THE EFFECT OF TULASI ON ALLOXAN INDUCED DIABETES IN RATS

BY

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INTRODUCTION

I INTRODUCTION

Diabetes mellitus can be characterized as an insufficiency of insulin relative to the requirement of the tissues for this hormone (David Martin et al., 1981). It is a disorder of the carbohydrate metabolism and is manifested by elevated blood glucose levels (hyperglycemia) and glycosuria and may be accompanied by changes in fat metabolism.

Diabetes is a colossal health problem. It is global in distribution and affects every race except some tribes in South America and to a lesser extent the Eskimos. It is estimated that in India 10 million people are known to have diabetes and yet another 10 million having diabetes but not knowing about it (Viswanathan, 1981). According to a survey at four centres made by the Indian Council of Medical Research, the average incidence of diabetes is 2.1 per cent in the cities and 1.5 per cent in villages. Every year the number of diabetics is increasing by 6.25 per cent in Western Countries and India will be no exception to it as longevity in India is also increasing fast every decade (Ajgaonkar, 1982).

In the tropics, peculiarily males are affected more in contrast to the prevalence in females in developed

countries i.e. in temperate Zones (Bajaj, 1982).

Diabetes may be discovered by all of the classical symptoms which are glycosuria, thirst, polydipsia, polyuria, nocturia, tiredness, loss of weight, delayed healing of wounds, failing strength, electrolyte imbalance, white marks on clothing, pruritus vulvae or balanitis.

As per the yard stick, control of sugar level in blood is control of diabetes (Simha, 1980). Three important factors that contributes to the effectiveness of good control are medication, diet and exercise. All things being equal the diabetic who knows the most about his disease lives the longest. This means that education is the foundation stone on which the control of diabetes depends and we can go a step further and say that without clear and proper instruction, the medical treatment of diabetes may not be effective (Memon, 1982).

Management of diabetes has been revoluationged by the discovery of insulin in 1921. The mortality because of ketacidosis and infection has declined remarkably. Insulin is still the best tool available against diabetes.

In milder cases oral hypoglycemic agents are a good substitute for insulin, on account of convenience of

administration. But there are studies which implicate oral drugs in some complications and mortality in diabetics. Hence oral hypoglycemic agents have been used diminishingly because of their contribution to the rare but often lethal condition of lactic acidosis (Cohen et al., 1976).

Some of the unpleasantness and side effects of insulin therapy are, insulin shock, insulin lipodystrophy, insulin allergy, insulin resistance and hypoglycaemia. To ameliorate the harmful effects of insulin therapy and oral hypoglycemia agents, there has been and eternal search for simpler, cheap, locally available and relatively non-toxic remedies from natural sources in the treatment of diabetes.

In the recent past, pharmacognostic classification of indigenous plants in relation to therapeutic application in diabetes and other clinical disorders have been compiled. The practitioners in Indian systems of medicinehave been using the plant materials and claiming success in the treatment of human diabetes even today. This is because a drug from a herbal source would have ready acceptability to the masses as it will suit the psychology of the people at large (Mukerjee, 1981).

More than 2000 plants have been screened for their blood sugar lowering activity, out of these, mild degree of blood sugar lowering activity was found with Szyzygium cumini (seed), Occinia grandis (root) and Quercus lancea folia (bark), in normal and experimental models.

A study by Giri et al., (1979) has proved the efficiency of Momordica Charanitia in lowering blood glucose level in diabetics. Another investigation by Giri et al., (1981) demonstrated the hypoglycemic effect of ginger in alloxanised rats. A recent study by Giri et al., (1983) has shown that jamiun seed extract is effective in lowering blood glucose in alloxan induced diabetic rats.

According to Dhar et al., (1968) Tulsi (Ocimum sanctum Linn) of family Labiatae has hypoglycemic action. But the actual degree of lowering of blood sugar level has not been reported. Hence the present study has been taken up to determine quantitatively the effect of Tulsi on blood sugar level.

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II REVIEW OF LITERATURE

The review of literature is discussed under the following headings:

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A. Definition of Diabetes Mellitus

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 (Simha, 1982). It is well known that diabetes is caused due to the deficiency of the available hypoglycemic hormone insulin in the metabolic tissues (Ramakrishnan et al., 1980).

Diabetes mellitus constitutes a genetically and clinically heterogeneous group of disorder which share glucose intolerance as a common factor (Thirumoorthi et al., 1983).

