Effect of Red Gram (*Cajanus cajan*) on Blood Glucose Level in Diabetic Rats

By

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Introduction

I. INTRODUCTION

Diabetes mellitus is an international disease effecting 1-2% of the population in most countries of the world (Bajaj, T.S. 1977). It is an important health problem in India with an overall prevalence rate of 1.5% amounting to no less than 12 million diabetics in the country (Gupta et al., 1983). According to a survey at four centres made by the Indian Council of Medical Research, the average incidence is 2.1% in the cities and 1.5% in villages.

Diabetes is global in distribution and effects every race except some tribes in South America and to a less extent, the Eskimos. Diabetes is found to be more common in males than females, more in older age group than younger age group (Govindraj et al., 1982).

Diabetes is a disease of great importance from the socio-medical point of view. It is characterised as an insufficiency of insulin relative to the requirement of the tissues for the hormone (David Martin et al., 1981). Diabetes itself is a complexity of syndromes of carbohydrate metabolism with consequent disturbance of protein and fat metabolism. Many diseases are associated with it (Patmabatar, 1983). The major complications of diabetes are atherosclerosis, diabetic retinopathy, neuropathy and nephropathy. The Classic symptoms include polyuria, polydypsia, polyphagia and ketosis (Diabetic coma). The types of coma associated with diabetes

are ketosis with high blood sugar, hypoglycemic, hyperosmolar hyperglycemic, non-ketotic and lactic acidosis (Antia, 1975).

Management of diabetes has been revolutionised by the discovery of insulin in 1921. The other three cornerstones of the therapy for diabetics are drugs, diet and exercise.

Insulin is still the best tool available against diabetes mellitus. But insulin therapy leads to certain complications-insulin allergy, insulin lipodystrophy and insulin oedema (Kumup, 1977).

The use of oral hypoglycemic agents is also controversial. Commonly used oral drugs are sulphonylureas and biguanide compounds. A study of sulphonylureas in the management of maturity onset diabetes indicated certain adverse effects affecting various organs of the body both in animals and humanbeings (Ashoja, R.S, 1977). Sulphonylureas have also shown interaction with various drugs in common use and hence due to the limited efficacy and possible toxicity it would be prudent to avoid the use of this drug.

The use of biguanide derivatives in the treatment of diabetes has shown side effects and complications. The possible adverse effects of these derivatives are drug intolerance and metabolic complications such as lactic acidosis and possible increase in the risk of cardio-vascular complications during treatment (Bajaj, 1976).

Hence it is necessary to find out an inexpensive, harmless remedy for diabetes. Diet therapy is considered to be

of most important in the control of diabetes (Halpern, L.S, 1979). Diabetes in many patients with maturity-onset diabetes may be controlled by diet alone without the addition of insulin or oral hypoglycemic agents.

Difference in blood glucose responses were observed with various legumes (Srinivasan, 1977). And it was suggested that protein in the food may alter the glycemic response.

In Leguminaceae, Cajanus cajan (Red gram) was found to be hypoglycemic (Dhar et al., 1968). But no systematic study has been conducted so far. Red gram is used in South India as a common food material. Hence it is proposed through this study to assess the effect of Red gram (Cajanus cajan) on blood glucose level in alloxan induced diabetic rats.

Review of Literature

II. REVIEW OF LITERATURE

Diabetes mellitus is an important health problem not only in India but in almost every country. It is not a single entity, but a heterogeneous, broad spectrum of clinical disorder characterized by hyperglycemia (Arky, 1983). It is a disease of great importance from the socio-medical point of view. The fundamental fault in diabetes mellitus is absolute or relative deficiency in pancreatic islet secretion, insulin (Robinson, 1979).

The review of literature pertaining to the study 'the effect of Red gram on blood glucose level' is discussed under the following headings:

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A. DEFINITION

Diabetes mellitus commonly known as diabetes is a disorder of the carbohydrate metabolism resulting in high blood sugar level and the presence of it in urine (Sinha, 1972).

B. HISTORY OF DIABETES

Aretaeus (A.D 70) described the disease and gave it the name 'Diabetes' which in Greek, meant 'to run through' - referring to polyuria which forms such an important diagnostic sign. However, it was not until a hundred years later that Thomas Willis (1660) described the major characteristic feature present namely, the sweetness of the urine - as if imbued with honey' - thus giving it the name 'mellitus'. Twenty years later Robinson (1700) demonstrated that this sweetness was due to the sugar content. The discovery of sugar in the urine led to a rational dietary approach and this was introduced by Rollo, twenty nine years later. Claude Bernard (1859) was the first to demonstrate an increased glucose content in diabetic blood, and he recognised 'hyperglycaemia' as a cardinal symptom of the disease.

C. PREVALENCE OF DIABETES

It is an important health problem in India with an overall prevalence rate of 1.5% amounting to no less than 12 million diabetics in the country (Gupta, A.P, 1983). It is one of the leading causes of death in the developed countries. It is found to be more common in males than females, more in older age group than younger age group (Govindraj et al., 1982). In India, the incidence of

diabetes has been reported to be 2.2% in Delhi, 2.9% in Bombay and 11.3% in Madras.

Countries with high incidence of diabetes are Germany, France, Soviet Union and South Africa. Recently U.S.A. showed an increase in incidence (Podolsky and Viswanathan, 1978) and countries with low incidence are U.K., Ireland, Australia, Israel, Czechoslovakia and Egypt.

D. AETIOLOGY

1. Hereditary Factor

It is established that diabetes is an inheritable disease. There is a statistically significant increase in the prevalence of diabetes among blood relatives of diabetic patients over that of a control group from the general population, the relative frequency being 6.7% and 1.22% respectively.

2. Age

The prevalence of diabetes in 1369 male subjects in the Al-Kharj area of Saudi-Arabia was studied by WHO criteria which showed that the prevalence increased with age (Bacchus et al., 1982). A study on the incidence of diabetes among different age groups conducted showed that majority of the cases (86.94%) occurred between the age of 41-70 years, highest (38.48%) being in the age group of 51-60 years (Patel, 1982).

3. Sex

Diabetes mellitus is found to be more common in males than females, more in older age group than younger age group (Govindraj et al., 1982).

4. Obesity

A strong predisposing factor in middle aged diabetes is obesity. Middle aged diabetes is due to increase in the size of fat cells and not the number. In all the clinical forms of diabetes characterised by the age of onset, obesity was more frequenty in males than females.

5. Endocrine factors:

The perfection of the symbiotic function of the various endocrine glands under normal conditions is that their collective activities seem as one in the maintenance of normal metabolism. When this harmony is disturbed the disease may proclaim itself in many ways. Among these may be the appearance of diabetes mellitus.

6. Infections

Infection, generally speaking, plays a minor part in the aetiology of diabetes. An infection causing pancreatitis may cause sufficient destruction of the islets to cause diabetes. Staphylococcal infections in particular are frequently associated with the development of clinical diabetes.

immediately after the onset of the disease, but in type IA they are transient. Autoimmune antibodies to other endocrine tissues are also found in type IB diabetes and it becomes certain that, although insulinopenia is present in both subgroups, their pathogenesis is entirely different. The classification adopted from National Diabetes Data group is shown in Table-I.

TABLE I

CLASSIFICATION OF DIABETES MELLITUS

TYPE	OTHER NAMES	CLINICAL CHARACTERISTICS
Type Ia	Insulin-dependent diabetes Juvenile onset diabetes Ketosis-prone diabetes	In early phase retain endogenous insulin secretion, and may have honey-moon phase. Latter have no endogenous insulin. Develop ketosis when insulin with drawn or with stress.
Type Ib	Same as type Ia	Same as type Ia*
Type II (Non-obese)	Non-insulin-dependent diabetes (NIDDM) Maturity-onset diabetes Maturity-onset diabetes of the young (MODY)	Always measurable insulin present. Tendency to insulin resistance Ketosis not provoked by insulin withdrawal but may become ketoacidotic with severe illness. Onset usually above age of 40
Type II (obese)	As for type II (non-obese)	Hyperinsulinaemic and insulin resistant. Rarely if ever ketotic.
Other types	Secondary diabetes	
A. Pancreatic disease	J type K type S type — Type III	Often underweight with history of malnutrition. Mostly restricted to tropical countries and non-caucasians. May have severe insulin resistance although pancreatectomised subjects are insulin sensitive
B. Hormonal		
C. Drug-induced		
D. Insulin receptor abnormalities		
E. Genetic Syndromes		
Impaired glucose tolerance (IGT)	Asymptomatic diabetes Chemical diabetes Borderline diabetes Latent diabetes Subclinical diabetes	Increased risk of macrovascular disease. Likely to be obese
Gestational diabetes		Diagnosis as for IGT or more severe forms of glucose intolerance

*Types Ia and Ib are differentiated by etiological factors.

F. SYMPTOMATOLOGY

Symptoms may suggest a diagnosis of diabetes. The varied symptomatology of diabetes in a typical case is at once indicative of some systematic disorder. General weakness, loss of weight, excessive appetite and thirst, the frequent passing of large quantities of urine without discomfort, rising at night to void, itching of the skin and backache are the most common symptoms of uncontrolled and complicated diabetes (Duncan, G.G, 1961).

1. General weakness

This symptom common to so many debilitating diseases gives little indication of the nature of the underlying disorder.

2. Rapid loss of body weight

A rapid loss of body weight in the afebrile patient, especially in the young patient, should suggest the possibility that diabetes may be the cause. Sugar is lost in the urine in amounts varying from mere traces to as much as 15%. Each gram of sugar lost represents a body deprivation of approximately four calories.

The weight loss is due in part to the loss of body water by the diuresis resulting from the effect which the high concentration of sugar in the blood exerts on renal epithelium and further loss is due to the presence of ketosis.

3. Polyuria

Is frequent urination of an abnormally large volume of urine.

4. Polydipsia

Is excessive thirst and is an effort to replace the lost fluids.

5. Polyphagia

Excessive appetite is a compensatory effort to replace lost nourishment.

6. COMPLICATIONS OF DIABETES

The major complications of diabetes mellitus are diabetic hyperlipidemia, diabetic coma, diabetic ketoacidosis, diabetic nephropathy and diabetic neuropathy (Menon, 1981). The overall incidence of complications among the diabetics was 38 percent, the same being 38% and 37.8% in the males and females respectively. Vascular complications which included retinopathy, hypertension, coronary artery disease, cerebro-vascular accidents and gangrene were the commonest. The others in order of frequency were septic complications, neuropathy, tuberculosis, acidosis and cataract (Patel et al., 1966).

SYNDROMES ASSOCIATED WITH DIABETES

A syndrome is a group of symptoms or signs occuring together to produce a pattern or symptom complex, typical

of a particular disease. Following are the syndrome which can occur with diabetes mellitus.

1. Achard - Thiers syndrome (Diabetic - bearded women syndrome)

In this syndrome there is a change in voice accompanied by scanty menstruation, acne of the face and hypertrichosis.

2. Bronze Diabetes (Diabetes haemochromatosis)

It is a rare inborn error of metabolism characterised by an excessive deposition of iron in the body.

3. Cushing's syndrome

It is a disorder clinically similar to cushing's disease. It is due to elevated level of plasma corticosteriod and is of two types (a) ACTH dependent and (b) due to adenoma.

4. Diabetes and adipose syndrome

Adiposity, imbecility, retarded genital development and overall irregular development - polydactylies, syndactylies, clinodactylies are the common signs of this syndrome.

5. Kimmelstiel Wilson syndrome

Intercapillary glomerulosclerosis is present in diabetes with hypertension, albuminuria and oedema.

