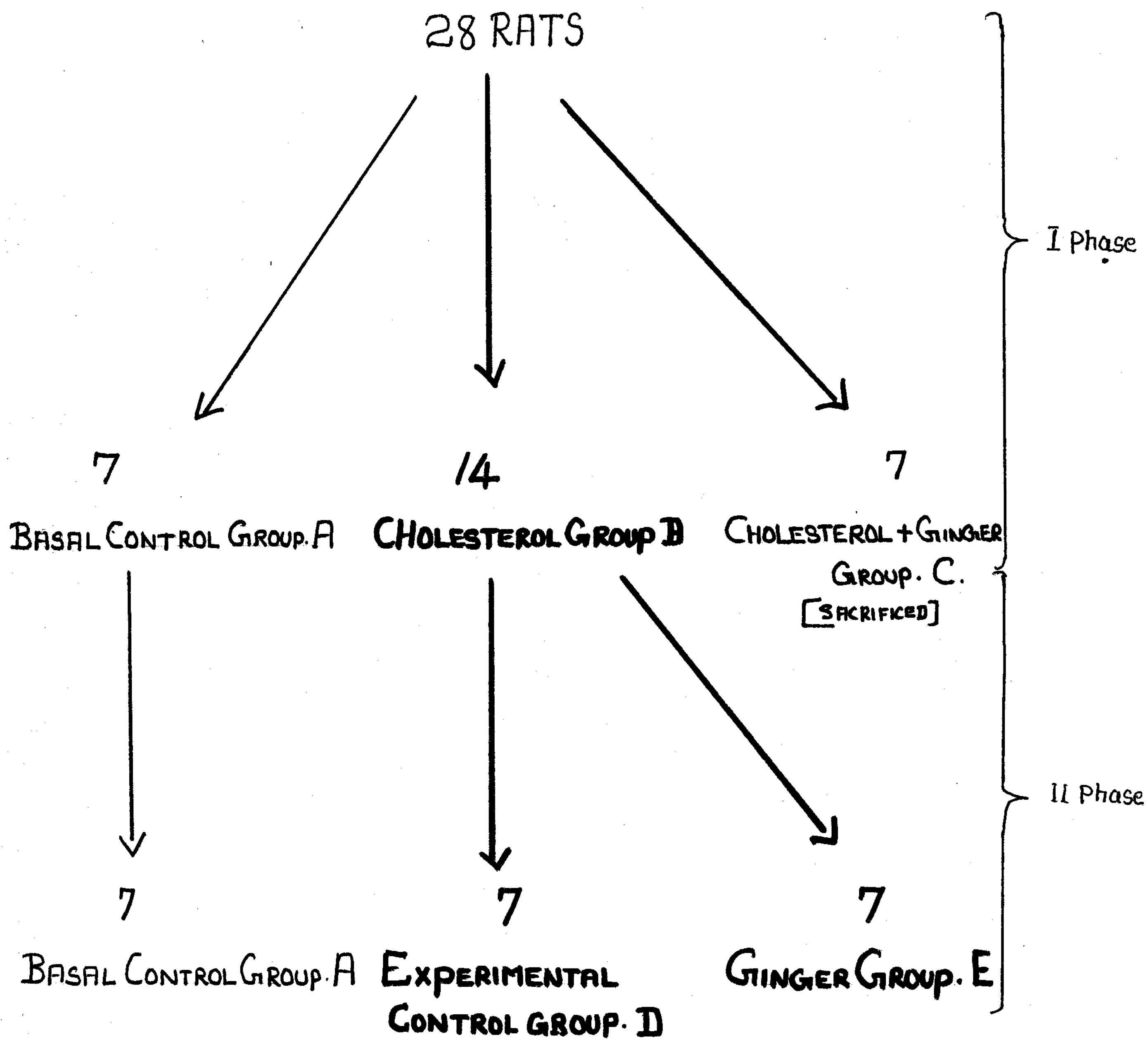
b. Selection and grouping of animals

c. Preparation of diets and feeding of rats

d. Collection of blood samples

e. Estimation of serum cholesterol.

# CHART 1



2. Phase II

In this phase of the experiment the effect of ginger on food intake, weight gain, serum cholesterol and liver cholesterol levels of hyper cholesterolemic rats were studied.

a. Grouping and feeding of rats

b. Collection and analysis of blood samples

c. Collection and analysis of liver samples.

B. Experiment II:

In this experiment the immediate effect of ginger on serum cholesterol was observed.

a. Grouping of animals

b. Feeding and collection of blood samples

c. Analysis of blood samples

C. Experiment III:

In this experiment the effect of ginger on blood glucose was studied.

a. Glucose tolerance test with glucose

b. Glucose tolerance test with glucose and ginger.

D. Statistical analysis:

A. Experiment I - Phase - 1

a. Formulation of Diets:

Three diets were formulated. These were:

1. Basal diet
2. Cholesterol diet
3. Cholesterol and ginger diet.

The details of the diets are presented in Table I. In all the diets skim milk powder was the main source of protein and groundnut oil was used as the source of fat at 10 per cent level (Achaya, 1978). The vitamin and mineral mixtures were supplied at 2 per cent and 4 per cent levels respectively, to provide adequate quantities of these nutrients. The rest of the weight was made up with corn starch. Cholesterol and ginger of the variety Riode janeiro were added at the cost of starch. Cholesterol was first dissolved in the oil and then mixed with the other ingredients. The ingredients of the diets were well mixed in dry form except for ginger, at the commencement of the experiment and stored in the deep freeze.

Preparation of ginger juice:

Fresh raw ginger of the variety Riode janeiro was washed well, the skin was scraped off with a clean knife

and cut into bits. 50g of this with just sufficient water was homogenized in a mixer and added to 90g of dry diet mixture as given in Table I.

Ginger contains 80 per cent of water (Gopalan, 1978) Hence 50g of fresh ginger is equivalent to 10g of dry ginger.

TABLE I

COMPOSITION OF DIETS IN PHASE I
(Per 100g)

| Components in the diet mixture | Control group (A) (g) | Cholesterol group (B) (g) | Ginger+cholesterol group (C) (g) |
|---|---|---|---|
| Skim milk powder | 47.37 | 47.37 | 47.37 |
| Groundnut oil | 10 | 10 | 10 |
| Vitamin mixture | 2 | 2 | 2 |
| Mineral mixture | 4 | 4 | 4 |
| Cholesterol | - | 1 | 1 |
| Ginger | - | - | (50g fresh ginger) 10 |
| Corn starch | 36.63 | 35.63 | 25.63 |

b. Selection and grouping of animals:

A number of scientists investigating the effects of different foods on the lipid profiles of rats had selected animals of different ranges of weight for their studies. For example Gutstein et al., (1973) used rats weighing between 200-250g, Yadav et al., (1977) between 100-120g, Marita et al., (1979) between 100-150g and Anusuya devi et al., (1979) between 100-120 g.

In this study twenty eight male albino rats of Wistar strain weighing 120-150g were selected from the laboratory stock colony and divided into three groups A, B and C.

Group A consisting of seven rats formed the control group and received the basal diet. Group B was made up of fourteen rats and they were fed the basal diet supplemented with 1% cholesterol. Group C had seven rats and received 1% cholesterol and 10% ginger (dry weight basis) in addition to the basal diet.

c. Preparation of diets and feeding of rats:

Food for control group (A) and cholesterol group (B) were directly weighed in separate cups, mixed with sufficient water, cooked by steaming, for 15 minutes. For ginger group

(C) the dry solid diet mixture already prepared was weighed in separate cups and fresh ginger juice equivalent to 10 per cent of the diet mixture on dry weight basis was added and steamed for 15 minutes.

The animals were given food and water ad libitum. The feeding was conducted for 24 days.

The food intake of the animals was assessed by recording the amount of food consumed everyday. The left over food was cleaned off hair and excreta, placed in separate watch glasses and heated in the oven at 100°C till completely dry. Overnight drying was found to be sufficient to completely dry the sample. Food intake was calculated by substracting the left over food from the quantity of food given to each animal. A record of the weight gain in all the rats was assessed by weighing the animals weekly.

d. Collection of blood samples:

At the end of the experimental period of 24 days, rats were fasted overnight and blood samples were collected from all the 28 rats. The tail was cut at the tip by about 0.25 cm with a sharp, sterile blade. Blood started flowing out in drops. The cut end was then lowered into a clean dry test tube (Plate I). A piece of cotton dipped in

Plate. 1. Collection Of Blood

xylene was rubbed over the whole length of the tail without touching the wound to facilitate an easy flow of blood. Xylene was used to dialate the major tail vein. After collecting the blood, the wound was plugged with a piece of cotton dipped in tincture iodine. Serum was separated and stored in a deep freeze.

After the collection of blood samples rats of ginger group (c) were sacrificed. The other two groups A and B were used for the II phase of the experiment.

e. Estimation of serum cholesterol:

Cholesterol was estimated in triplicates in all the samples by Zak's method (Varley, 1975). The details are given in Appendix I.

2. Phase II:

a. Grouping and feeding of rats:

Rats in group A of phase I were continued to be fed with the same basal diet and served as basal control group (A) in the II phase. The fourteen rats in the cholesterol group (Group B) were divided equally into two groups D and E. Group D was fed with basal diet.

This formed the experimental control. The remaining seven rats of Group E were fed the basal diet supplemented with 10 per cent ginger on dry weight basis. The composition of diets is given in Table II.

Feeding was done as in phase I of the experiment and continued for another 24 days. The daily food consumption and the weekly gain in body weight of rats were recorded carefully.