B. History of Diabetes

Diabetes is as old as humanity. The earliest reference is in the Egyptian papers by Ebers Papyrus in 1500 B.C. There is a detailed description of diabetes, dietary advice and exercise in Ayurvedic texts by Charaka. Arabic physicians also knew about this malady(Talwalkar, 1981). The word "Diabetes" (to flow through) was coined by the Greek Physician Aeretaeus in the first century A.D. (Gupta

et al., 1981). The Indian Physician Sushruta (about 500 A.D.) described the disease as "Madhumeha" (passing honey urine) with symptoms of thirst, foul breath, voracious appetite and languor (Park & Park, 1976).

C. Prevalence of Diabetes

Diabetes is one of the leading causes of death in the developed countries. World wide population surveys for diabetes indicate that in urban socities 20 to 60 people per 1000 or 2 - 6 per cent have diabetes (Park and Park, 1976). In India, the incidence of diabetes mellitus has been reported to be 2.2 per cent in Delhi, 2.9 per cent in Bombay and 11.3 per cent in Madras (Vakil, 1973). Recently however, a multicentric study, Sponsored by the I.C.M.R. has revealed that the overall prevalence of diabetes in rural India, is 1.5 per cent (Varying from 1.1 to 1.9 per cent in different parts of the country) (Bansilal Vigg et al., 1981).

Diabetes mellitus is an important health problem in India with an overall prevalence of 1.8 per cent (Ahuja, 1979).

D. Aetiology of Diabetes

Although several contributing factors are known to be involved, the precise aetiology is still uncertain. A

summary of some of the factors that might cause diabetes is as follows:

1. Heredity

Genetic factors have a definite role in the aetiology of diabetes. At least in one form of diabetes i.e. the maturity onset diabetes of young (MODY) a precise mode of transmission has been suggested. Tattersal and Fajans (1975) have proposed that MODY is transmitted as an autosomal dominant disorder.

2. Age:

The disease may appear at any age. The juvenile onset diabetes has its highest incidence starting at age 4 or 5 and reaching a peak in early adolescence, and the older onset diabetes has a high incidence after age 40 (Krall, 1978).

3. Sex

There are rather more young male diabetics than female, in middle age women are more often affected. In temperate zones diabetes is more commonly seen in females while in the tropics the reverse is true. Pregnancy and increasing parity may add to the likelihood of developing diabetes (Stanley Davidon et al., 1975).

4. Obesity

Obesity and diabetes appear to be related in that over weight is associated with a decrease in the activity of insulin in various tissues (eg., fat and muscle cells).

5. Infections

These may unmask latent diabetes. Staphylococcal infections in particular are frequently associated with the development of clinical diabetes.

6. Stress

Stressful events have been shown to result in increased sympathetic and peripheral nervous system activity and in increased plasma glucose (Lustman et al., 1983). Males have higher influence of the disease state than females, as the changes in acetyl choline, histamine and cortisol are greater in males than in females (Pathak and Singh, 1983).

7. Diet

An interesting correlation exists between the types of food eaten and the onset of diabetes. A change in diet, particularly an increased consumption of refined carbohydrates causes increased incidence of diabetes. The use of fibre in

the diet has been suggested as a possible factor, since high fibre content decreases the incidence of diabetes.

8. Viruses

A variety of viruses possess the potential of damaging beta cells directly or triggering host's immune mechanism towards beta cell destruction. English scientists noted a relationship between a strain of viruses known as COXSACKIE and diabetes in childreh. Many other viruses including measles, rubella, polio and cytomegalovirus can be incriminated indirectly in Sporadic cases of insulin dependant diabetes mellitus. There is only an isolated report of direct recovery of viruses from the pancreas of a child with insulin dependent diabetes mellitus (Yoon - Ji - Won et al., 1979).

9. Drugs and Medication

Drugs, for example corticosteroids and thiazide divretics, may precipitate diabetes in those genetically susceptible. Toxins like DDT and Fluoride can cause hyperglycemia.

10. Auto Immunity

Diabetes is known to be associated withmany autoimmune disorders, like Addison's disease thyrotoxicosis

and pernicious anaemia. This autoimmune mechanism may operate in concert with the genetic factors as determined by HLA antigens.

11. Trace Elements

The most important trace element with a known relationship to diabetes and Vascular disease is chromium (Dorsy, 1978). Chromium is needed for sugar and fat metabolism in which insulin takes part (Schroeden, 1973). Zinc is necessary for insulin production and zinc deficiency may be a contributory factor to some diabetic states (Alexander, 1979). It has been observed that diabetics excrete increased amounts of zinc in their urine and that their plasma, leucocyte and erythrocyte zinc levels are reduced (Quarterman et al., 1972).