6. Transitory diabetes associated with meningitis syndrome

The syndrome is observed in 20% of meningococcal meningitis as well as in tuberculosis meningitis. Etiologic agents affect the endocrine regulation of carbohydrate

metabolism due to damage of thalamus, hypothalamus, pituitary, adrenal, pancreas and liver.

Signs

Blood : Hyperglycemia and acidosis

Urine : Glycosuria and acetone

C.S.F. : Findings of meningitis

H. CLINICAL DIAGNOSIS

The diagnosis of diabetes mellitus is made by the appropriate consideration of the patients symptoms, family and past history, physical findings and laboratory data. The clinical signs, and symptoms are of utmost value as they arouse the suspicion of the presence of diabetes and its complications.

1. Urine

a. Glycosuria

b. Specific gravity

The specific gravity of the urine usually increases in direct proportion to the amount of sugar present.

c. Urine volume

In untreated diabetes the 24 hour urine volume characteristically exceeds the average normal of 1500ml.

2. Blood

Sugar content

Hyperglycemia is the most decisive sign of diabetes.

3. Glycosylated Haemoglobin

In most long-term studies, judging the control of diabetes has been difficult. GHb is an excellent parameter in judging long-term control of diabetes (Chandalia, 1984). Glycosylated Hb (HbA1) could reflect the actual metabolic state in patients with a stable clinical evolution (Reynals et al., 1978). In addition, it has become clear that Hb is not unique and that non-enzymatic glycosylation is in fact a common post-translational modification of many body proteins (Kennedy and Baynie, 1984).

4. Special test

Glucose tolerance test

I. METABOLISM IN DIABETES

A deficient supply of functioning insulin affects the metabolism of carbohydrate, fats, proteins, electrolytes, water and the consequences of the impairments are complex.

1. Carbohydrate

When insulin is not being produced or is ineffective, the formation of glycogen is decreased and the utilization of glucose in the peripheral tissue is reduced. As a consequence

the glucose that enters the circulation from various sources is removed more slowly and hyperglycaemia follows. Liver glycogen was found to be diminished in alloxan induced diabetic rats (Prasannan, 1972).

2. Protein

Gluconeogenesis occurs through which about 58% of the protein molecule and 10% of the fat molecule can yield glucose.

When the blood glucose exceeds the renal threshold (about 160-180mg/100ml) glycosuria occurs. The loss of glucose in urine represents a wastage of energy and entails and increased elimination of water and sodium.

A slightly insignificant negative correlation between urea and blood sugar levels of male diabetes was observed and contrarily insignificant but positive correlation was found between the same levels of opposite sex (Singh et al., 1982).

3. Lipids

With a deficiency of insulin lipogenesis decreases and lipolysis is greatly increased, these effects being of both immediate and long range consequence. The rapid release of fattyacids in to the blood circulation often results in hyperlipidemia and the blood stream may have a milky opalescent appearance.

4. Triglycerides

Most of the fat in the body is present as triglyceride molecules containing three fattyacids which are major source of energy (Sinha, 1982). Insulin deprivation results in increased formation of triglyceride in the liver. Hypertriglyceridemia in diabetic a rats (60% insulin deficiency) is caused by slower removal of lipoprotein triglycerides from the plasma space, owing to reduced lipolytic activity of the peripheral tissues (Bobek et al.,1981). The blood levels of cholesterol are usually increased either because of increased synthesis or because of decreased destruction by the liver (Robinson, 1979).

5. Ketone Bodies

The fattyacids released from adipose tissue or available by absorption from the intestinal tract are oxidised by the liver to form 'ketone bodies' including acetoacetic acid, B-hydroxy butyric acid and acetone. A possibility exists for developing ketoacidosis whenever the urine test indicates uncontrolled diabetes particularly during periods of any other illness. Under such conditions acetone could be found in urine (Sinha, 1982).

6. Vitamins

Carbohydrates are not completely metabolised when there is a deficiency of vitamin B. It is postulated that products of partial carbohydrate metabolism like pyruvic

acid, accumulate due to the deficiency of vitamin B and damage to nerves results in peripheral neuropathy. A diabetic requires supplements of vitamin B. It is also advisable to supply vitamin A, as the liver which is the store house of this vitamin may be damaged in diabetes (Antia, 1976).

7. The essential trace elements

Elements which are necessary for biological function are known as essential, that is, without them life does not exist. Some trace elements in wild animals and man are iron, zinc, copper, manganese, chromium etc.,

a. Chromium

Is needed for sugar and fat metabolism in which insulin takespart. In 1959, Klaus Schwarz and Walter Mertz, who had been studying a dietary deficiency in rats manifest a reduced tolerance to glucose, after an exhaustive search found that the deficient factor was chromium (Schroeder, A.H, 1976). Several studies have shown that increasing the chromium intake of people with wildly impaired glucose tolerance has resulted in normalization of glucose metabolism (Walter Mertz, 1983).

b. Manganese

It maintains structure and function of the insulin producing β-cells of the pancreas and hence its deficiency leads to impaired insulin secretion.

C. Zinc

Zinc content of liver tissue increased in diabetic than in controls (Goyal et al., 1981). Possible mechanisms of zinc deficiency may produce diabetes (Gupta et al., 1983).

J. TREATMENT OF DIABETES MELLITUS

The object of the treatment should be to restore and maintain physiological blood sugar and cholesterol values, to correct and prevent glycosuria and aetonuria, to secure normal nutrition, and by virtue of these accomplishments, to restore the patient to a normal sense of well being with courage, ambition and ability to carry on a useful existence.

1. The methods of treatment of diabetes

a. Insulin Administration

Management of diabetes has been revolutionized by the discovery of insulin in 1921. The commercially available insulins are extracted from bovine and porcine pancreas and are well known to be associated with several problems.

i. Complications of insulin therapy

a. Insulin allergy

Allergy to insulin may consist of a local reaction at the site of injection associated with redness, swelling and heat, resulting in the formation of a subcutaneous nodule.

b. Insulin lipodystrophy

A more frequent result of insulin injection is lipodystrophy. These constitute therapeutically harmless but cosmetically distressing changes in the subcutaneous fat at the site of injection.

c. Insulin oedema

This is an infrequently recognised complication of insulin therapy. It is characterised by generalised retention of fluid (Kreeseler et al., 1971).

ii. Types of newer insulins

The newer insulins may be divided in to three groups.

a. Highly purified insulins

They are i) Single peak insulin (SP, Eli Lilly) produced by removal of high molecular weight contaminants. ii) Mono component insulin (MC, Novo) by further purification by anion exchange chromatography and iii) rarely immunogenic insulin (Lee insulin, Mordich) through other purification procedures.

b. Altered insulin molecules

These include i) Sulphated insulin ii) Desphenyl-alanine insulin (Desphe, Hoechst) iii) Leu B-30 semisynthetic insulin.

iii. Future insulins

Efforts are being made to commercialise homologous human insulin for control of diabetes mellitus and to reduce the complications due to circulating insulin antibodies (Saini and Dash, 1982).

b. Oral hypoglycemic drugs

The oral drugs which have proved of value are sulphonylureas and guanide compounds. But the use of these drugs in the management of diabetes have shown some adverse effects.

i. Sulphonylureas

a. Drug interaction

Sulphonylureas have shown interaction with various drugs in common use, such as sulphonamides, salicylates, barbiturates etc., One cannot therefore use these compounds casually as is commonly being done.

b b. Adverse effects

Adverse effects of sulphonylureas affecting various organs of the body have been reported (Prout, 1974). Sorenstain et al , (1975) demonstrated increased incidence and severity of coronary artery disease in monkeys given sulphonylurea (Raheja, 1977).

Therefore in view of the limited efficacy and possible toxicity it would be prudent to avoid the use of sulphoylureas.

ii. Biguanide derivatives

Possible adverse effects of biguanidederivatives are:

a. Drug intolerance

Symptoms of drug intolerance such as metallic taste in the mouth, anorexia, nausea, vomiting, gastric pains, flatulence and diarrhoea are more frequent when high doses of biguanide derivatives are initially administered(Artur, 1977).

b. Lactic acidosis

Is rare but an important problem. The mechanism by which biguanides cause lactate to accumulate in the blood and tissues is not yet completely understood, but it is attributed to overproduction from increased anaerobic glycolysis (Steiner and Williams, 1958) or to under utilization in the liver or peripheral tissues (Searle and Silverstein, 1975).

The development of lactic acidosis and a possible increase in the risk of cardiovascular complications during treatment of diabetes with biguanide derivatives caused some authors to question the usefulness of these drugs.

c. Exercise

Exercise is a very useful measure in the management of diabetes. It utilises carbohydrate (or energy) and reduces the requirement of insulin or antidiabetic tablets if these are being taken (Antia, 1975).

Walking for a mile or two, swimming and jogging are some good exercises. Exercise should not produce difficulty in breathing or tiredness.

Yoga is a kind of exercise meant for both mind and body. Various yogic asanas compress abdomen and improve liver function. Pancreas is also stimulated and there is increase in blood circulation (Divekar, 1983). Yoga is not a cure but it is part of treatment for diabetes.

D. Dietary management

Diet has long been regarded as the cornerstone of management for all types of diabetes. Many patients with maturity-onset diabetes may be controlled by diet alone without the addition of insulin or oral hypoglycaemic agents.

Aim of dietary treatment

The first aim with diet as part of the overall therapeutic approach to diabetes is, to procure the relief of symptoms - the excessive thirst, polyuria, visual changes, the cramps, etc., so characteristic of untreated diabetes.

A further aim of increasing importance as the life expectation of the diabetic continues to increase is, to try dietary means to delay the appearance and to minimize the frequency of the "long-term complications" of diabetes, which includes major arterial occlusive disease, disruption

and obstruction of the microvascular circulation of the retina, kidney and other tissues (Keen and Krinsy Thomas, 1979).

2. Use of indigenous drugs in diabetes

a. 'The effect of Sarkarai Kalli (Gymnema sylvestre) on blood glucose level' was carried out in diabetic patients (Nageswari, 1978). The response to treatment by the oral administration of about 1000 mg of this drug was found to vary with sex, weight, age and duration of diabetes. The mean blood sugar reduction ranged from 2-44 mg% indicating that though Gymnema sylvestre does not decrease the blood sugar level remarkably, definitely it has blood sugar lowering effect and antidiabetic property.

b. A study on the 'effect of bittergourd juice (Momordica charantia) on blood glucose level of diabetics' was conducted (Cynthia, 1979). Depending upon the urinary glucose levels of diabetics, different quantities of fresh bittergourd juice was administered. The reduction in blood glucose during fasting and post prandial states were found to be 139mg% and 240 mg% respectively.

c. The hypoglycemic effect of ginger was studied on normal albino rats (Giri et al., 1981). Fresh ginger juice extracted from 1.0g of ginger was administered to rats. In ginger fed rats, the blood glucose level was found to be decreased. Thus in addition to its hypocholesteremic effect, ginger also acts as a hypoglycemic agent.

d. A study on the effect of Thulasi (Ocimum sanctum) on blood glucose level in rats was conducted by Giri et al in 1985. On treatment with Thulasi leaf extract the blood sugar, blood urea, serum cholesterol and serum triglyceride levels were found to be decreased and after discontinuing the treatment, the mean blood sugar, blood urea, cholesterol levels were increased. Thus thulasi was proved to have antihyperglycaemic qualities.

e. The effect of bittergourd juice (Momordica charantia) on blood glucose level and absorption of glucose was studied in alloxanized rats (Giri et al., 1982, 1985). According to this study on 10 days administration of bittergourd juice to diabetic rats the blood glucose level came down to 65mg from 198mg. Bittergourd juice was found to lower the blood glucose level by inhibiting the glucose absorption from the intestine. The liver glycogen was depleted in diabetic rats treated with bittergourd juice indicating that prolonged administration of bittergourd juice in some way prevented the formation of liver glycogen.

f. In the Ayurvedic system of Medicine, Jamun seed had been reported as an antidiabetic drug. When alloxan induced diabetic rats were fed with jamun seed extract, the increased blood glucose, blood urea, serum cholesterol, serum triglyceride levels were found to decrease significantly (Giri et al., 1983).