TABLE II

COMPOSITION OF DIETS IN PHASE II
(Per 100g)

| Ingredients | Basal control Group (A) (g) | Experimental control Group (B) (g) | Ginger Group (E) (g) |
|---|---|---|---|
| Skim milk powder | 47.37 | 47.37 | 47.37 |
| Groundnut oil | 10 | 10 | 10 |
| Vitamin mixture | 2 | 2 | 2 |
| Mineral mixture | 4 | 4 | 4 |
| Ginger | - | - | 10 |
| Starch | 36.63 | 36.63 | 26.63 |

b. Collection and Analysis of blood samples:

After 24 days, fasting blood samples were collected as before and the serum was separated and cholesterol was estimated as described earlier.

c) <u>Collection and analysis of liver samples:</u>

After the collection of blood samples all the 21 rats were sacrificed, their livers collected, stored in a deep freeze and hepatic cholesterol estimations were done by Tschugaeff reaction (Hanel and Dam, 1955). The details of the procedure are given in Appendix II.

B. <u>Experiment II</u>

In experiment II the immediate affect of ginger on serum cholesterol was studied.

a. <u>Grouping of animals:</u>

Eighteen male albino rats of Wistar strain weighing 120-150g were taken and divided equally into three groups I, II and III. Group I received 0.2g of cholesterol dissolved in 2ml of groundnut oil. Group II received 2ml of fresh ginger juice containing 1g of fresh ginger and 0.2g of cholesterol dissolved in 2ml of groundnut oil. Group III received 2ml of fresh ginger juice containing 1g of fresh ginger.

b. <u>Feeding and collection of blood:</u>

On the day of the experiment fasting blood was collected early in the morning from all the rats. Immediately after that the rats of different groups

were fed cholesterol (Group I), ginger plus cholesterol (Group II), and ginger juice (Group III) by forced tube feeding (Plate II). The time of feeding was noted and after 2 hours and 4 hours of feeding, blood was collected and serum was separated for cholesterol estimation.

c. Analysis of blood samples:

The serum was analysed for its cholesterol content as before by Zak's method.

C. Experiment III.

In this experiment the effect of ginger on blood glucose level was studied.

a. Glucose tolerance test with glucose:

Twenty four male albino rats (Wistar strain) weighing 190-200g were taken for the experiment, divided into two groups and glucose tolerance test was carried out. These rats were previously fed with stock diet. The composition of which is given in Table III. Each rat in the first group was fed with 0.4 g (2g/kg body weight) of glucose (Varley, 1975).

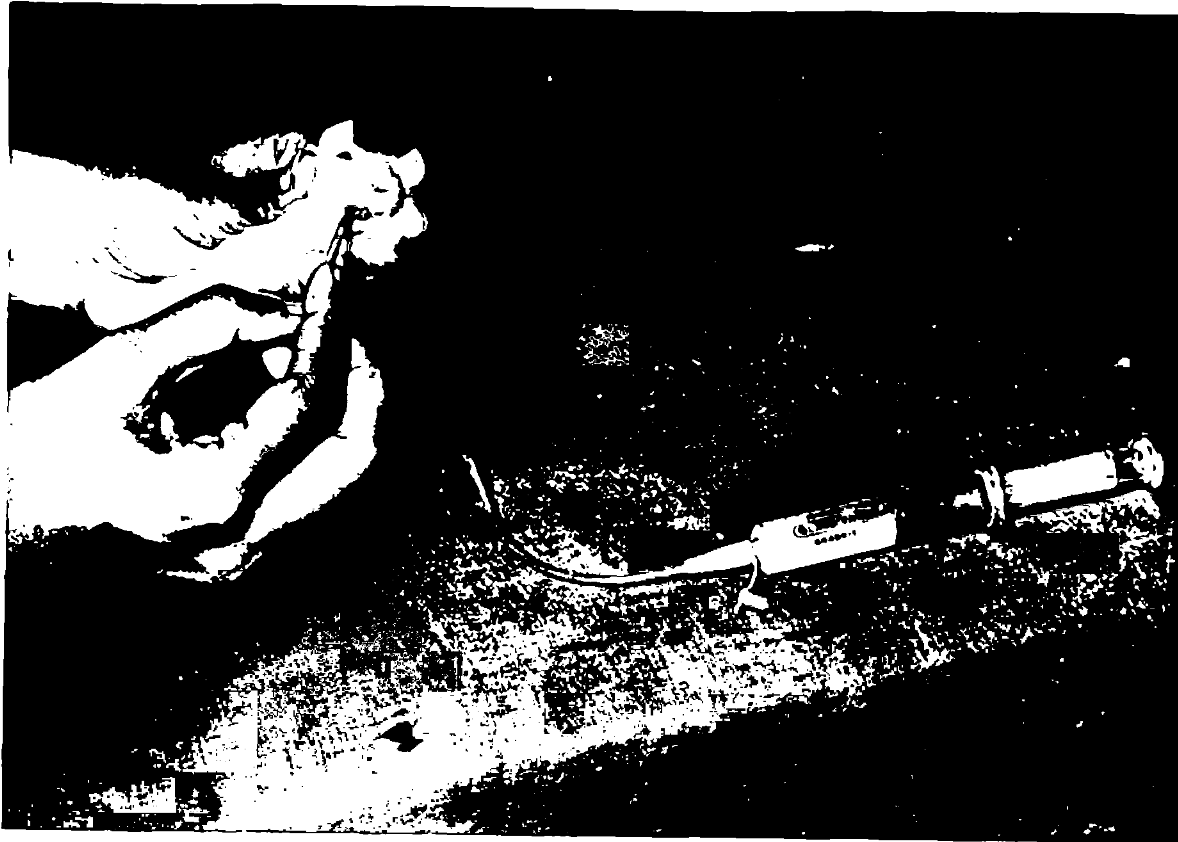


Plate II. Feeding Of The Animal

This was dissolved in 4ml of water and fed orally using a special polythene feeding tube. Blood samples were collected initially just before administration of glucose and after feeding of glucose, at intervals of half an hour, for 2 hours, from the tail as described earlier. The blood glucose was estimated immediately by Folin-Wu method (Appendix III).

b. <u>Glucose tolerance test with glucose and ginger:</u>

The second group of rats were used to find out the effect of ginger on blood glucose level.

1 g of fresh ginger (free from skin) and 0.4g of glucose were ground well with little of water to give a homogenous solution and made upto 4ml. Fasting blood samples were collected. Then the rats were fed with glucose and ginger juice. The time of feeding was noted. The blood samples were collected from then every half an hour upto two hours. Blood glucose was estimated as before by Folin-Wu method.

TABLE III

COMPOSITION OF STOCK DIET

| Ingredients | Amount per 100g |
|---|---|
| Wheat flour | 35 |
| Bengal gram flour | 16 |
| Green gram flour | 15 |
| Ground nut oil | 10 |
| Milk | 15 |
| Greens | 5.0 |
| Yeast | 1.0 |
| Cod liver oil | one drop |
| Salt mixture | 0.9 |
| Beef liver | 1.2 |

D. <u>Statistical analysis:</u>

For Food intake, weight gain and immediate effect of ginger on serum cholesterol analysis of variance was done. For serum cholesterol liver cholesterol and blood glucose 't' test was carried out. The details of statistical analysis is given in appendix IV.

# IV. RESULTS AND DISCUSSION

The findings of the present study are discussed under the following headings.

A. Experiment I

1. Phase I:

a) Food intake and weight gain

(i) Food intake (ii) weight gain

b) Effect of ginger on blood cholesterol level

2. Phase II

a) Food intake and weight gain

b) Effect of ginger on blood cholesterol levels of hypercholesterolemic rats.

B. Experiment II - Immediate effect of ginger on blood cholesterol

B. Experiment III

Effect of ginger on blood glucose levels.

A. ExperimentI

1. Phase I

a) Food intake and weight gain

The rats are divided into three groups in the I phase. Group A consisting of seven rats formed the control group and received the basal diet. Group B was made up of fourteen rats and they were fed the basal diet supplemented with 1% cholesterol. Group C had seven rats and received 1% cholesterol and 10% ginger (dry weight basis) in addition to the basal diet.

Effect of ginger on the food intake and weight gain of the rats were studied and the observations are discussed below:

(i) <u>Effect of ginger on food intake</u>

Table I gives the mean values of the food intake in the three stages, 1-8 (1st stage), 9 to 16 (2nd stage) and 17-24 (3rd stage) days. Fig.1 gives diagrammatically the effect of ginger on food intake.

TABLE I

FOOD INTAKE OF RATS DURING THE I PHASE OF THE EXPERIMENTAL PERIOD

| Stages / Groups | Average food intake in stages (g/day/rat) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Mean |
| Basal control group (A) | 10.51 | 12.88 | 14.18 | 12.52 |
| Cholesterol group (B) | 10.30 | 12.58 | 14.25 | 12.38 |
| Cholesterol + Ginger group(C) | 7.05 | 10.14 | 13.85 | 10.35 |
| Mean | 9.29 | 11.87 | 14.09 | |

| | SE | CD |
|---|---|---|
| <u>Stages</u> | 0.08246 | 0.2327 |
| | SED | CD |
| <u>Groups</u> | | |
| A Vs B | 0.1166 | 0.2327 |
| A Vs C | 0.1347 | 0.2686 |
| B Vs C | 0.1166 | 0.2327 |

| **Stages x Groups** | SE | CD |
|---|---|---|
| Group A | 0.02[illegible] | 0.07674 |
| Group B | 0.1166 | 0.3290 |
| Group C | 0.0272 | 0.07674 |

| **Stages** | SED | CD |
|---|---|---|
| A Vs B | 0.2020 | 0.4030 |
| A Vs C | 0.2332 | 0.4653 |
| B Vs C | 0.2020 | 0.4030 |

**Conclusion:**

| | | | | | |
|---|---|---|---|---|---|
| (i) | Stages | | 1 | 2 | 3 |
| (ii) | Groups | | A (line over A–B) | B | C |
| (iii) | Stages x Groups | | | | |
| Groups | Groups | A | 1 | 2 | 3 |
| | Group | B | 1 | 2 | 3 |
| | Group | C | 1 | 2 | 3 |