12. Hormones

Excessive amounts of growth hormone (from the pituitary) will counteract the activity of insulin and may produce a diabetes-like condition in addition to excess growth.

i) Growth Hormone

This if administered to dogs, produces permanent

diabetes and about 30 percent of patients with acromegaly are diabetic.

ii) Thyroid Hormones

Thyroxine if given in excess aggrevates the diabetic state and some patients with hyperthyroidism show impaired glucose tolerance.

iii) Adrenaline

This raises blood glucose by increasing breakdown of liver glycogen and by suppressing secretion of insulin.

iv) Adreno Cortial Hormones

Cortisol and other cortico steroids raise the blood glucose by increasing protein breakdown and by inhibiting utilisation of glucose by peripheral tissues.

13. Pregnancy

The Gestational diabetes refers to the hyperglycaemia which may occur temporarily during pregnancy in women with an inherited predisposition I Stanley Davidson, 1975).

B. Classification of Diabetes Mellitus

1. Diabetes mellitus can be broadly classified into two groups.

a) Primary, Idiopathic or Essential diabetes and

b) Secondary diabetes

a) Primary Idiopathic or Essential Diabetes

Primary diabetes has been classified in to 4 sub groups depending on the degree of severity of the disease.

Potential Diabetic (Prediabetic)

A subject with normal glucose tolerance test but with a family history of the disease i.e., one of the parents suffered from diabetes.

Latent Diabetic (Suspected Diabetic)

A subject with a normal glucose tolerance test but who had a diabetic type of glucose tolerance curve under stress conditions such as after cortisone administration pregnancy or sever infection.

Asymptomatic Diabetic (Latent or Chemical Diabetic)

A subject with a diabetic glucose tolerance curve but, without symptoms of diabetes.

Primary Clinical Diabetic (Overt Diabetic)

The clinical diabetes mellitus can be divided into two groups. Juvenile - on set type and adult or maturity onset type.

Juvenile - onset type

This condition usually develops during the first 40 years of life in subjects of normal or less than normal body weight. Insulin administration is essential for their survival, as insulin secretion is very low. The cells soon become exhausted of insulin and the cells become atrophied resulting in total diabetes mellitus.

Adult or Maturity onset type

This condition usually develops in middle - aged or elderly subjects over 40 years of age who are often obese. This is a mild or moderate diabetes which can be controlled by diet or oral hypoglycemic drugs. The β-cells contain moderate amounts of insulin but the secretion of insulin is less than normal.

b) Secondary Diabetes

Secondary diabetes has been classified as follows.

Pancreatic

Absolute insulin deficiency is due to pancretic destruction in chronic pancreatitis and pancreatic carcinoma or total pancreatectomy.

Presence of Insulin Antagonists

Excess growth hormone secretion (acromegaly) or excess glucocorticoid secretion (cushing's syndrome) act as antagonists to insulin.

Inhibition of Insulin Secretion

Due to excess secretion of adrenalin and thyroxine which inhibits insulin secretion by the β cells and promotes breakdown of liver glycogen (Swaminathan, 1981).

2. Revised Classification of Diabetes Mellitus

The criteria for diagnosis and classification of diabetes have been revised in the past two years. Only three overt type of diabetes have been recognised.

a) Insulin dependent diabetes mellitus or type I (Devendra Sareen et al., 1982)

b) Non-insulin dependent diabetes mellitus or Type II and

c) Secondary diabetes mellitus (Chandalia, 1982).

F. Symptoms of Diabetes

The classical symptoms include glycosuria, excessive urination (polyuria) great thirst, weight loss despite a good appetite, polydisia, nocturia, tiredness, delayed

healing of wounds, failing strength, electrolyte imbalance, white marks on clothing, pruritus vulvae or balanitis. In addition, in some instances there may be itching and infexctions of the genitalia and the skin, eye symptoms such as blurred or misty vision, menstrual disorders, impotence, occasional dehydration and diabetic coma (Luft, 1979).

G. Physiological Changes in Diabetes Mellitus

There is excretion of large volumes of urine, upto 5 to 6 litres of urine may be passed daily. A paleurine with a high specific gravity is characteristic of the condition.

There is a decrease in the blood PH or in the acid-buffering capacity of the blood buffers. The plasma volume reduces, and the blood pressure is lowered. In most severed cases, ketonemia (elevated blood ketone bodies) Ketonuria, and acidosis occurs (Lehninger, 1982). The sense of taste can change in diabetes (Neville colman, 1982).