K. LEGUMES IN DIABETES

Legumes are commonly used in Indian diets. Legumes contain a large amount of carbohydrate varying from 50-60% and protein 15-25%.

Difference in blood glucose responses were observed with various legumes (Arky, 1983). A lower rise in blood glucose was observed when glucose was ingested with a legume and it was suggested that protein in the food may alter the glycemic response (Srinivasan, 1987).

L. EFFECT OF RED GRAM (CAJANUS CAJAN) ON DIABETES

A programme for the screening of plant extracts for a wide range of biological activity was conducted by Khan et al., 1968. In Leguminaceae, Red gram (Cajanus cajan) was found to be hypoglycemic.

The nutritive value of Cs. cajan seed is quite high at the 20% protein level (Ahsan et al., 1968). Red gram was found to be rich in lysine. Aminoacid analysis of the different globulin fractions of Cs. cajan showed that the γ - fraction was comparatively rich in sulphur containing aminoacids (Gopalakrishna et al., 1977). The nutrient content of Red gram is given in Table XI (Gopalan et al., 1984).

TABLE - II

a. NUTRIENT CONTENT OF RED GRAM.

NUTRIENT	AMOUNT PER 100 g of EDIBLE PORTION	NUTRIENT	AMOUNT PER 100g of EDIBLE PORTION
Moisture	13.4(g)	Phosphorus	304mg
Protein	22.3(g)	Iron	5.8mg
Fat	1.7(g)	Caretene	132ug
Minerals	3.8(g)	Thiamine	0.45mg
Fibre	1.5(g)	Riboflavin	0.19mg
Carbohydrate	57.4(g)	Niacin	2.9 mg
Energy	350(Kcal)	Vitamin C	0 mg
Calcium	73(mg)		

b. TRACE ELEMENTS

Mg	Na	K	Cu	S	Cl
mg/100g of edible protion					
138	28.9	1.104	1.25	177	9

c. ESSENTIAL AMINOACIDS

Total N gm%	Arg	His	Lys	Trp	Phe	Tyr	Met	Cys	Thr	Leu	Ile
3.57	0.36	0.25	0.48	0.04	0.46	0.13	0.06	0.06	0.20	0.25	0.26

Hypolipidemic activity of the protein isolated from Ca. ciliata in high fat, cholesterol diet was proved (Prem and Kurup, 1973). But no systematic study has been conducted for the hypoglycemic activity of Red gram. Hence the present study is undertaken.

Experimental Procedure

III. EXPERIMENTAL PROCEDURE

The experimental procedure followed for the present investigation is described in the following sequence:

A. EXPERIMENT - I

1. Formulation of diet
2. Selection and grouping of animals
3. Induction of diabetes
4. Preparation of diet and feeding
5. Preparation and feeding of Red gram juice
6. Collection of blood and liver samples
7. Biochemical analysis of blood/serum
   a. Estimation of glucose
   b. Estimation of urea
   c. Estimation of cholesterol
   d. Estimation of triglyceride
8. Estimation of liver glycogen

B. EXPERIMENT - II

An *in vitro* study of the effect of red gram on the absorption of glucose by the intestine.

C. STATISTICAL ANALYSIS

A. EXPERIMENT-I

1. Formulation of Diet

The stock diet was formulated and fed to all the rats. Its composition is given in Table - III

TABLE - III

COMPOSITION OF STOCK-DIET

INGREDIENTS	AMOUNT IN g/100g
Wheat flour	35
Bengal gram flour	16
Green gram flour	15
Whole milk	15
Yeast	1
Greens	5
Cod liver oil	1 drop
Salt Mixture	0.9
Beef (cooked)	2.1
Groundnut oil	10

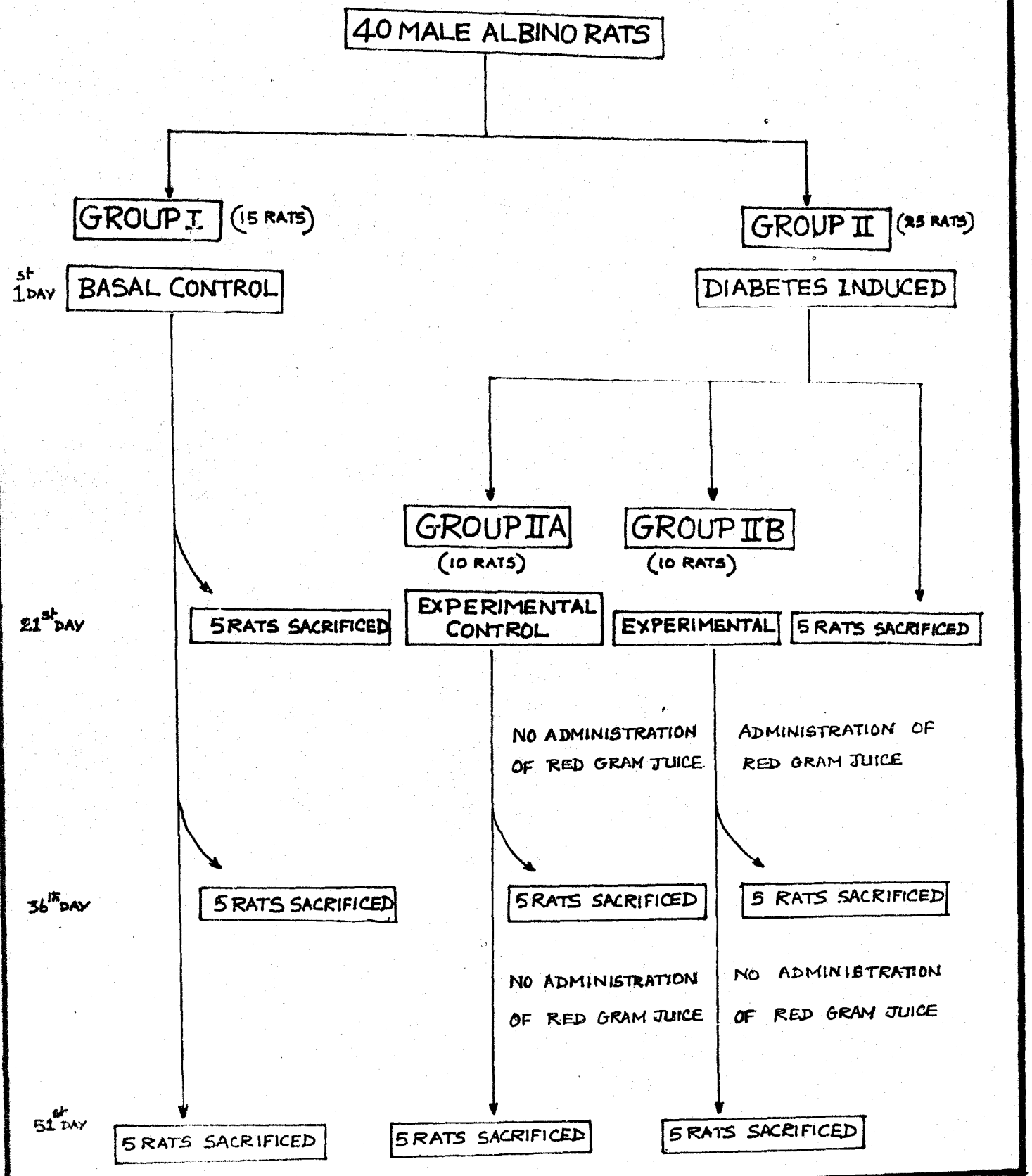
2. SELECTION AND GROUPING OF ANIMALS

Forty male albino rats of Wister strain weighing 110-130g were selected from the stock colony and divided in to group I and II as shown in figure I. Both group I and group II rats were fed the stock diet throughout the experimental period. Group I consisting of 15 rats were not given either alloxan or Red gram juice. Group II consisted of 25 rats. This group was given alloxan subcutaneously (140mg/kg of body weight). Diabetes was found to be induced in two days. But the rats were left for 20 days without any treatment to make sure that the diabetes was permanently induced. After 20 days that is, on the 21st day five rats of group II were sacrificed. Blood and liver samples were collected for analysis. The remaining 20 rats of group II were divided in to sub-groups A and B each consisting of 10 rats.

Group II A rats were used as experimental control while group II B rats formed the experimental rats. Group II B rats were given daily red gram juice by force feeding for 15 days (2ml. of 100% solution). At the end of this period (ie., the 36th day) five of group II B rats were sacrificed by decapitation. For the remaining five, administration of Red gram juice was discontinued and they were sacrificed after another 15 days, that is, on the 51st day.

FIG-1

GROUPING OF RATS



As for the experimental control (Group II A) rats, they were not given any Red gram juice. Five of them were sacrificed on the 36th day along with group II B rats and the remaining 5 rats were sacrificed on the 51st day along with group B rats for analysis of blood and liver.

Group I which formed the basal control group was not given alloxan or Red gram juice. Five of them were sacrificed on the 21st day, five on the 36th day and the remaining five on the 51st day along with group B rats. All the 40 rats (Group I and Group II) were fed the stock diet during the entire period of this study and given water ad libitum.

3. INDUCTION OF DIABETES

The rats were fasted for 24 hours and then quickly given a single subcutaneous injection of alloxan (140mg/kg body weight dissolved in physiological saline). Diabetes was induced in two days, but they were left as such for 20 days to make sure that diabetes was induced permanently.

4. PREPARATION OF DIET AND FEEDING

The food was weighed as given in Table III mixed with sufficient water and cooked by steaming for 30 minutes. All the rats were fed the same amount of food. The daily food intake varied between 30-40g/rat from the beginning to the end of the experiment and water was fed ad libitum.

5. PREPARATION AND FEEDING OF RED GRAM JUICE

Fresh raw seeds of Red gram (Cajanus cajan) were taken and homogenised in a waring blender using distilled water such that a 100 percent solution was got. This was filtered through a muslin cloth and 2ml of the Red gram juice was given to the experimental rats (Group II B) by force feeding daily in the morning, before feeding the stock diet.

6. COLLECTION OF BLOOD AND LIVER SAMPLES

The rat was removed from its cage, gently to avoid exciting it, was stunned by a blow on the head and immediately blood was collected from the jugular vein. The liver was removed quickly, 0.1g of it was transferred to 6.0ml of 30 percent potassium hydroxide.

7. BIOCHEMICAL ANALYSIS OF BLOOD/SERUM

All estimations were done in duplicates.

a. Estimation of Glucose in blood

The blood glucose level was estimated by Folin-Wu method (Varley, 1976). The details of the method are given in Appendix-I.

b. Estimation of Urea

The blood urea level was estimated by DAM-TSC method (Varley, 1976). The details of the method are given in Appendix-II.