Stages

| | | | |
|---|---|---|---|
| Stage 1 | A (line over A–B) | B | C |
| Stage 2 | A (line over A–B) | B | C |
| Stage 3 | A (line over A–C) | B | C |

**Stages:**

When the 3 stages are compared food intake is significantly different. Feed intake is maximum in the third stage and minimum in the first stage. This may be

Fig. 1

Food Intake Of Rats During The I Phase Of The Experiment

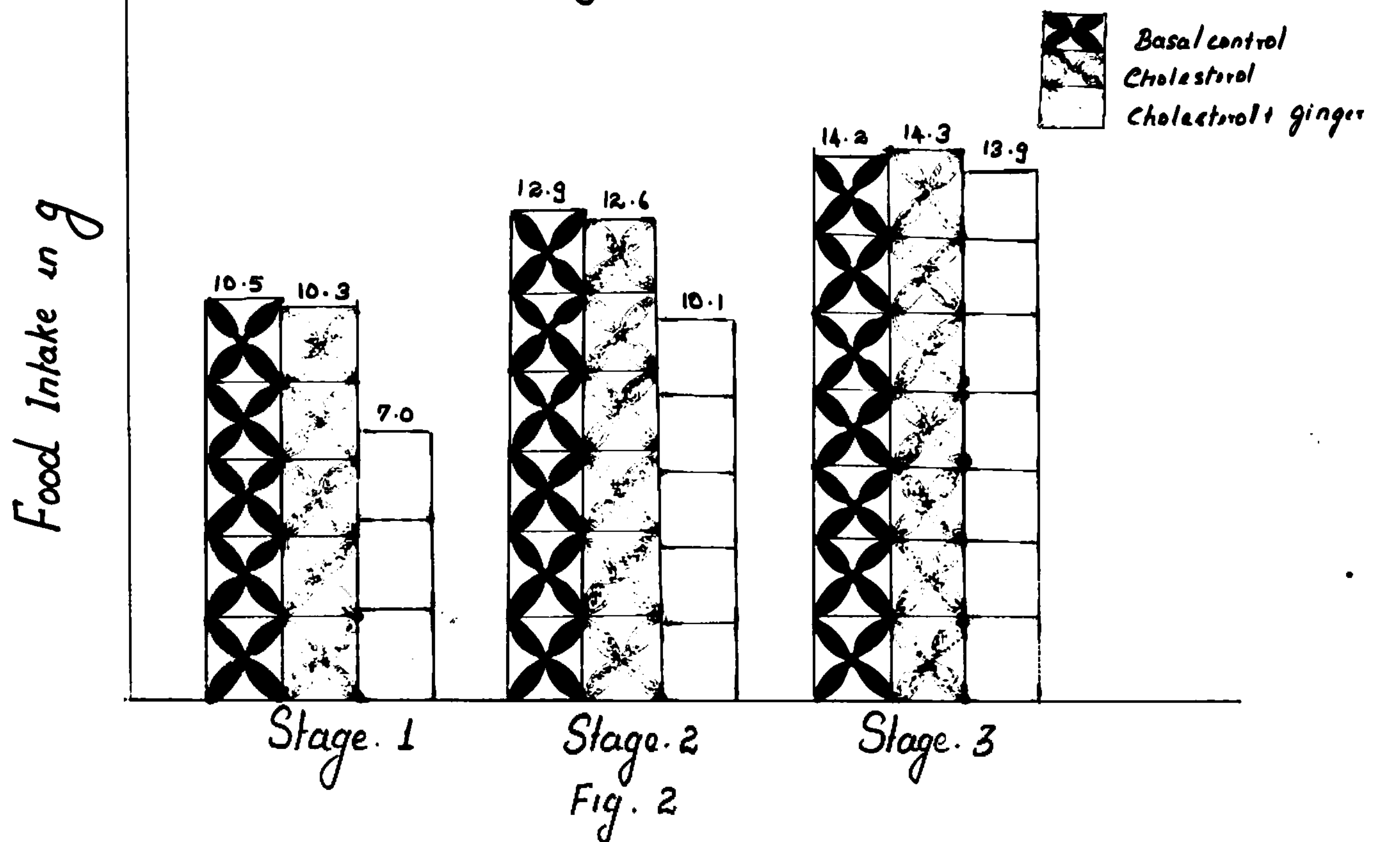



Fig. 2

Weight Gain Of Rats During The I Phase Of The Experiment

Basal control
Cholesterol
Cholesterol + ginger

Weight Gain in g

21.9 21.7 11.5

35.6 33.9 22.1

34.5 33.7 32.0

Stage. 1 Stage. 2 Stage. 3

due to the increasing age and exposure of rats to the new diet in the case of groups A and B. In group C it is mainly due to the hot nature of the ginger present in the diet.

Groups:

When the 3 groups are compared the maximum food consumption is recorded in the basal control group, Followed by the cholesterol group and the ginger group in the order. The decrease is statistically significant between the basal control group and ginger group, but not between the basal control group and cholesterol group. The decreased food intake in the ginger group may be due to the hot nature of the ginger added in the diet, eventhough the rats received the ginger in the cooked form they could not eat much in the first stage, but in the subsequent stages their consumption improved and in the 3rd stage it is equal to groups A and B.

Stages x groups

In groups A, B and C when the stages are compared maximum food intake is in stage 3, then 2nd stage and then comes the 1st stage.

In stage 1 and 2 there is no significant difference between groups A and B. That is the added cholesterol does not bring about any significant change in the food intake, Whereas the ginger added in the diet decreased the food intake significantly. But in stage 3, there is no significant difference in the food intake of all the three groups.

ii. Weight gain

The weight gain of rats in the I phase of the experiment are discussed below:

Table II gives the mean values of the weight gain in the three stages, 1-8 (1st stage), 9-16 (2nd stage) and 17-24 (3rd stage) days. Fig.2 diagrammatically represents the effect of ginger on body weight gain.

TABLE II

WEIGHT GAIN OF RATS DURING THE I PHASE OF THE EXPERIMENTAL PERIOD

| Groups \ Stage | Average weight gain in stages (g/rat) | | | Mean |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Basal control group (A) | 21.91 | 35.64 | 34.49 | 30.68 |
| Cholesterol Group (B) | 21.72 | 33.90 | 33.66 | 29.43 |
| Cholesterol + Ginger Group (C) | 11.47 | 22.11 | 31.97 | 21.85 |
| Mean | 18.37 | 30.22 | 33.37 | |

| | | SE | CD |
|---|---|---|---|
| **Stages** | | 0.4419 | 1.2468 |
| | | SED | CD |
| **Groups** | | | |
| A Vs B | | 0.62.50 | 1.2468 |
| A Vs C | | 0.7217 | 1.4397 |
| B Vs C | | 0.6250 | 1.2468 |
| | | SE | CD |
| **Stages x Groups** | | | |
| Groupm | A | 0.8839 | 2.4936 |
| Group | B | 0.6250 | 1.7633 |
| Group | C | 0.8839 | 2.4936 |
| **Stages** | | SED | CD |
| A Vs B | | 1.0824 | 2.1596 |
| A Vs C | | 1.2500 | 2.4940 |
| B Vs C | | 1.0824 | 2.1596 |

**Conclusion**

| | | | | |
|---|---|---|---|---|
| (i) | Stages | 1 | 2 | 3 |
| (ii) | Groups | $\overline{A \quad\quad B}$ | | C |

(iii) Stages x Groups

Groups

| | | | | |
|---|---|---|---|---|
| Group | A | 1 | $\overline{2 \quad\quad 3}$ | |
| Group | B | 1 | $\overline{2 \quad\quad 3}$ | |
| Group | C | 1 | 2 | 3 |

Stages

| | | | | |
|---|---|---|---|---|
| Stage | 1 | $\overline{A \quad\quad B}$ | | C |
| Stage | 2 | $\overline{A \quad\quad B}$ | | C |
| Stage | 3 | $\overline{A \quad\quad B \quad\quad C}$ | | |

**Stages:**

With increasing age the weight gain also increases.

**Groups:**

When the groups are compared the basal control group recorded the maximum weight followed by cholesterol group and ginger group in the order. The difference in weight gain between basal control group and cholesterol group is not significant.

Stages x Groups:

In groups A and B stages 2 and 3 do not show any difference. That is in group A and B, there is no difference in weight gain in stages 2 and 3. Weight gain in stage 1 is lower than the weight gain in stages 2 and 3.

In group C, weight gain is in the following order stage 3 > stage 2 > stage 1.

In stages 1 and 2 groups A and B show higher weight gain than group C. In stage 3 weight gain is not statistically significant in all the 3 groups.

b. Effect of ginger on serum cholesterol level

Table III gives the effect of ginger on serum cholesterol levels during the 1 phase of the experiment. Fig. 3 diagrammatically represents the same.