PLATE 1

FORCE FEEDING OF
RED GRAM JUICE

c. Estimation of Cholesterol

The serum cholesterol level was estimated by Zak's method (Varley, 1976). The details of the method are given in Appendix-III.

d. Estimation of triglycerids

The serum triglyceride level was estimated by Chin et al method (Varley et al., 1980). The details of the method are given in Appendix-IV.

e. ESTIMATION OF LIVER GLYCOGEN

Glycogen was estimated according to the method of Remmentgoneny (1959). The details of the method are given in Appendix-V.

f. EXPERIMENT-II

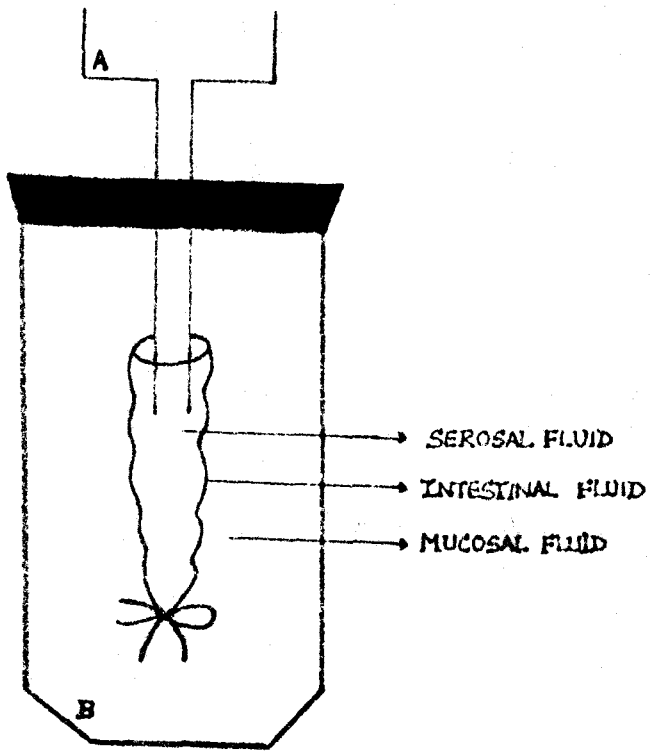
In this experiment an in vitro study of the absorption of glucose by the intestine was conducted to find out the effect of Red gram juice on glucose absorption. The method followed is that of Wilson (1950) with slight modifications.

Diabetic rats were fasted for 30 hours and then anesthetised with chloroform before the experiment. The intestine was separated from the mesenterum and cut in to 5cm long sections. They were then washed with Krebs Ringer phosphate buffer solution (Umbreit et al., 1972) at 30° C. The intestine was everted with a round tipped glass rod. One end was ligated and the other was attached to the tube as shown in figure 2. 1.0ml of Ringer solution was introduced

FIG-2

EVERTED SAC METHOD

INTESTINAL ABSORPTION OF GLUCOSE



in to the intestinal sac through the tube A, avoiding air bubbles, and the intestine was lowered in to tube B, which contained 10ml of Krebs Ringer phosphate buffer containing 0.5 percent glucose. The intestinal fluid was collected at different intervals of 10, 20, 30, 40 and 50 minutes and used for the estimation of glucose by Folin-Wu method. A similar experiment was conducted taking in tube B, 10ml of Krebs Ringer phosphate buffer containing 0.5 percent glucose and red gram juice at 100 percent concentration (Red gram was homogenised with Ringer solution to produce 100 percent solution).

C. STATISTICAL ANALYSIS

't' tests were conducted wherever necessary to check if the results were significant using the formula

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S} \times \sqrt{\frac{n_1 \; n_2}{n_1 + n_2}}$$

$\bar{X}_1$ = Mean of the first sample

$\bar{X}_2$ = Mean of the second sample

S = Combined standard deviation

n_1 & n_2 = Number of observations of the first and second samples

$$S = \sqrt{\frac{\Sigma (X_1 - \bar{X}_1)^2 + \Sigma (X_2 - \bar{X}_2)^2}{n_1 + n_2 - 2}}$$

Results and Discussion

IV. RESULTS AND DISCUSSION

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

EXPERIMENT-I

1. The effect of Red gram juice on blood glucose level
2. The effect of Red gram juice on blood urea level
3. The effect of Red gram juice on serum cholesterol level
4. The effect of Red gram juice on serum triglyceride level
5. The effect of Red gram juice on liver glycogen content

EXPERIMENT-II

The effect of Red gram juice on glucose absorption by the intestine.

EXPERIMENT-I

1. The effect of Red gram juice on blood glucose level

The IV gives the mean blood glucose level in alloxan induced diabetic rats. Figure 3 diagrammatically represents the same.

TABLE IX

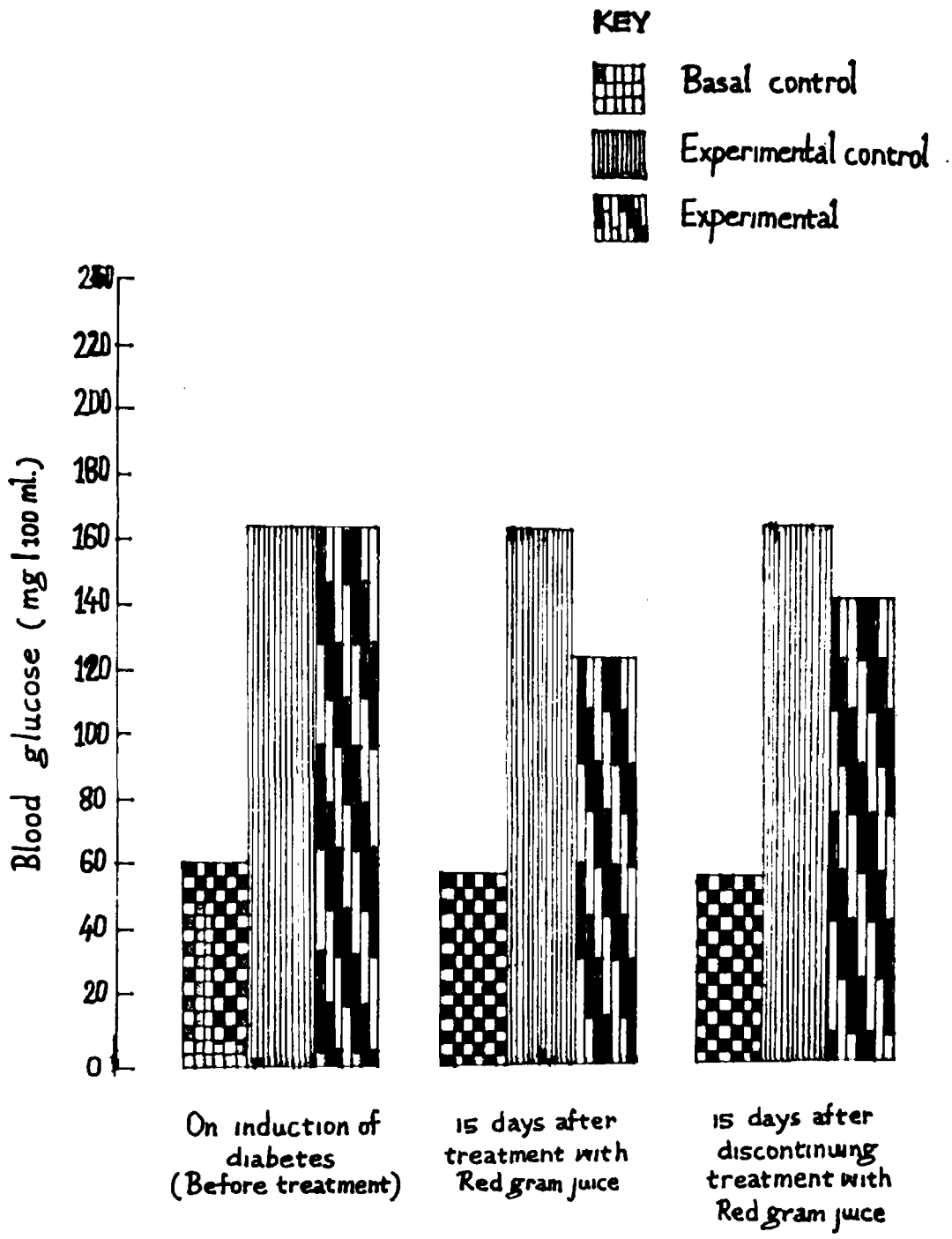
BLOOD GLUCOSE VALUES OF RATS IN mg/dl

DIFFERENT EXPERIMENTAL CONDITIONS	BASAL CONTROL MEAN ± S.D	EXPERIMENTAL CONTROL MEAN ± S.D	EXPERIMENTAL MEAN ± S.D	GROUPS COMPARED	STATISTICAL SIGNIFICANCE
DIABETIC CONDITION INITIAL VALUE	60.3 ± 3.19 (a_1)	162.8 ± 3.15 (b_1)	162.8 ± 3.15 (c_1)	a_1 Vs b_1	29.94**
				a_1 Vs c_1	29.95**
				b_1 Vs c_1	0.0 NS
				a_1 Vs a_2	0.73NS
				a_1 Vs a_3	0.53NS
				a_2 Vs a_3	0.26NS
15 DAYS AFTER TREATMENT WITH REDGRAM JUICE	57.8 ± 4.56 (a_2)	162.25 ± 4.96 (b_2)	123.14± 3.79 (c_2)	a_2 Vs b_2	25.12**
				a_2 Vs c_2	18.19**
				b_2 Vs c_2	12.21**
				b_1 Vs b_2	0.14NS
				b_1 Vs b_3	0.71NS
				b_2 Vs b_3	0.38NS
15 DAYS AFTER DISCONTINUING THE FEEDING OF RED GRAM JUICE	58.8 ± 3.97 (a_3)	162.0 ± 4.99 (b_3)	140.3 ± 2.89 (c_3)	a_3 Vs b_3	27.42**
				a_3 Vs c_3	29.48**
				b_3 Vs c_3	6.91**
				c_1 Vs c_2	12.36**
				c_1 Vs c_3	7.58**
				c_2 Vs c_3	7.07**

NS - NOT SIGNIFICANT
** - SIGNIFICANT AT 1% LEVEL.

FIG.3

EFFECT OF RED GRAM JUICE ON BLOOD GLUCOSE LEVEL



It can be seen from the values presented that the mean blood glucose levels of the experimental control and experimental group rats were raised more than two fold on induction of diabetes when compared to the basal control group which were not injected alloxan.

It is clear from the table that the mean blood glucose level of the experimental group after 15 days treatment with Red gram juice was reduced by 24.4% (from 162.8 ± 5.15 to 123.14 ± 3.79mg/100ml) while in the experimental control group the mean blood sugar value did not undergo any significant change. This shows that Red gram juice has hypoglycemic effect.

On discontinuing the administration of Red gram juice to diabetic rats for 15 days, the mean blood glucose level increased (from 123.14 ± 3.79 to 146.3±2.89mg/100ml) in the experimental group. But the value was still lower than that of the experimental control group. This confirms that Red gram juice has blood sugar lowering effect.

2. <u>Effect of Red gram juice on blood urea level in diabetic rats</u>

Table V indicates the mean urea level in blood of diabetic rats. Fig 4 diagrammatically represents the same.

It can be seen from the table that the mean blood urea level of the experimental control and experimental group rats were raised almost three fold (2.8 times) compared

TABLE V

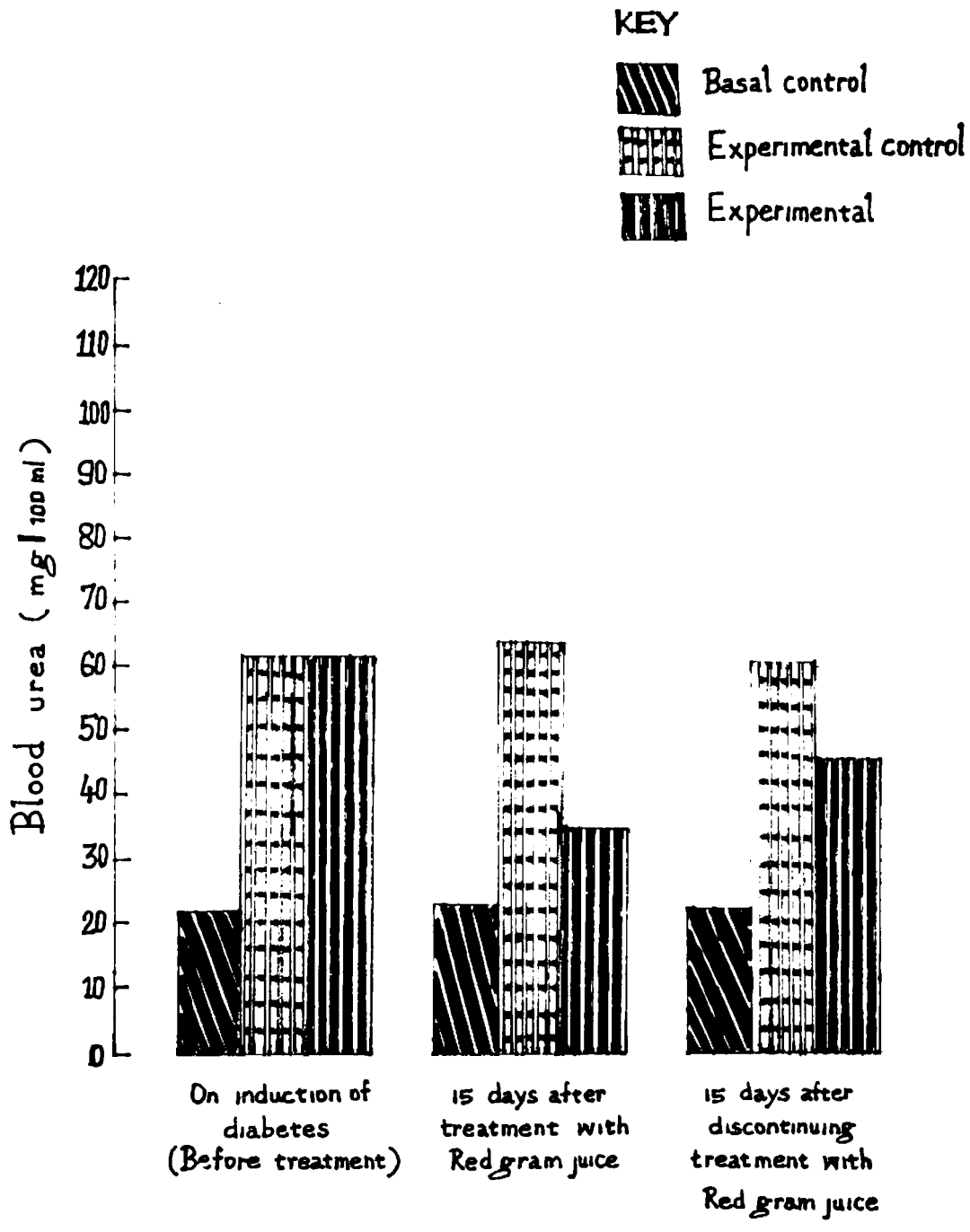
BLOOD UREA VALUES OF RATS IN mg/dl

DIFFERENT EXPERIMENTAL CONDITIONS	BASAL CONTROL MEAN ± S.D	EXPERIMENTAL CONTROL MEAN ± S.D	EXPERIMENTAL MEAN ± S.D	GROUPS COMPARED	STATISTICAL SIGNIFICANCE
DIABETIC CONDITION INITIAL VALUE	21.6 ± 2.024 (a_1)	61.1 ± 3.74 (b_1)	61.1 ± 3.74 (c_1)	a_1 Vs b_1	18.32**
				a_1 Vs c_1	18.32**
				b_1 Vs c_1	0.0 NS
				a_1 Vs a_2	0.3185
				a_1 Vs b_2	0.1785
				a_1 Vs c_2	0.3285
				a_2 Vs b_3	14.16**
15 DAYS AFTER TREATMENT WITH REDGRAM JUICE	22.7 ± 3.78 (a_2)	62.25 ± 3.71 (b_2)	34.4 ± 2.87 (c_2)	a_2 Vs b_2	3.71**
				a_2 Vs c_2	12.86**
				b_2 Vs c_2	0.7885
				a_1 Vs b_2	0.3185
				a_1 Vs b_3	1.1885
				a_2 Vs b_3	
15 DAYS AFTER DISCONTINUING THE FEEDING OF RED GRAM JUICE	21.91 ± 2.72 (a_3)	60.37 ± 4.74 (b_3)	48.0 ± 1.81 (c_3)	a_3 Vs b_3	20.16**
				a_3 Vs c_3	12.85**
				b_3 Vs c_3	9.82**
				a_1 Vs c_2	12.15**
				a_2 Vs c_3	7.46**
				a_2 Vs c_3	8.02**

NS - NOT SIGNIFICANT
** - SIGNIFICANT AT 1% LEVEL.

FIG4

EFFECT OF RED GRAM JUICE ON BLOOD UREA LEVEL



to the basal control group on induction of diabetes. The increase in blood urea in each case is statistically significant at 1% level when compared to the normal rats.

The mean blood urea value of the experimental group after 15 days treatment with Red gram juice was reduced by 31.9% (from 61.1 ± 3.74 to 34.4 ± 2.87mg/100ml) while in the experimental control group the mean urea level did not undergo any significant change. This shows that Red gram juice brings down the urea level in the blood of diabetic rats.

On discontinuing the administration of Red gram juice to diabetic rats for 15 days, the mean blood urea level increased (from 34.4 ± 2.87 to 45.8 ± 1.81mg/100ml) in the experimental group. But the value was still lower than that of the experimental control group. This confirms the fact that Red gram juice lowers blood urea level.

3. <u>Effect of Red gram juice on serum cholesterol level</u>

Table VI gives the mean cholesterol level in serum of diabetes induced rats. Figure 5 diagrammatically represents the same.

It can be seen from the values presented that the mean serum cholesterol level of the experimental control and experimental group rats were raised three fold when compared to the basal control group.

TABLE VI

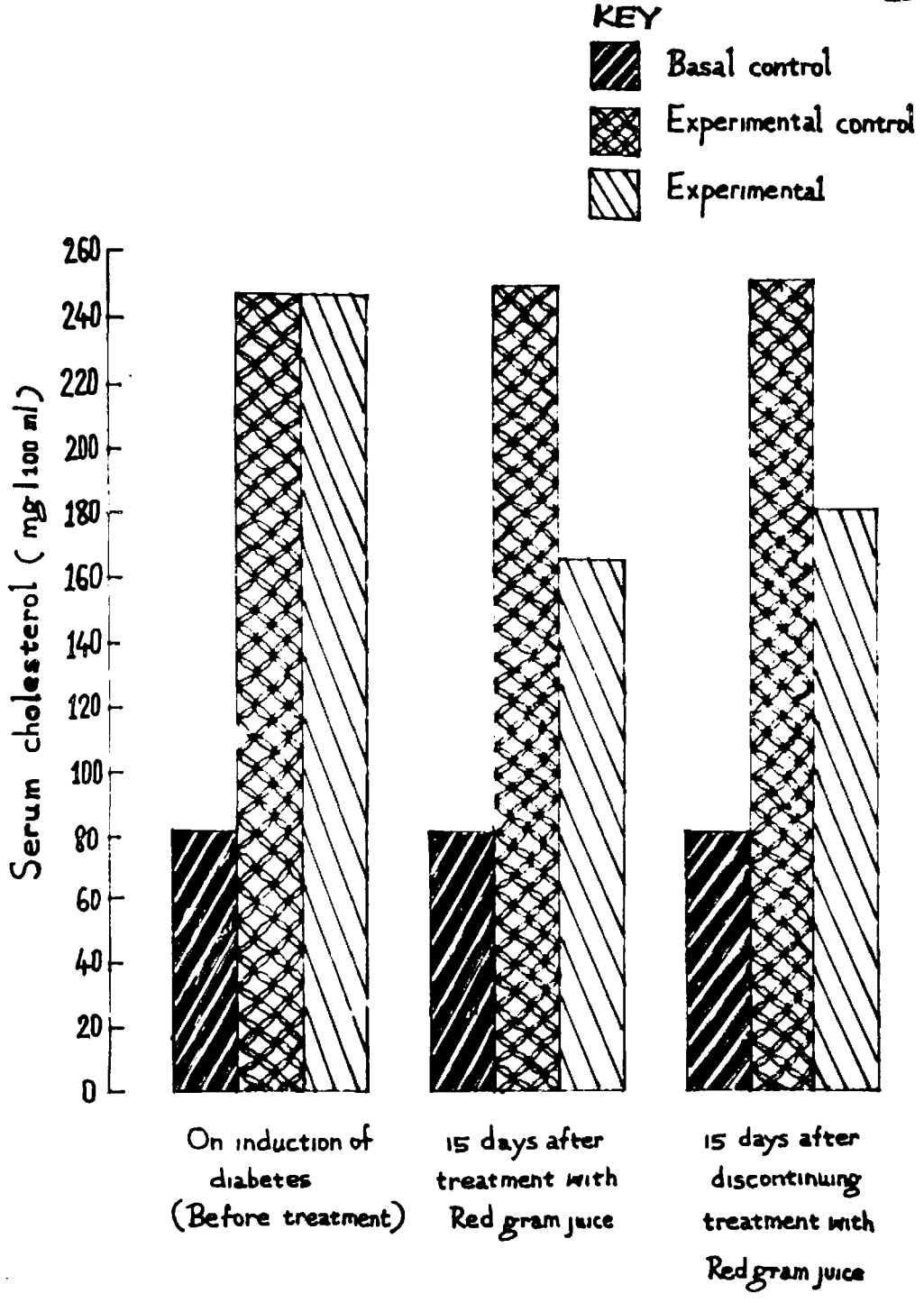
SERUM CHOLESTEROL VALUES OF RATS IN mg/dl

DIFFERENT EXPERIMENTAL CONDITIONS	BASAL CONTROL MEAN ± S.D	EXPERIMENTAL CONTROL MEAN ± S.D	EXPERIMENTAL MEAN ± S.D	GROUPS COMPARED	STATISTICAL SIGNIFICANCE
DIABETIC CONDITION INITIAL VALUE	81.3 ± 3.41 (a_1)	246.7 ± 6.12 (b_1)	246.7 ± 6.12 (c_1)	a_1 Vs b_1	47.95**
				a_1 Vs c_1	47.95**
				b_1 Vs c_1	0.0 NS
				a_1 Vs a_2	0.0 NS
				a_1 Vs a_3	0.0 NS
				a_2 Vs a_3	0.05NS
15 DAYS AFTER TREATMENT WITH RED GRAM JUICE	81.4 ± 3.44 (b_1)	248.5 ± 4.94 (b_2)	165.4 ± 4.27 (c_2)	a_2 Vs b_2	51.47**
				a_2 Vs c_2	29.94**
				b_2 Vs c_2	23.83**
				b_1 Vs b_2	0.43NS
				b_1 Vs b_3	1.24NS
				b_2 Vs b_3	0.83NS
15 DAYS AFTER DISCONTINUING THE FEEDING OF RED GRAM JUICE	81.3 ± 2.50 (c_1)	251.3 ± 3.02 (c_2)	180.0 ± 4.04 (c_3)	a_3 Vs b_3	81.82**
				a_3 Vs c_3	53.65**
				b_3 Vs c_3	32.53**
				c_1 Vs c_2	22.09**
				c_1 Vs c_3	20.12**
				c_2 Vs c_3	6.01**

NS - NOT SIGNIFICANT
** - SIGNIFICANT AT 1% LEVEL

FIG.5

EFFECT OF REDGRAM JUICE ON SERUM CHOLESTEROL LEVEL



The mean serum cholesterol value of the experimental group after 15 days treatment with Red gram juice was reduced (from 246.7 ± 6.12 to 168.4 ± 4.27mg/100ml) while in the experimental control group the mean serum cholestrol value did not undergo any significant change which shows that Red gram juice has hypocholesteraemic effect.