TABLE III

EFFECT OF GINGER ON SERUM CHOLESTEROL LEVELS (mg/100ml)

| Groups | initial cholesterol level | Cholesterol level at the end of the I phase | Increase in blood cholesterol during I phase | 't' value |
|---|---|---|---|---|
| Basal control group (A) | 84.71 ± 5.52 | 95.43 ± 4.44 | 10.72 ± 1.67 | A Vs B-72.937** |
| Cholesterol group (B) | 82.21 ± 5.69 | 286.36 ± 10.16 | 204.15 ± 6.57 | A Vs C-34.1005** |
| Cholesterol + Ginger Group(C) | 81.00 ± 5.29 | 187.43 ± 8.09 | 106.43 ± 6.67 | B Vs C-30.404** |

**significant at 1% level.

Fig. 3

Effect Of Ginger On Serum Cholesterol

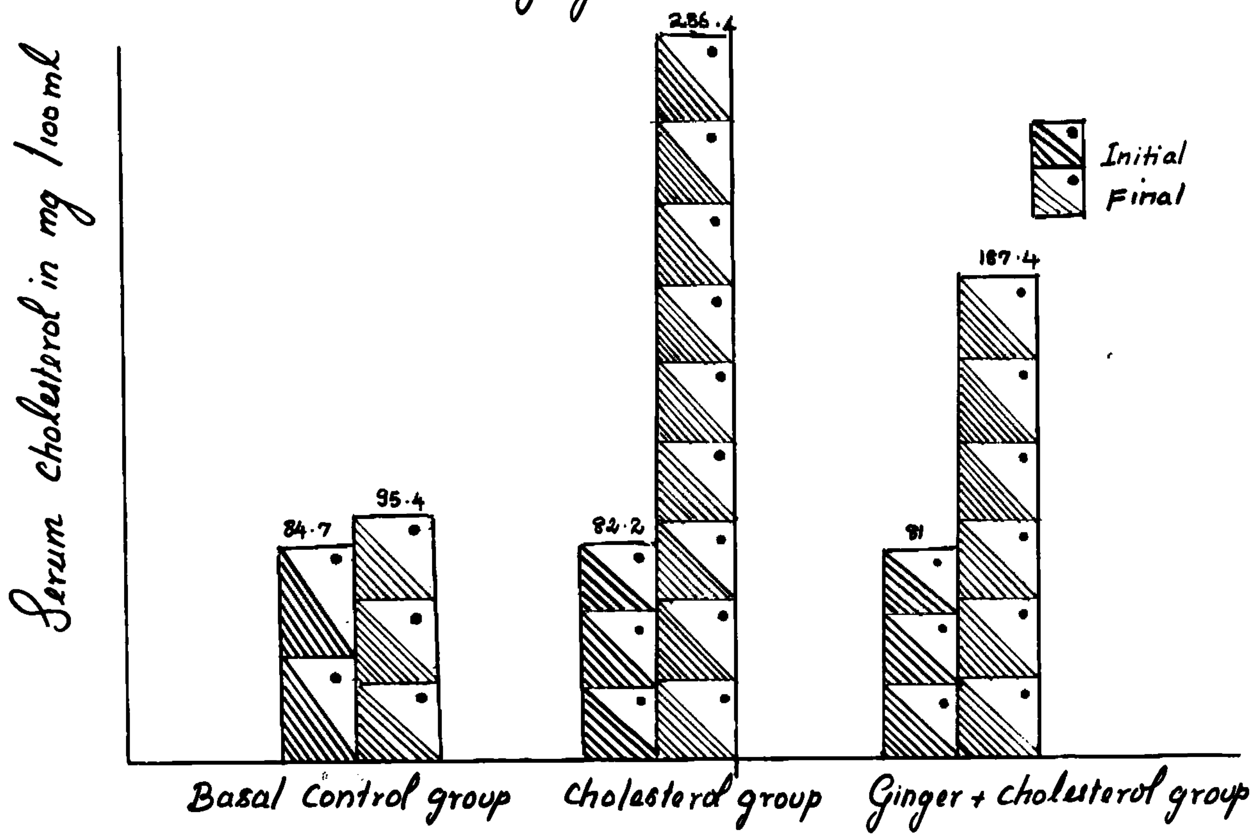
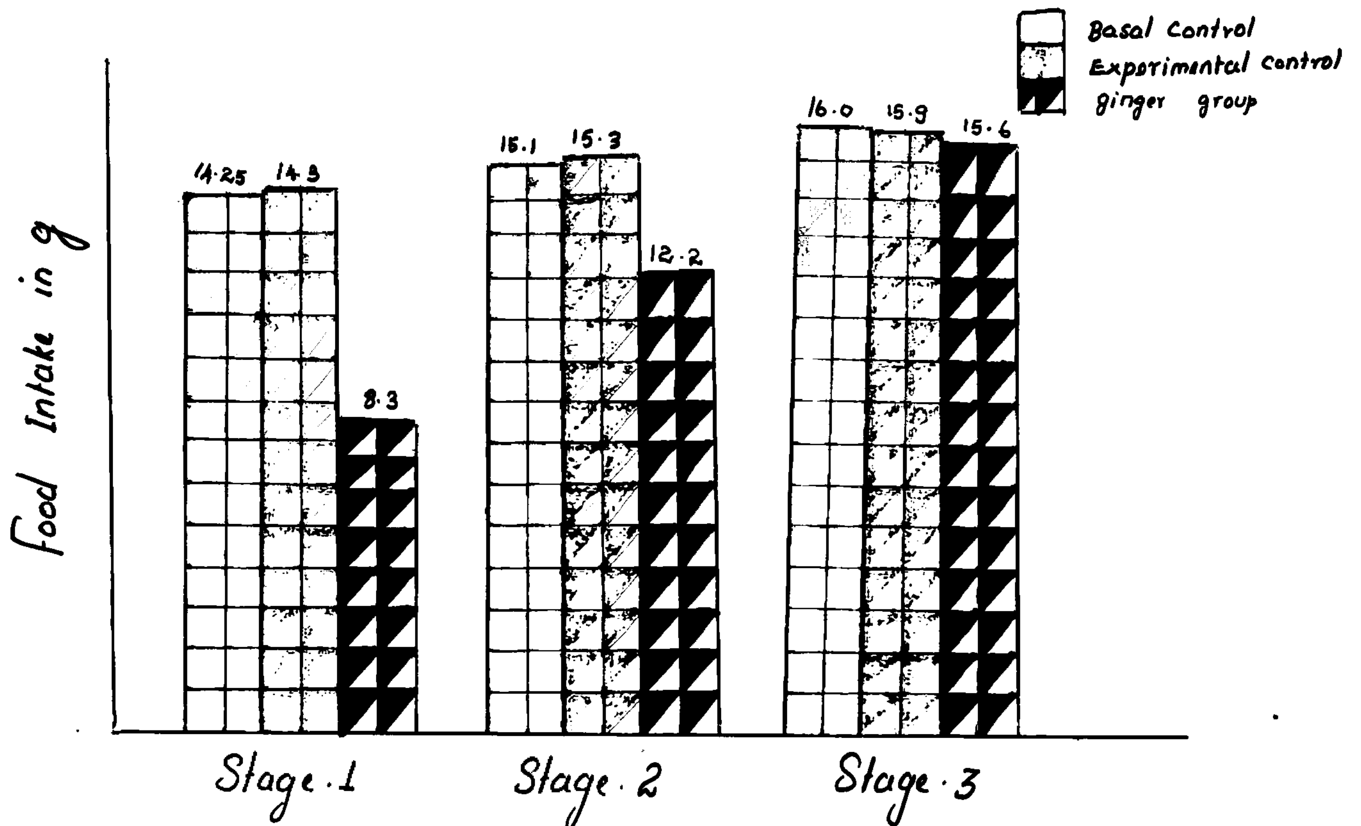



Fig. 4

Food Intake Of Animals During The II Phase Of The Experiment



When the mean increase in the cholesterol levels of the three groups are compared, significant difference are noted between the groups. Basal control group shows 10.72 mg/100ml and this increase may be due to the increase in age during the experimental period. Group B, which received 1% cholesterol shows a mean increase of 204 mg/100ml. Cholesterol group recorded three fold (286.36) increase compared to the basal group (95.43). The hyper cholesterolemia observed is due to the 1% cholesterol which was added to the diet at the cost of starch. Group C which received ginger along with 1% cholesterol shows only an increase of 106.4mg/100ml. This increase id less than in group B, which received only cholesterol and no ginger. It is evident that the given ginger decreased the effect of added cholesterol, by 50 per cent nearly, though it could not prevent completely the rise in cholesterol level due to added cholesterol. Ginger decreased cholesterol level by 97.6 mg/100ml, which is significant at 1% level. From the results of this study it is clear that when cholesterol is included in the diet at 1% level it increases the serum cholesterol significantly but when ginger is administered along with cholesterol, the added ginger prevents the increase of blood cholesterol levels significantly. The fibre content of ginger may have contributed to some of

cholesterol lowering properties. Similar observations were made by Gujral et al., (1978) with ginger oleoresin.

2. Phase II

a. Food intake and weight gain

(i) Food intake

Table IV gives the mean values of food intake by different groups of rats at various stages of the experimental period. Fig.4 diagrammatically represents this.

TABLE IV

FOOD INTAKE OF ANIMALS DURING THE II PHASE OF THE EXPERIMENT

| Groups \ Stages | Average food intake in stages (g/day/rat) | | | Mean |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Basal control group (A) | 14.26 | 15.06 | 15.97 | 15.10 |
| Experimental control (D) | 14.3 | 15.26 | 15.87 | 15.14 |
| Ginger group (E) | 8.32 | 12.23 | 15.58 | 12.04 |
| Mean | 12.29 | 14.18 | 15.81 | |

<u>Stages x groups:</u>

In stages 1 and 2 there is no significant difference between the food intake of groups A and D. But group E recorded a significant decrease in food intake when compared to A and D. In stage 3 there is no significant difference in the food intake of all the 3 groups.

In groups A, D and E food intake in 3 stages are significantly different. The order of increase of food intake is stage 1 < stage 2 < stage 3. This gradual increase may be due to increase in age as the stage increase. Fig.IV diagrammatically represents the effect of ginger on food intake of the animals at different periods, of the experiment.

(ii) <u>Weight gain</u>

Table V gives the mean values of weight gain in the II phase of the experiment.

## TABLE V

WEIGHT OF RATS DURING THE II PHASE OF THE EXPERIMENT

| Groups \ Stages | Average weight gain of rats in stages (g/rat) 1 | 2 | 3 | Mean |
|---|---|---|---|---|
| Basal Control Group (A) | 30.9 | 33.6 | 33.9 | 32.8 |
| Experimental control group (D) | 30.32 | 32.26 | 33.5 | 32.03 |
| Ginger group (E) | 14.2 | 22.66 | 30.6 | 22.49 |
| Mean | 25.14 | 29.51 | 32.67 | |

| | SE | CD |
|---|---|---|
| (i) Stages | 0.6318 | 1.7924 |
| (ii) Groups | 0.6318 | 1.7924 |
| (iii) Stages x Groups | 1.0943 | 3.1045 |

**Conclusion**

(i) Stages 1 2 3

(ii) Groups A D E

(iii) Stages xGroups

Stages 1 A D E

Stage 2 A D E

Stage 3 A D E

Group A 1 2 3

Group D 1 2 3

Group E 1 2 3

**Stages**

When Stages are compared, stage 1 shows the least and stage 3 the maximum weight gain.