On discontinuing the administration of Red gram juice to diabetic rats for 15 days, the mean serum cholesterol level increased (from 168.4 ± 4.27 to 180 ± 4.04mg/100ml) in the experimental group. But the value was still lower than that of the experimental control group. This proves that Red gram juice definitely has hypocholesteraemic effect.

4. <u>Effect of Red gram juice on serum triglyceride level</u>

Table VII indicates the mean serum triglyceride level of diabetic rats before and after treatment of the Redgram juice. The mean serum triglyceride level of the experimental control and experimental group rats were raised almost three fold when compared to the basal control on induction of diabetes. Figure 6 diagrammatically represents the same.

From the values presented, it is clear that the means serum triglyceride value of the experimental group after 15 days treatment with Red gram juice was reduced by 31.8% (from 111.96 ± 6.4 to 90. ± 3.6mg/100ml) while in the experimental control group the mean serum triglyceride level did not undergo any significant change. This shows that Red gram juice has got serum triglyceride lowering effect.

TABLE - XII

SERUM TRIGLYCERIDE VALUES OF RATS IN mg/dl

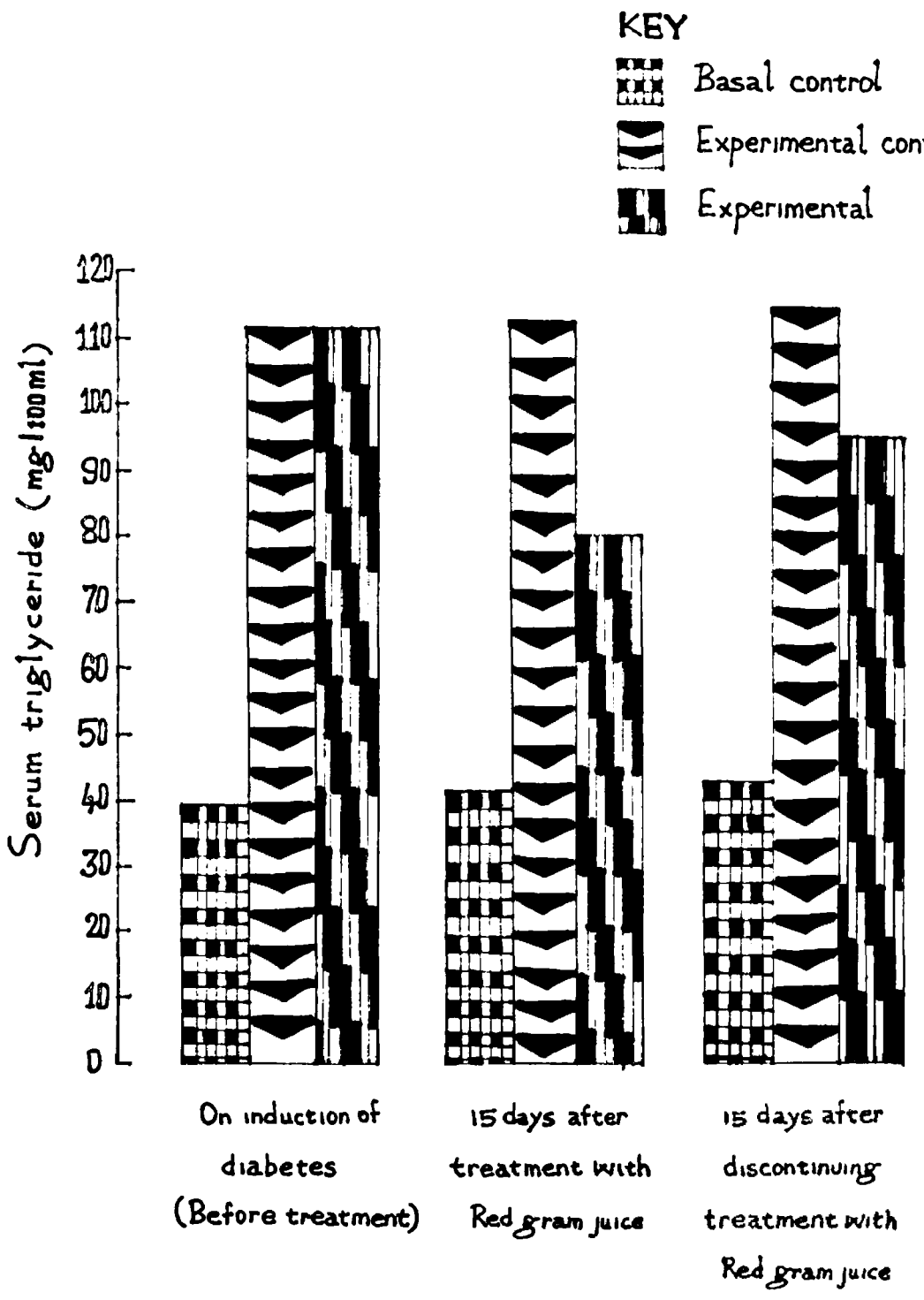
DIFFERENT EXPERIMENTAL CONDITION	BASAL CONTROL MEAN ± S.D	EXPERIMENTAL CONTROL MEAN ± S.D	EXPERIMENTAL MEAN ± S.D	GROUPS COMPARED	STATISTICAL SIGNIFICANCE
DIABETIC CONDITION INITIAL VALUE	39.8 ± 1.99 (a_1)	111.36 ± 6.4 (b_1)	111.36 ± 6.4 (c_1)	a_1 Vs b_1	22.36**
				a_1 Vs c_1	22.36**
				b_1 Vs c_1	0.0 NS
				a_1 Vs a_2	0.0NS
				a_1 Vs a_3	0.0NS
				a_2 Vs a_3	0.0NS
10 DAYS AFTER TREATMENT WITH RED GRAM EXTRACT	41.0 ± 2.58 (a_2)	112.12 ± 3.4 (b_2)	80 ± 2.6 (c_2)	a_2 Vs b_2	28.31**
				a_2 Vs c_2	14.26**
				b_2 Vs c_2	10.28**
				b_1 Vs b_2	0.13NS
				b_1 Vs b_3	0.72NS
				b_2 Vs b_3	0.46NS
15 DAYS AFTER DISCONTINUING THE FEEDING OF RED GRAM JUICE	41.7 ± 3.08 (a_3)	113 ± 5.38 (b_3)	94.6 ± 2.74 (c_3)	a_3 Vs b_3	19.43**
				a_3 Vs c_3	22.36**
				b_3 Vs c_3	5.92**
				a_1 Vs c_2	8.36**
				a_1 Vs c_3	4.86**
				c_2 Vs c_3	6.38**

NS - NOT SIGNIFICANT

** - SIGNIFICANT AT 1% LEVEL.

FIG.6

EFFECT OF RED GRAM JUICE ON SERUM TRIGLYCERIDE LEVEL



On discontinuing the administration of Red gram juice to experimental rats for 15 days, the serum triglyceride level increased (from 80 ± 3.6 to 94.6 ± 2.74) in the experimental group. But the value was still lower than that of the experimental control group. This confirms that Red gram juice lowers significantly the triglyceride level in serum.

9. Effect of Red gram juice on liver glycogen content

Table VIII gives the mean liver glycogen values in diabetic rats before and after treatment with Red gram juice. Fig. 7 diagrammatically represents the same.

It is clear from the results presented that the mean liver glycogen value of the experimental group after 15 days treatment with Red gram juice reduced by 21.37% (from 1.31 ± 0.06 to 1.03 ± 0.07mg/100ml) while in the experimental control group the value increased only slightly (from 1.222 to 1.31). However the increase was not statistically significant.

It is also seen from the values given that in the experimental group the mean liver glycogen content shows an upward trend (from 1.034 ± 0.07 to 1.36 ± 0.07) on discontinuing Red gram juice administration for 15 days. This confirms the fact that Red gram juice in some way prevents liver glycogen formation.

TABLE - VIII

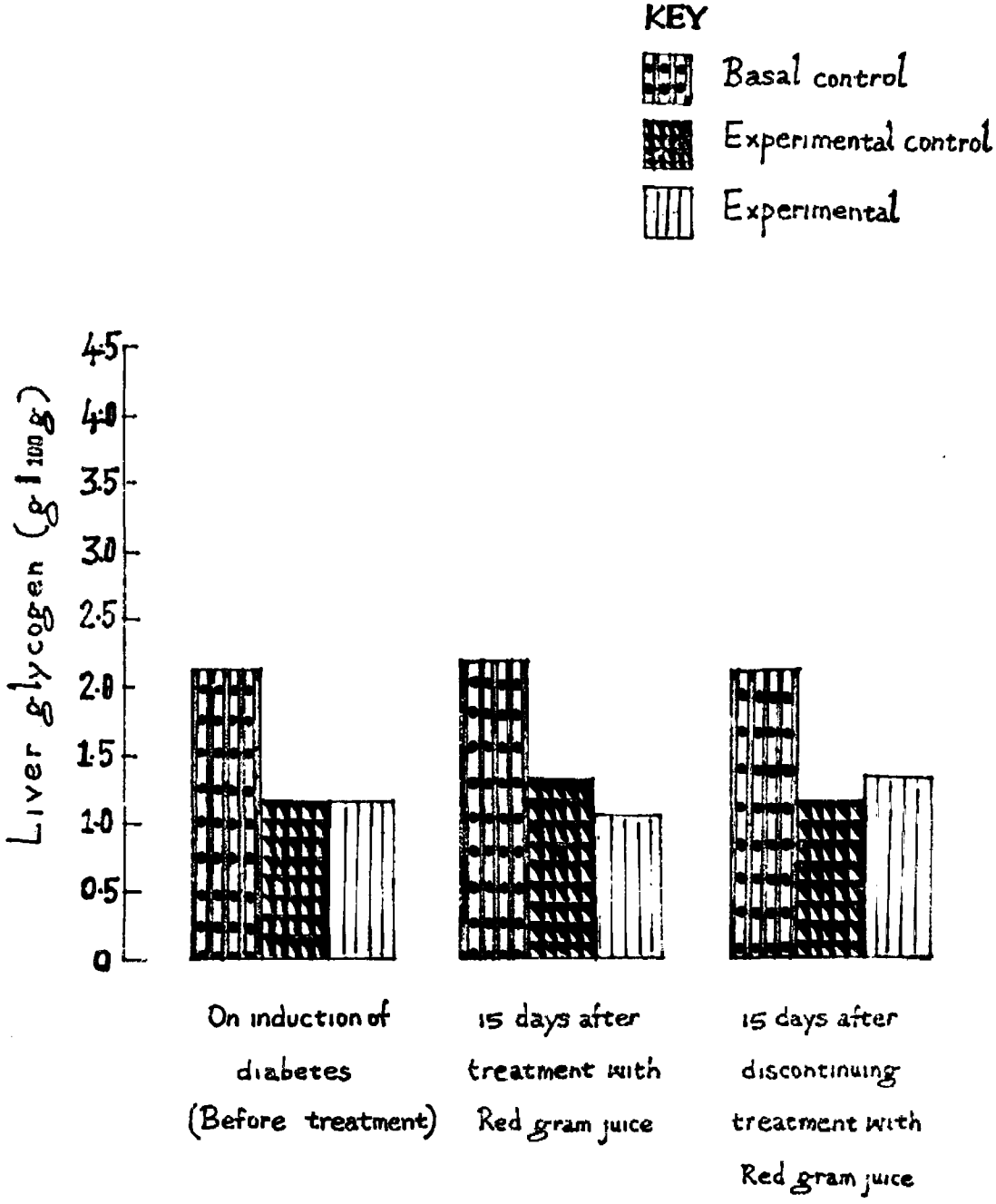
LIVER GLYCOGEN VALUES OF RATS IN g/100g.

DIFFERENT EXPERIMENTAL CONDITION	BASAL CONTROL MEAN ± S.D	EXPERIMENTAL CONTROL MEAN ± S.D	EXPERIMENTAL MEAN ± S.D	GROUPS COMPARED	STATISTICAL SIGNIFICANCE
DIABETIC CONDITION INITIAL VALUE	2.16 ± 0.144 (a_1)	1.22 ± 0.12 (b_1)	1.22 ± 0.12 (c_1)	a_1 Vs b_1	3.68**
				a_1 Vs c_1	3.68**
				b_1 Vs c_1	0.0 NS
				a_1 Vs a_2	3.94**
				a_1 Vs a_3	0.0 NS
				a_2 Vs a_3	3.93**
15 DAYS AFTER TREATMENT WITH RED GRAM JUICE	2.20 ± 0.103 (a_2)	1.31 ± 0.06 (b_2)	1.03 ± 0.07 (c_2)	a_2 Vs b_2	12.06**
				a_2 Vs c_2	16.32**
				b_2 Vs c_2	4.47**
				b_1 Vs b_2	1.10NS
				b_1 Vs b_3	0.02NS
				b_2 Vs b_3	0.02NS
15 DAYS AFTER DISCONTINUING THE FEEDING OF RED GRAM JUICE	2.16 ± 0.194 (a_3)	1.21 ± 0.02 (b_3)	1.36 ± 0.03 (c_3)	a_3 bVs b_3	2.73*
				a_3 Vs c_3	4.21**
				b_3 Vs c_3	3.21*
				c_1 Vs c_2	2.41
				c_1 Vs c_3	1.19NS
				c_2 Vs c_3	2.26NS

NS - NOT SIGNIFICANT
** - SIGNIFICANT AT 1% LEVEL
*** - SIGNIFICANT AT 5% LEVEL

FIG. 7

EFFECT OF RED GRAM JUICE ON LIVER GLYCOGEN CONTENT



Treatment with Red gram juice reduces the blood glucose level but this reduction does not seem to be due to conversion of glucose to glycogen in liver.

This needs further investigation. According to Giri et al (1961) when diabetic rats were treated with bittergourd juice there was reduction in blood glucose level and simultaneously depletion of liver glycogen stores. The explanation given by them was that absorption of glycose in the intestine was reduced leading to lower blood glucose level and consequent reduction in the synthesis of liver glycogen. It may be that by similar mechanism the blood glucose and liver glycogen values are reduced on treatment with Red gram juice. However, to test this, experiment - II was conducted.

EXPERIMENT-II

The effect of Red gram juice on glucose absorption by the intestine

Table IX gives the intestinal absorption of glucose in diabetic rats in presence and in absence of Red gram juice at various, time intervals. Figure 8 gives the diagrammatic representation of the same.

A comparison of absorbed glucose value of A and B at different time intervals indicate a lowering of the glucose value (absorbed) when Red gram juice vaccided to

TABLE-IX

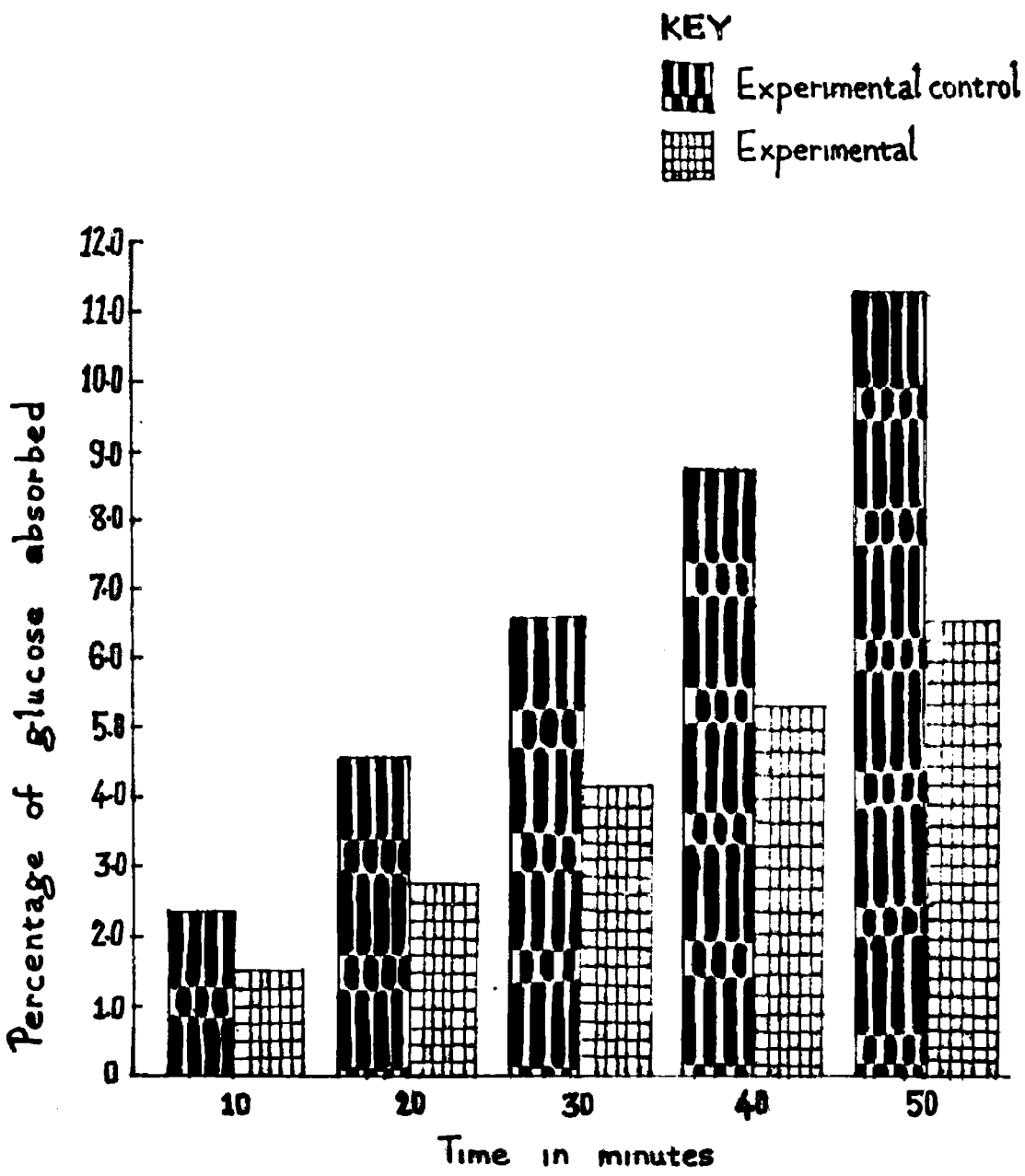
GLUCOSE VALUES IN SEROSAL FLUID WITH AND WITHOUT RED GRAM JUICE

Time in minutes	Amount of glucose absorbed in mg/ml in presence of		Percentage of glucose absorbed in presence of	
	0.5% Glucose A	0.5% Glucose + RGJ (100% solution) B	Glucose A	Glucose + RGJ B
10	0.13	1.2	2.8	1.44
20	1.25	2.05	4.5	2.7
30	3.25	[illegible]	6.5	4.1
40	4.35	[illegible]	8.7	5.26
50	5.0	3.28	11.2	6.56

RGJ=RED GRAM JUICE

FIG.8

EFFECT OF RED GRAM TUICE ON THE INTESTINAL ABSORPTION OF GLUCOSE



glucose in the mucosal fluid. In this study the percentage of glucose absorbed in the absence and presence of Red gram juice was 2.3 to 11.2 and 1.44 to 6.36 during 10 to 30 minutes. The above findings indicate that Red gram juice has got an inhibitory effect on the absorption of glucose.

Red gram juice definitely has hypoglycemic, hypocholesteremic, hypolipidemic and blood urea lowering effects. But it somehow prevents the formation of glycogen in the liver, the mechanism of which is not yet fully understood. Hence further investigation needs to be done regarding this aspect. Red gram is commonly used in our daily diet, hence it can be recommended for diabetics as a source of protein because of its hypoglycemic effect.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

The present study was undertaken to find out the effect of Red gram on blood glucose level in rats. The results of the study are summarised below.

EXPERIMENTAL

Forty male albino rats of Wister strain weighing 110-120g were selected from the stock colony and divided in to the two groups I and II. Both groups were fed the stock diet throughout the experimental period. Group I which formed the basal control consisted of 15 rats. Group II consisted of 25 rats. Group II rats were given alloxan (140mg/kg of body weight) subcutaneously. Diabetes was found to be induced in two days. But the rats were left for twenty days without any treatment to make sure that the diabetes was permanently induced. After 20 days, that is, on the 21st day, 5 rats of group I and 5 of group II were sacrificed. The remaining 20 rats of group II were divided into group II A and II B each consisting of 10 rats. Group II A was considered as experimental control and not given any treatment. Group II B rats formed the experimental group and were treated by administering Red gram juice for 15 days and on the 36th day, 5 rats from each of group I and group II and group II B were sacrificed and after discontinuing the treatment with Red gram juice, for further 15 days, the remaining rats were sacrificed on the 51stday. Each time blood and liver samples were collected for analysis.

Blood glucose level of diabetic rats before and after treatment with Red gram juice was estimated and compared with the control values.

The mean blood glucose levels of the experimental control and experimental group (162.8 ± 3.19mg%) rats were raised more than two fold on induction of diabetes when compared to the basal control (60.3 ± 3.19mg%) which were not injected alloxan. The mean blood glucose of the experimental group after 15 days treatment with Red gram juice was reduced by 24.4% (from 162.8 ± 3.19 to 122.9 ± 3.79mg/100ml) while in the experimental control group the blood sugar value did not undergo any significant change. On discontinuing the administration of Red gram juice for 15 days, the mean blood glucose level was increased (from 123.9 ± 3.79 to 140.3 ± 2.89mg/100ml) in the experimental group. This confirms that Red gram juice has blood sugar lowering effect.

The mean blood urea level of the experimental control and experimental group (61.1 ± 3.74mg%) rats were raised almost three fold (2.8 times) compared to the basal control group (21.6 ± 2.03mg%) on induction of diabetes. The mean blood urea value of the experimental group after 15 days treatment with Red gram juice was reduced by 31.9% (from 61.1 ± 3.74 to 34.4 ± 2.87mg/100ml). On discontinuing the administration of Red gram juice to diabetic rats for 15 days, the blood urea level increased (from 34.4 ± 2.87 to 45.0 ± 1.81mg/100ml) in the experimental group. This confirms that confirms that Red gram juice lowers blood urea level.