**Groups:**

When groups are compared weight gain in E is less than in A and D, But there is no statistically significant difference in the weight gain between A and D. This is due to the fact that the same diet is fed to both the groups. The decrease noted in the ginger group may be due to the addition of 10% ginger in the diet.

**Stages x groups:**

In stages 1 and 2, group E recorded less weight gain than A and D. But there is no significant difference between the weight gains of A and D. In stage 3 there is no significant difference in the weight gain between the three groups. This may be due to the fact that the rats got accustomed to the given ginger diet and consumed the required amount. In groups A and D no significant difference is noted between stages 1, 2 and 3. But in group E there is gradual increase in weight gain from stages 1 to 3. Fig.5 diagrammatically represents the effect of diet composition on weight gain.

Fig. 5.

Weight Of Rats During The II Phase Of The Experiment.

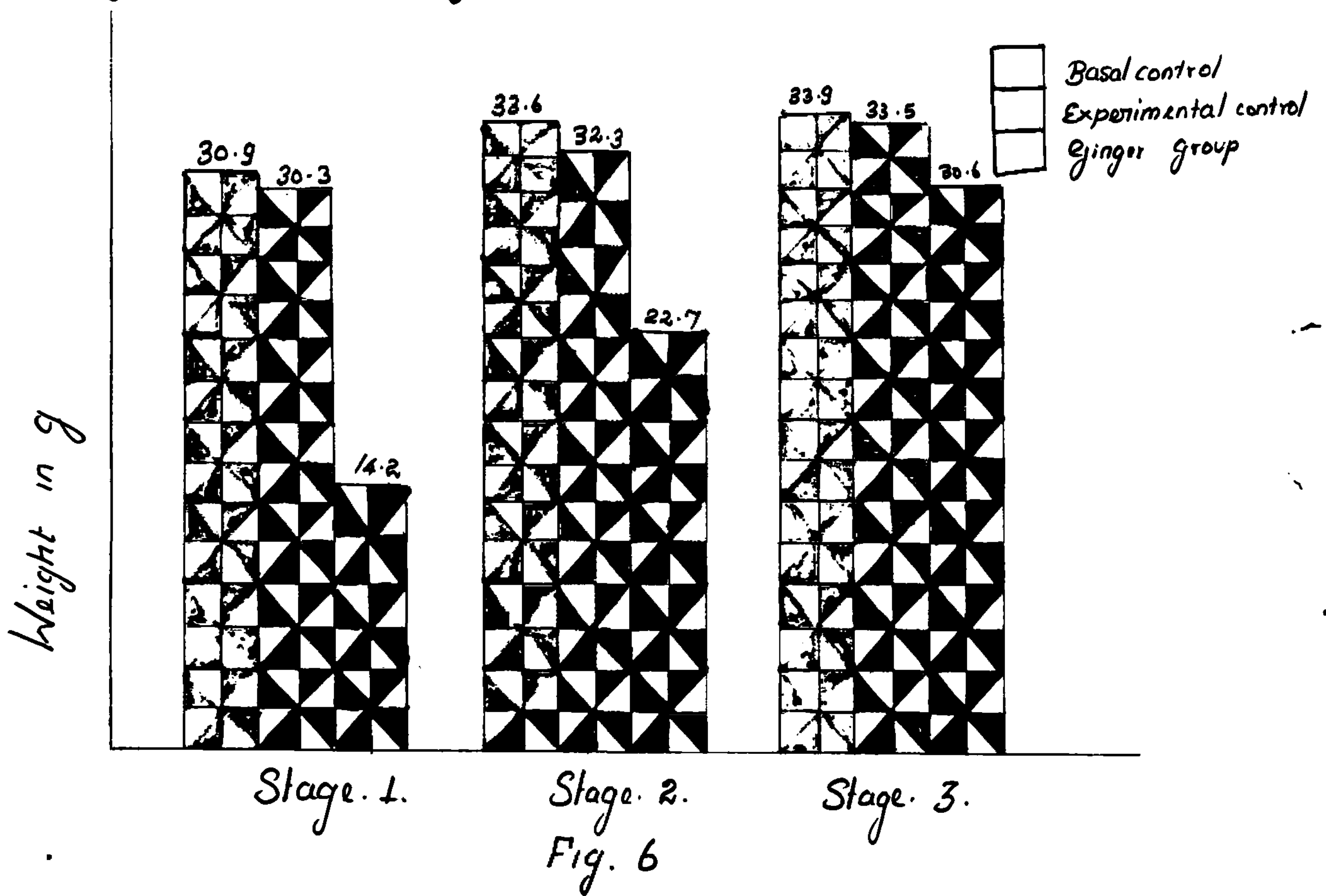
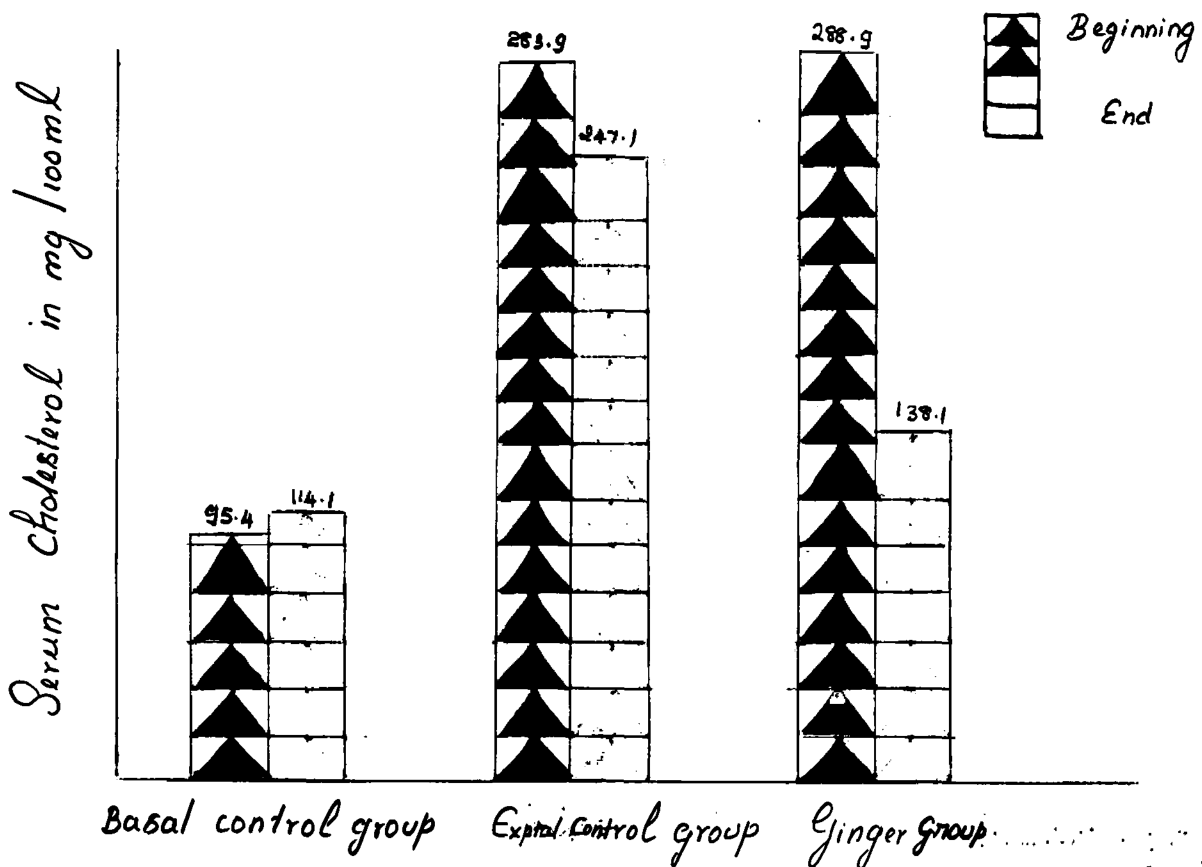



Fig. 6

Effect Of Ginger On Serum Cholesterol Level Of Hypercholesterolemic Rats



b. Effect of ginger on serum cholesterol levels of hyperchclesterolemic rats.

Table VI gives the effect of ginger on serum cholesterol levels of hypercholesterolemic rats.

TABLE VI

EFFECTS OF GINGER ON SERUM CHOLESTEROL LEVEL OF HYPERCHOLESTEROLEMIC RATS (mg/100ml)

| Groups | Cholesterol level at the beginning of II Phase | Cholesterol level at the end of II phase | Difference (bet. Initial and final) | 't' Value |
|---|---|---|---|---|
| Basal control group (A) | 95.43 ± 4.44 | 114.14 ± 5.67 | 18.71 ± 3.1 | A Vs D = 6.64** |
| Experimental control group (D) | 283.86 ± 7.02 | 247.14 ± 8.69 | 36.71 ± 5.87 | A Vs E = 39.05** |
| Ginger group (E) | 288.86 ± 11.67 | 138.14 ± 8.58 | 150.72± 7.67 | D Vs E = 20.93** |

** Significant at 1% level

When the mean difference of the three groups are compared there is significant difference between the groups. In group A which received basal diet the cholesterol level increased during the II phase of experimental period. In groups D and E cholesterol level decreased during the same period. Their (group D & E) cholesterol levels were high at the end of the I phase. The increase in serum cholesterol level caused by the dietary cholesterol, is decreased by ginger (group E); but in group D

also cholesterol levels are found to be decreased. This group, in the II phase received only basal diet. Therefore this decrease (group D) reveals the spontaneous decrease of cholesterol with time in the absence of cholesterol and ginger. However, the difference in the cholesterol levels between groups D and E are statistically significant. Therefore this indicates that the given ginger reduced the blood cholesterol level significantly. Fig.6 diagrammatically represents the effect of ginger on hypercholesterolemic rats.

e. Effect of ginger on liver cholesterol levels of hypercholesterolemic rats.

Table VII gives the effect of ginger on liver cholesterol of hypercholesterolemic rats. Fig.7 diagrammatically represents the same.

TABLE VII

EFFECTS OF GINGER ON LIVER CHOLESTEROL LEVEL OF HYPERCHOLESTEROLEMIC RATS

| Groups | Liver cholesterol in mg/g | Groups compared | 't' value |
|---|---|---|---|
| Basal control group (A) | 5.98 ± 0.54 | A Vs D | 24.92** |
| Experimental control group (D) | 19.46 ± 1.21 | A Vs E | 7.76** |
| Ginger group (E) | 8.47 ± 0.57 | D Vs E | 20.13** |

** Significant at 1% level.

Fig. 7.

Effect Of Ginger On Liver Cholesterol Levels Of Hypercholesterolemic Rats

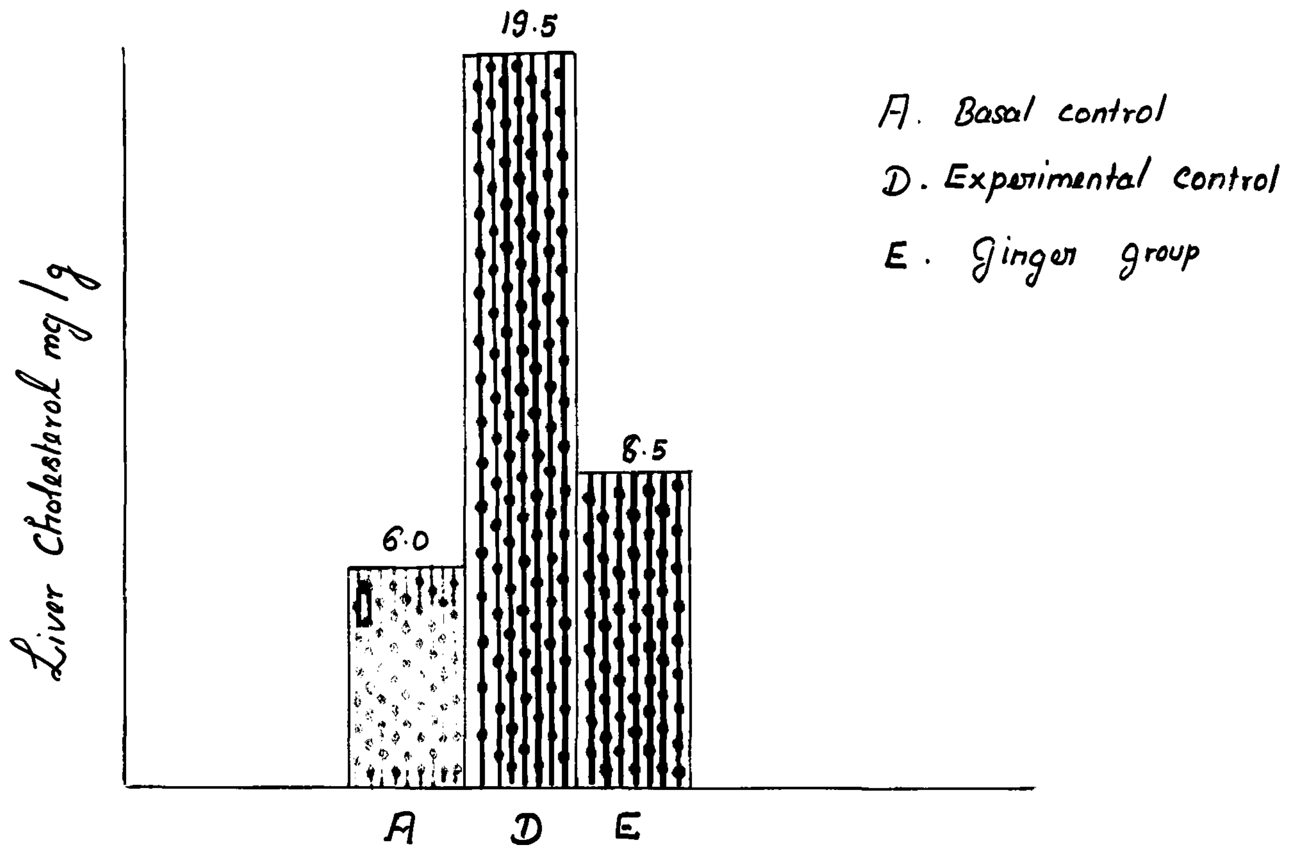
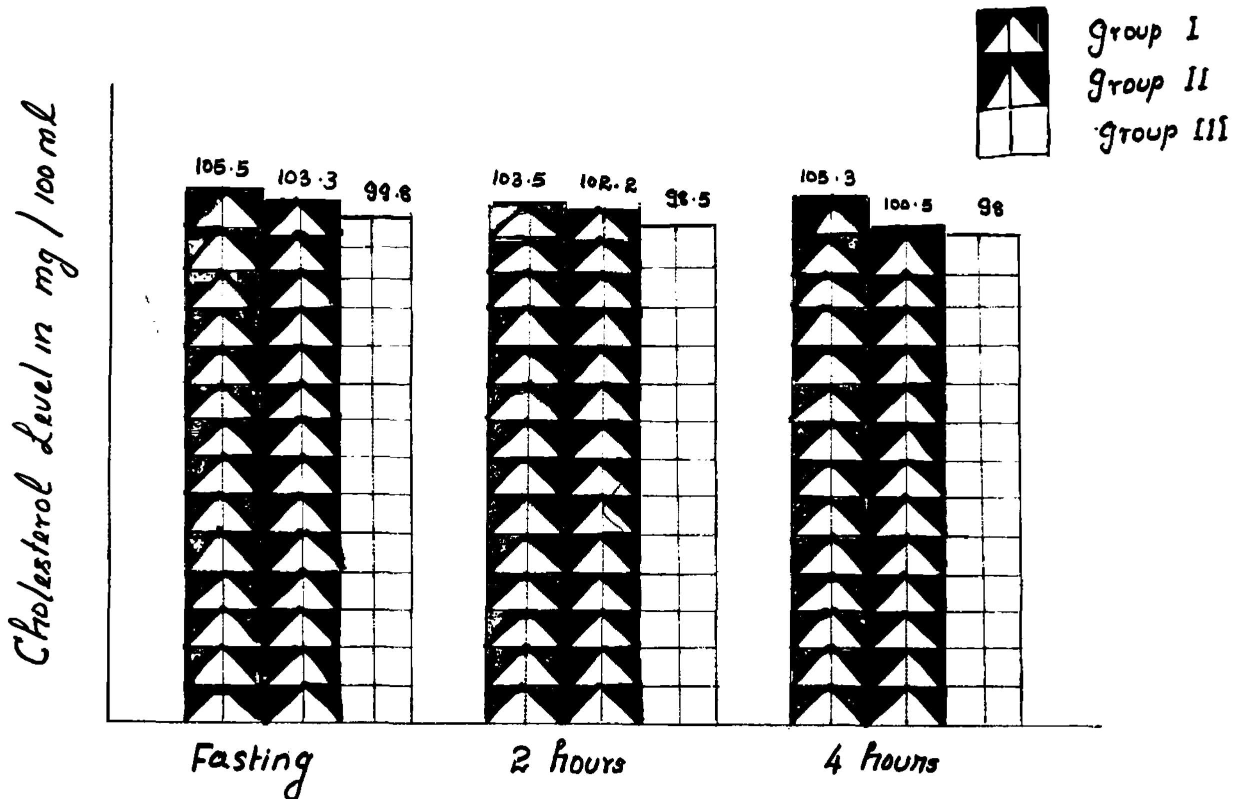



Fig. 8

Immediate Effect Of Ginger On Serum Cholesterol



When liver cholesterol values of the three groups are compared, group A recorded the lowest values followed by group E and Group D in the increasing order. Group D as already explained received basal diet (in the II phase) after an intake of cholesterol rich diet for 24 days in the I phase. Group E which received hypercholesterolemic diet in the I phase was kept on a ginger diet in the II phase. And this addition of ginger to group E reduced the liver cholesterol significantly (at 1% level) when compared to group A. This may be due to the increased metabolism of cholesterol or decreased synthesis of cholesterol.

B. Experiment II

Immediate effect of ginger on serum cholesterol.

Table VIII gives the immediate effect of ginger on serum cholesterol. Fig.8 diagrammatically represents the same.

TABLE VIII

IMMEDIATE EFFECT OF GINGER ON SERUM CHOLESTEROL

| Time \ Groups | Mean serum cholesterol in mg/100ml | | | |
|---|---|---|---|---|
| | Group I (cholesterol) | Group II (cholesterol + Ginger) | Group III (Ginger) | Mean |
| Fasting | 105.50 | 103.33 | 99.83 | 102.89 |
| 2 hours | 103.50 | 102.17 | 98.50 | 101.39 |
| 4 hours | 105.33 | 100.50 | 98.00 | 101.28 |
| Mean | 104.78 | 102.00 | 98.78 | |

| | | SE | CD |
|---|---|---|---|
| (i) | Groups | 1.4377 | 4.0990 |
| (ii) | Time | 1.4377 | 4.0990 |
| (iii) | Group x time | 2.4902 | 7.0997 |

**Conclusion**

(i) Groups — I II III

(ii) Time — 0 2 4

(iii) Groups x Time

Time 0 — I II III

Time 2 — I II III

Time 4 — I II III

Group I — 0 2 4

Group II — 0 2 4

Group III — 0 2 4

(i) Groups:

When cholesterol level in different groups are compared it is clear that there is no significant difference between them.