The increase in blood urea level in diabetic rats before the treatment was due to the high rate of protein catabolism. And the decreased blood urea level from 61.1 ± 3.74 to 34.4 ± 2.87mg/100ml confirms the fact that Red gram juice has got blood urea lowering effect.

The mean serum cholesterol level of the experimental control and experimental group (246.7 ± 6.12mg%) rats were raised three fold when compared to the basal control group (81.3 ± 3.41mg%) after induction of diabetes. After 15 days treatment with Red gram juice, the mean cholesterol level of the experimental group reduced (from 246.7 ± 6.12 to 168.4 ± 4.27mg/100ml) on discontinuing the treatment for 15 days, the mean serum cholesterol level of the experimental group was increased (from 168.4 ± 4.27 to 180 ± 4.04mg/100ml) which confirms the fact that Red gram juice has got antihypercholesterolemic effect.

The mean serum triglyceride level of the experimental control and experimental group (111.36 ± 6.4mg%) rats were raised almost three fold compared to the basal control group (39.8 ± 1.99mg%) after the induction of diabetes. And this is due to increased mobilisation of fat in diabetic rats. In severe cases more fat is used for supplying the energy requirements of the body and hence serum triglyceride level will be raised in diabetics.

After 15 days treatment with Red gram juice the mean serum triglyceride level of the experimental group was reduced by 31% (from 111.86 ± 6.4 to 80 ± 3.6mg/100ml). On discontinuing the treatment for 15 days, the serum triglyceride level increased from 86.9 ± 3.6 to 94.6 ± 2.74mg/100ml in the experimental group. This confirms that Red gram juice lowers significantly the serum triglyceride level.

Liver glycogen values of the experimental control and experimental group (1.22 ± 0.12g%) rats were decreased when compared to the basal control group (2.16 ± 0.14g%) on induction of diabetes, the decreased being significant at 1% level. After 15 days treatment with Red gram juice the mean liver glycogen value of the experimental group increased only slightly (from 1.22 to 1.31g/100g). However, the increase was not statistically significant. On discontinuing the treatment with Red gram juice for 15 days the mean liver glycogen content in the experimental group showed an upward trend (from 1.03 to 1.36g%) which confirms that Red gram juice in some way prevented liver glycogen formation. Further investigationis needed in order to understand the actual mechanism involved in this process.

However, Experiment-II was conducted to understand better the results of experiment-I.

EXPERIMENT-II

Intestinal absorptionof glucose was conducted in presence and in absence of Red gram juice at various time intervals. The percentage of glucose absorbed in presence and in absence of Red gram juice indicated a lowering tin the glucose absorption

when the Red gram juice was added to glucose in the mucosal fluid. This indicates that Red gram juice has got an inhibitory effect on the absorption of glucose from the intestine.

Red gram has been used from time immemorial in Indian diets. It is non toxic and has no other adverse effects. Since the present investigation indicates that Red gram is hypoglycemic, hypocholesteremic, hypolipidemic in action and it also lowers blood urea level, it is suggested that it can be profitably used daily in diabetic diets.

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Appendices

APPENDIX - I

ESTIMATION OF BLOOD GLUCOSE BY FOLIN-WU METHOD (VARLEY, 1976)

PRINCIPLE:

Blood was deproteinised and treated with alkaline copper reagent in Folin-Wu tubes and heated. Cuprous oxide formed was treated with an acid molybdate when a blue colored solution was got. The color was compared with that of the standard colorimetrically at 660mμ.

REAGENTS

1. 10% sodium tungstate
2. Phosphomolybdic acid reagent

Dissolved 35g of molybdic acid and 5.0g of sodium tungstate in 100ml of 10% sodium hydroxide and 200ml of water and boiled to remove the ammonia present in molybdic acid. This usually takes 30-40 minutes. Cooled, transferred with washings to a one litre flask, diluted to 750ml. Then added 125ml of phosphoric acid and made up to a litre with water.

3. Alkaline copper reagent

Dissolved 40g of pure anhydrous sodium carbonate in about 400ml of water and transferred to a litre flask. Added 7.5g of tartaric acid. When the latter had dissolved added 4.5g of crystalline $CuSO_4 - 5H_2O$ and made up to a litre.

4. Stock standard glucose solution

Dissolved 0.2g of glucose in saturated benzene solution and made up to 100ml.

5. Working standard solution

10.0ml of the stock standard was diluted to 100ml with distilled water. 1.0ml of this solution contains 200γ of glucose.

PROCEDURE

Took 3.4ml of water, added 0.2ml of blood and 0.2ml of 2/3 N sulphuric acid. Then added 0.2ml of 10% sodium tungstate solution. Kept for ten minutes and then centrifuged. Pipetted out 2.0ml of supernatant in to a Folin-Wu tube.

0.2 - 1.0ml of the standard glucose solution was taken in to a series of Folin-Wu tubes and added 2.0ml of a alkaline-copper solution. Heated the tubes in a boiling water bath for 8 minutes. Cooled and then added 2.0ml of phosphomolybdic acid reagent. Mixed and then made up to 12.5ml with distilled water. The blue colour developed was read colorimetrically using a red filter (660mμ).

APPENDIX-II

ESTIMATION OF UREA BY DAM - TSC METHOD (WATANEY, 1978)

PRINCIPLE

Urea directly reacts with diacetyl monoxime in the presence of thiosemicarbazide to form a red coloured product which is measured colorimetrically at 540nm.

REAGENTS

1. **Acid reagent**

Water	-	100ml
Concentrated H_2SO_4	-	6ml
Phosphoric acid	-	20ml
5% Ferric chloride	-	1.0ml

2. **Colour reagent**

Acid reagent	-	30ml
Water	-	20ml
2.5% DAM	-	1.0ml
0.25% TSC	-	0.25ml

Colour reagent was prepared just before use since the solution is not stable for more than one hour.

3. **Stock standard**

100mg of urea per 100ml. Dissolved 100mg of urea in 0.25% benzoic acid solution and made up to 100ml with the same.

4. Working Standard

2.0ml of the stock standard was diluted to 100ml with water. 1.0ml of this solution contains 20µg of urea.

PROCEDURE

Took 1.0ml of 3% TCA and 0.2 ml of blood, centrifuged after 10minutes. 0.5ml of the supernatant was pipetted out into a test tube.

In to a series of test tubes took 0.5-2.5ml of standard urea solution corresponding to 10-50µg values. The volumes of all the tubes were made up to 3.0ml with water. Added 5.0ml of the colour reagent. Mixed well, corked and heated in a vigorously boiling waterbath for twenty minutes. Along with this a blank was also conducted. Removed the tubes and cooled. The readings were taken in a colorimeter at 540µ against a reagent blank.

APPENDIX-III

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD (YARLEY, 1978)

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 540mμ in a spectrocolorimeter.

REAGENTS

1. Stock Ferric Chloride Reagent

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

2. Ferric Chloride Precipitating Reagent

10.0ml of stock ferric chloride reagent was taken in a 100ml standard flask and made up to the mark with pure glacial acetic acid.

3. Ferric Chloride Diluting Reagent

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

4. Standard Cholesterol Solution

100mg of pure dry cholesterol was taken in a clean dry 100ml standard flask and dissolved in glacial acetic acid. Then made up to the mark with pure gacial acetic acid.

5. Working standard

10ml of the stock standard was taken in a 100ml standard flask containing 0.5ml of stock ferric chloride reagent and made up to the mark with pure glacial acetic acid. 1.0ml of this solution contains 100µg of cholesterol.

PROCEDURE

0.5 - 2.5ml of working standard 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 0.1ml of serum added 4.9ml of ferric choleride precipitating reagent and mixed well. Allowed to stand for a while and centrifuged. Transferred 2.5ml of the clear supernatant into a dry test tube and added 2.5ml of ferric chloride diluting reagent, mixed well. The tubes were kept in cold water and to each tube added 4.0ml of concentrated sulphuric acid drop by drop. The solutions were mixed well. The tubes were allowed to come to room temperature. A blank was also simultaneously 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 against the reagent blank.

APPENDIX - XV

ESTIMATION OF SERUM TRIGLYCERIDES BY CHEN et al METHOD (VARLEY 1980

PRINCIPLE

Triglyceride is measured after hydrolysis by estimating its glycerol content. The commonest procedure involves oxidation of glycerol to formaldehyde which is measured colorimetrically with chromotrophic acid. The lipid extract of serum must be freed from other sources of glycerol, in particular phospholipid, which on oxidation yields formaldehyde. Florisil may be used for this purpose.

REAGENTS

1. Chloroform — Redistilled grade
2. Florisil — Activated by heating for 4 hours at 124° C. stored in a tightly stoppered bottle. Reactivated with 1N hydrochloric acid.
3. Alcoholic Potassium Hydroxide — Dissolved reagent grade potassium hydroxide (about 1/4th of a pellet) in 2.0ml of redistilled 95% ethyl alcohol. Diluted 0.5ml stock potassium hydroxide solution to 2.5ml with 95% ethanol (This was freshly prepared before use).
4. 0.2 N Sulphuric acid
5. Sodium Arsenite (0.5%) — Dissolved 2.25 g of NaOH and 5g of reagent grade arsenious trioxide in distilled water and diluted to 100ml with distilled water.

colour appeared and vanished in a few minutes. Added 5.0ml of chromotropic acid reagent to each tube. Mixed well and heated in a boiling waterbath for 10 minutes. After cooling determined the optical density at 570 nm.

CALCULATION

Milligram of triglyceride per decilitre

$$= \frac{\text{Reading of Test}}{\text{Reading of standard}} \times \text{Concentration of standard} \times \frac{100}{\text{volume of serum taken}}$$

$$= \frac{\text{Test}}{\text{standard}} \times 100\text{mg percent}$$

APPENDIX - V

ISOLATION (OSER, 1965) AND ESTIMATION OF LIVER GLYCOGEN (ROSENTHOLEBERY, 1950).

ISOLATION

Removed a rat from its cage gently to avoid exciting it, stunned it by a blow on the head and decapitated it quickly. Immediately removed the liver, weighed quickly 0.1g. Minced the liver portion immediately and transferred samples of the minced liver to a centrifuge tube containing 6.0ml of 30% Potassium hydroxide. Heated the tubes for 15-20 minutes agitating the solution occasionally to ensure through disintegration.

Added 7.0ml of 95% alcohol to each tube mixed by tapping and immersed in the water both until boiling just begins. Allowed the tubes to cool at room temperature for 2 hours, centrifuged, decanted the supernatant fluid, drained an d washed the precipitate with 5.0ml portion of 60% alcohol by centrifuging and draining as before. Expelled the last traces of alcohol by immersing the tubes, in boiling water just long enough to dry the glycogen.

ESTIMATION

REAGENTS

1. **Stock standard** weighed 20mg of glycogen and dissolved in distilled water and made up to 100ml with water.

2. **Working standard** 10ml of the stock standard was diluted to 100ml with water.

3. 80% Phenol Purified the phenol by distillation and 80ml of it was dissolved in 20ml of water by adding water to the phenol with constant stirring.

PROCEDURE

The glycogen prepared from 0.1g of rat liver was dissolved in 100ml of water from which 0.5 was taken for the experiment.

In to a series of test tubes aliquotes of the standard 0.5 - 2.0ml corresponding to 10-80 ug of glycogen were taken. To all the tubes added distilled water to make up the volume to 2.0ml. Then added 0.1 ml of 80% phenol and finally added 5.0ml of concentrated analar sulphuric acid. Shook and allowed for 30 minutes for the pink colour to develop. Took the readings at 490nm against a reagent blank.