(ii) Time:

When cholesterol level at different time intervals say 0, 2 and 4 hours are compared, there is no significant difference between their cholesterol levels.

(iii) Groups x Timings:

In groups I, II and III, when the cholesterol level at 0, 2 and 4 hours are compared there is no significant difference. In the same way there is no significant difference between groups at different time intervals. This clearly indicates that the given ginger or ginger plus cholesterol has no immediate effect on serum cholesterol level.

c. Experiment III

Effect of ginger on blood glucose level.

Table IX gives the effect of ginger on blood glucose level. Fig.9 diagrammatically represents this.

TABLE IX

EFFECT OF GINGER ON BLOOD GLUCOSE LEVEL

| Time | Blood glucose in mg/100ml | | | | 't' Value |
|---|---|---|---|---|---|
| | Ginger and glucose fed rats | | Glucose fed rats | | |
| Fasting | A. | 75.75 ± 3.06 | F. | 76.33 ± 3.87 | A Vs F 0.4296 NS |
| 1/2 hour | B. | 82.83 ± 3.95 | G. | 92 ± 7.82 | B Vs G 3.4711 ** |
| 1 hour | C. | 100.08 ± 5.81 | H. | 124.58 ± 4.92 | C Vs H 7.0002 ** |
| 1 1/2 hours | D. | 88 ± 5.83 | I. | 101.5 ± 8.41 | D Vs I 4.375 ** |
| 2 hours | E. | 76.08 ± 5.69 | J. | 86.67 ± 6.98 | E Vs J 3.9167 ** |

** Significant at 1% level. NS - not significant.

Table IX gives the blood sugar levels of rats fed with ginger plus glucose and glucose at various time intervals including fasting sugar level. There is no statistically significant difference in the fasting blood sugar of the two groups. Comparison of 1/2 an hour, 1 hour, 1 1/2 hour and 2 hour samples reveals a significant difference that is, the rats receiving ginger plus glucose recorded low blood sugar values compared to their counterparts receiving only glucose. The decrease is found to be significant

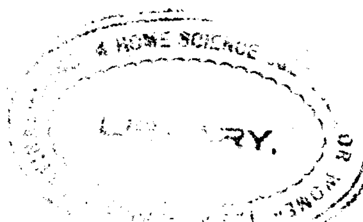
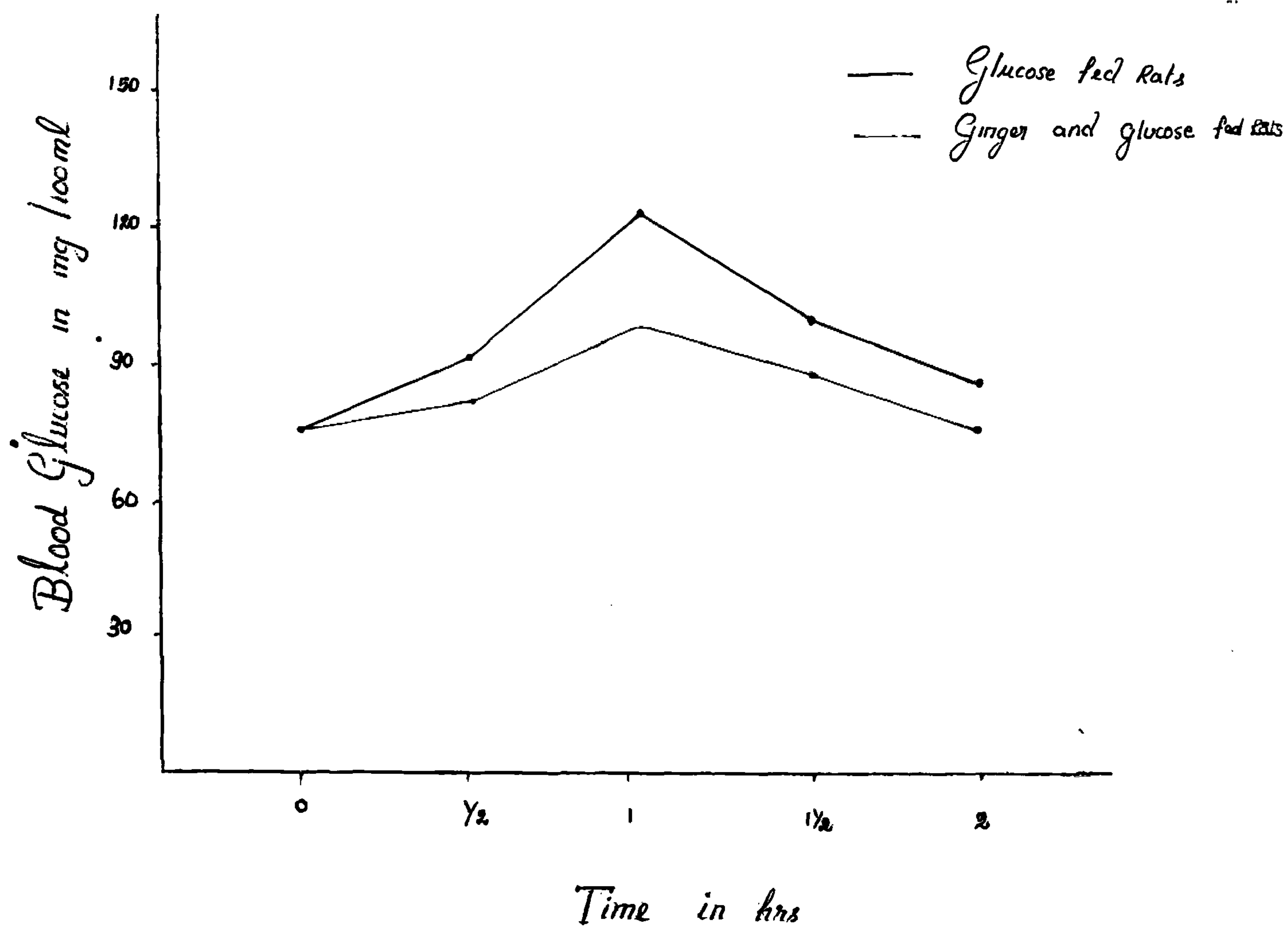


Fig. 9

Effect Of Ginger On Blood Glucose Level



at 1% level. Hence it is evident that ginger also acts as hypoglycemic agent in addition to its role as hypocholesterolemic agent. So ginger can be adviced for diabetic patients in whom both blood sugar and cholesterol are elevated. The mechanism of this action may be due to (1) increased utilisation of glucose (2) decreased synthesis of glucose (3) increased secretion of insulin which might decrease the glucose level. Ginger lowers glucose immediately, but it has no immediate effect on cholesterol. This finding agrees with the report of Sharma and Shukla (1978). The values recorded during this investigation was given in Appendix V.

## V. SUMMARY AND CONCLUSION

In the present investigation an attempt has been made in rats to study (1) the effect of ginger on (a) food intake (b) weight gain (c) serum cholesterol level and (d) liver cholesterol (2) the immediate effect of ginger on serum cholesterol and (3) effect on blood glucose level. The findings of the study are presented below:

A. Experiment I

Phase I:

The study consisted of three experiments. Experiment I was conducted in two phases. In phase I the effect of ginger on food intake, weight gain and serum cholesterol levels of rats fed ginger for 24 days was studied. In phase I twenty eight male albino rats of Wistar strain, weighing 120-150g were selected and divided into three groups A, B and C. Group A consisting of seven rats formed the control group and received the basal diet. Group B was made up of fourteen rats and they were fed the basal diet supplemented with 1 per cent cholesterol. Group C had seven rats and received 1 per cent cholesterol and 10 per cent ginger (dry weight basis) in addition to the basal diet.

At the end of the experimental period of 24 days fasting blood samples were collected from all the twenty eight rats. The serum was separated and cholesterol was estimated.

After the collection of blood samples rats of ginger group (c) were sacrificed. The other two groups A and B were used for the II phase of the experiment.

The results of phase I indicated an increase in food intake with stage in the following order stage 3 > stage 2 > stage 1. (Stage 1-9.29g, stage 2-11.87g and stage 3-14.09g). In stages 1 and 2, groups A and B showed higher food intake than C. In stage 3, there was no significant difference in food intake of the three groups. In all the three groups, there was an increase in food intake with stage. This may be due to increasing age. In groups A and B the food intake was higher than in group C (group A - 12.52g, group B - 12.30g. and group C - 10.35g). The decreased food intake in the group C may be due to the hot nature of ginger added in the diet.

Weight gain was found to increase with stage in the order stage 1 < stage 2 < stage 3.

When groups were compared, weight gain in groups A and B were higher than in group C. (group A - 30.68g, group B - 29.43g and group C - 21.85g). This may be due to the decreased food intake in group C. In groups A and B, stages 2 and 3 showed higher weight gain than stage 1. In group C the weight gain was in the order stage 1 < stage 2 < stage 3 and the difference between these groups were significant. In stage 1 weight gain in groups A and B were higher than in group C. In stage 2 the weight gain was in the order group A > group B > group C. In stage 3 the weight gain in all the 3 groups were almost equal.

When the mean increase in the serum cholesterol levels of the three groups in the I phase were compared, significant differences were noted between the groups. The basal control group recorded a mean increase of 10.72 mg of cholesterol per 100ml. of serum and this increase might be due to the increase in age, during the experimental period. Group B, which received 1 per cent cholesterol showed a mean increase of 204 mg of cholesterol per 100ml of serum which was three fold, compared to the basal control group. The hypercholesterolemia observed was due to the 1 per cent cholesterol added in the diet. Group C which received ginger along with 1 per cent cholesterol

showed only an increase of 106.4 mg of cholesterol per 100ml of serum. This increase was less than in group B, which received only cholesterol and no ginger. It was evident that the given ginger decreased the effect of added cholesterol by 50 per cent nearly, though it could not prevent completely the rise in cholesterol level due to added cholesterol. Ginger decreased cholesterol level by 97.6 mg/100ml. From the results of this study, it was clear that when cholesterol was included in the diet at 1 per cent level, it increased the serum cholesterol significantly, but when ginger was administered along with cholesterol, the added ginger prevented the increase of blood cholesterol level significantly.

Phase II

In phase II the effect of ginger on food intake, weight gain and serum and liver cholesterol levels of hypercholesterolemic rats were observed. In this phase the rats of group A of phase I were continued to be fed with the same basal diet and served as basal control group (A) in the II phase. The fourteen rats in the cholesterol group (B) were divided equally into two groups D and E.

Group D was fed with basal diet. This formed the experimental control group (D) in phase II. The remaining seven rats of group E were fed the basal diet supplemented with 10 per cent ginger on dry weight basis and this formed the ginger group (E) in the II phase.

At the end of II phase of 24 days, fasting blood samples were collected from all the 21 rats and the serum was separated and cholesterol was estimated.

After the collection of blood samples all the rats were sacrificed, their livers collected and hepatic cholesterol estimated.

Food intake in phase II showed an increasing trend from stage 1 to 3 stage 1 < stage 2 < stage 3. When the three groups were compared food intake in groups A and D were higher when compared to group E (group A - 15.10g, group D - 15.14g and group E 12.04g). Thus in stage 1 and 2 food intake in groups A and D were almost equal while in E the value was lower. But in stage 3 the food intake in all three groups were nearly equal and did not show any statistically significant variation. In all the three groups the food consumption increased with increase in stage (stage 1- 12.29g, stage 2 - 14.18g and stage 3 - 15.8g).

When the mean weight gain in phase II was compared, weight gain increased in the order stage 1 $<$ stage 2 $<$ stage 3. This was probably due to increased consumption of food with increase in age. Weight gain in groups A and D were higher when compared to group E (group A - 32.8g, group D - 32.03g and group E - 22.49g). In all the 3 stages weight gain in groups A and D were almost equal while that in E was significantly less. In all the 3 stages the weight gain increased with increase in stage (stage 1 - 25.14g, stage 2 - 29.51g and stage 1 - 32.67g).

In group A cholesterol level increased by 18.71mg. This increase during the II phase was due to increase in age. In groups D and E cholesterol level decreased during the II phase. The decrease in group E may be due to the ginger in the diet. In group D which did not receive any ginger also recorded a significant decrease. But this decrease may be due to the removal of cholesterol, from the diet.

The liver cholesterol values recorded by group A (5.98mg/g) group D (19.46mg/g) and group E (8.47 mg/g) showed that liver cholesterol values varied in the following

order: group A < group E < group D. Thus maximum value was observed in group D which received no ginger followed by group E which was kept on ginger diet in the II phase (but in the I phase both the groups D and E were on atherogenic diet). Group A which was kept in both phases under basal diet recorded the minimum value .

Experiment II

In experiment II the immediate effect of ginger on serum cholesterol was studied. In this experiment eighteen male albino rats of Wistar strain weighing 120-150g were taken and divided equally into three groups (I, II and III). Group I received 0.2g of cholesterol dissolved in 2ml of groundnut oil. Group II received 2 ml of fresh ginger juice containing 1g of fresh ginger and 0.2ml of cholesterol dissolved in 2ml of groundnut oil. Group III received 2 ml of fresh ginger juice containing 1g of fresh ginger.
On the day of the experiment, fasting, 2 hour and 4 hour blood samples were collected from all the groups and serum was separated and cholesterol was estimated.

The results of this experiment showed that there was no significant difference in cholesterol values between groups and between time intervals in the various groups and within groups. This showed that ginger had no immediate effect on serum cholesterol.

C. Experiment III

In this experiment the effect of ginger on blood glucose level was studied. 24 male albino rats (Wistar strain). weighing 190-200g were taken for the experiment and divided into two groups and glucose tolerance test was carried out by giving glucose to one group and glucose plus ginger to another group.

The glucose group recorded 76.33± 3.27, 92 ± 7.82, 124.58 ± 4.92, 101.5 ± 8.41 and 86.67 ± 6.98 mg/100ml of blood in fasting, 1/2, 1, 1 1/2 and 2 hour blood samples respectively. The glucose plus ginger group recorded 75.75 ± 3.06, 82.83 ± 3.95, 100.08 ± 5.81, 88.0 ± 5.83, 76.08 ± 5.69 mg/100ml blood in fasting, 1/2, 1, 1 1/2 and 2 hour blood samples. By comparing the blood glucose values of the two groups it was evident that the glucose values in the glucose plus ginger group was definitely lower compared to the corresponding values of the glucose group. The decrease was found to be statistically significant at 1 per cent level.

Thus the results of the present study indicates that ginger, a commonly used spice in Indian homes lowers both cholesterol and glucose level in blood. Its effect on cholesterol seems to be rather slow compared to its effect on glucose. However, further studied need to be done to understand its mechanism of action.

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# A P P E N D I C E S

# APPENDIX - I

## ESTIMATION OF SERUM CHOLESTEROL BY ZAK'S METHOD

### Principle

Cholesterol reacts with ferric chloride in the presence of concentrated sulphuric acid to give a pink colour. The intensity of the colour developed is directly proportional to the amount of cholesterol present and is read at 540 m/μ in a colorimeter.

### Reagents:

1. Stock ferric chloride reagent:

840 mg of pure dry ferric chloride was weighed and dissolved in 100ml of glacial acetic acid.

2. Ferric chloride precipitating reagent:

10 ml of stock ferric chloride reagent is placed in 100 ml of standard flask and made upto the mark with glacial acetic acid.

3. Ferric chloride diluting reagent:

8.5 ml of stock ferric chloride reagent is diluted to 100ml with pure glacial acetic acid in a 100ml standard flask.

4. Standard cholesterol solution:

100 mg of pure dry cholesterol is placed in a clean dry 100ml standard flask and dissolved in glacial acetic acid and then made up to the mark with the same acid.

5. Working standard:

10ml of the stock standard was placed in a 100ml standard flask containing 0.85 ml of ferric chloride stockreagent and made upto the mark with pure glacial acetic acid. 1.0ml of this solution contains 100µg of cholesterol.

Procedure:

0.5 2.5 ml of working cholesterol solution were pipetted out into a series of clean dry test tubes. The total volume of each tube was made up to 5.0ml with ferric chloride diluting reagent.

To 0.1 ml of the serum, added 4.9 ml of ferric chloride precipitating reagent and mixed well. Allowed to stand for a few minutes and centrifuged. Transferred 2.5ml of the supernatant to a dry test tube and added 2.5ml of ferric chloride diluting reagent and mixed well. The tubes were kept in cold waterbath; to each added 4.0ml of concentrated sulphuric acid drop by drop. The contents

were shaken to mix well. Allowed the solutions to come to the room temperature. A blank was also simultaneiously prepared by taking 5.0ml of the diluting reagent and 4.0ml of concentrated sulphuric acid. After 30 minutes the intensity of the colour developed was read at 540 mμ using the blank as control.

# APPENDIX II

## ESTIMATION OF LIVER CHOLESTEROL

**Reagents:**

1. Aqueous potassium hydroxide solution 33%
2. Petroleum ether boiling point below 50°C
3. Ethanol absolute
4. Sodium sulphate anhydrous
5. Glacial acetic acid
6. Acetyl chloride
7. Chloroform
8. Zinc chloride anhydrous (sticks).

Zinc chloride solution in glacial acetic acid.

40g anhydrous zinc chloride (sticks analytical grade) was quickly crushed and weighed in a dry 250ml bottle and mixed with 153 ml of glacial acetic acid. The bottle was shaken, stoppered and kept at 80°C for 2½ hours with occassional shaking. After cooling to room temperature the solution was filtered by suction through a glass filter.

The zinc chloride reagent was kept in a dark bottle with a tight glass stopper. It could be used until it took up sufficient moisture to become turbid when shaken with chloroform. The reagent was useable for atleast 3 months.

Procedure:

A. Extraction:

100 mg of liver was placed in a 25 ml Erlenmeyer flask, then 33% potassium hydroxide and alcohol were added to make the mixture 17% with respect to potassium hydroxide and 50% with respect to ethanol. The mixture was heated on a steam bath either with the flask sealed with a glass ball or when the amount of cholesterol was particularly small, in a nitrogen atmosphere. The tissue was heated 2 to 3 hours according to the fat content and time required for homogenization.

After cooling to room temperature, each sample was extracted three times with 5 ml portions of petroleum ether (B.P. below 50°C). The extracts (top layer) were combined and washed with water until neutrality to litmus. The combined extract was now dried over anhydrous sodium sulphate and filtered by suction, the sodium sulphate and filtered by suction, the sodium sulphate being washed with petroleum ether. The anhydrous solution was evaporated in vacuo at 40-50°C and the residue transferred with chloroform into a 10ml test tube. The main part of the chloroform was then evaporated on the steam bath to give a final volu me of about 2 ml.

B. Colour reaction:

To each of the above tubes containing about 2 ml chloroform solution was added 1 ml of the zinc chloride reagent and 1 ml of acetyl chloride. The addition of acetyl chloride was most conveniently done with a burette. The contents of the tubes were then mixed with a glass rod and the tubes were placed in a water bath at precisely 65°C for exactly 15 minutes. Thereafter, the tubes were cooled in ice water and the reaction mixture was transferred to a 5 ml volumetric flask, the tubes were washed several timesxwith small amounts of chloroform to bring the volume in the flask up to the 5ml mark. The eosin-red solution was then placed in a 10ml cuvette and light absorbance (O.D.) was measured within 30 minutes at 528 mμ in a spectrocolorimeter. The same reaction was carried out with 2ml of standard solution of cholesterol in chloroform (e.g 0.02mg/ml) and with 2 ml chloroform as a blank.

Because of the sensitivity of the reaction to variations in the permissible low concentration of water, it is advisable to use a standard with cholesterol for each series of determinations.