H. Biochemical Changes

The characteristic features of diabetes mellitus is a diminshed glucose tolerance with a raised blood sugar level. A study of Singh et al., 1982 has shown that

diabetics exhibit increased level of blood urea. In severe cases of diabetes, ketonemia is present. It is observed that there is a raise in blood histamine in the case of diabetes (Bhattacharya et al., 1982).

Among the diabetics there is an increased rate of reduction of pyruvate to lactate and hence lactate level is increased. Elevated lactate and pyruvate levels are shown in uncontrolled diabetes mellitus (Thirumalai Kolundu Subramanian et al., 1981).

Disturbance in lipid metabolism is an essential component of deranged metabolism in diabetes mellitus. Serum cholesterol, triglyceride and free fatty acid levels were found to be significantly raised in patients of uncontrolled diabetes without ketosis as compared to those of controlled diabetes (Agarwal et al., 1980).

Serum magnesium levels are found to be significantly higher in dibetes mellitus (Chandra et al., 1981).

Serum chromium, plasma bicarbonate, sodium chloride, inorganic phosphorus levels are decreased, while cartone, potassium and lipoprotein levels are elevated.

Gastric secretary functions are known to be effected in diabetes. Electrolytes in gastric juice are said to be disturbed among diabetes. (Khosla et al., 1983). Cerebrospinal fluid sugar is increased considerably.

Both glycosylated hemoglobin and glycosylated plasma proteins were raised 2-3 times in diabetic cases. (Rastogi et al., 1983). Hyper coagulability of blood was demonstratable in diabetetics by way of increased stickiness of platelets (Rishi et al., 1983).

Mucoprotein level was found to be increased. In uncontrolled diabetes, the amount of 2:3 diphosphoglycerate is reduced because of decreased glycolysis. Glucokinase enzyme is decreased and erythrocyte glyceraldehyde reductase level is increased. Liver copper content is higher while zinc content is same.

I. Metabolic Abnormalities in Diabetes Mellitus

Since diabetes mellitus is primarily due to deficiency in insulin production, the metabolism of carbohydrates, fats and proteins is affected as insulin is essential for the utilisation of glucose in the body. The characteristic findings of the disease are:

i) hyperglycemia

ii) glycosuria and

iii) decreased glucose tolerance. The fasting blood glucose level may range from 120-150mg/100ml as against 75-90 mg/100ml in normal persons.

a) Carbohydrate Metabolism

Insulin lack produces many fundamental changes in carbohydrate metabolism which leads to hyperglycemia

i) Reduced entry and oxidation of glucose in muscle and other tissues

ii) Decreased formation of glycogen in liver

iii) Decreased synthesis of fat from carbohydrates and

iv) Release of glucose into blood from the increased breakdown of glycogen in liver.

Consequently the glucose level in the cells are low and are high in blood and extracellur fluids.

b) Fat Metabolism

Since the tissues cannot oxidise sufficient quantities of glucose to meet the energy needs, the body has to use fats as a source of energy. Consequently fat from adipose tissue is mobilised on a large scale and passes

as free fatty acids into the circulation. The concentration of free fatty acid and triglycerides in blood increases considerably.

c) Protein Metabolism

Since the diabetic cannot meet the energy requirements from the oxidation of glucose and fat, there is breakdown of tissue proteins leading to negative energy balance. The synthesis of tissue proteins from dietary proteins requires insulin. Since, insulin production is inadequate in diabetics, the synthesis of tissue proteins from dietary proteins does not take place. The concentration of amino acids in blood and liver increases considerably. The amino acids are deaminated in liver leading to the production of glucose from the keto acids by the process of gluconeogenesis. The urea produced is excreted in urine. Consequently diabetics need more protein (about 50 per cent more) than normal individuals (Swaminathan, 1981).

J. Complications of Diabetes

Because the incidence and prevalence of diabetes mellitus increases with age, the elderly are the single largest group affected, and have the greatest frequency of

diabetic complications (Sergio Mather et al., 1983).

i) Diabetic Ketoacidosis

Diabetic ketoacidosis is usually characterised by hyperglycaemia, hyperketonaemia and a metabolic acidosis that can be attributed in major part to the elevated levels of acetoacetate, acetone and B-hydroxy-butyrate. It is now accepted that diabetic ketoacidosis is due to an absolute or relative deficiency in the insulin levels as compared to the levels of glucagon, cortisol, growth hormone and the catecholamines.

ii) Diabetic Retinopathy

Diabetic retinopathy is one of the most challenging problems to ophthalmologists (Bhatia et al., 1981). Diabetic retinal disorders can be classified into non proliferative and proliferative retinopathy (Ronald Michels, 1977).

Laser Photocogulation, vitrectomy endophoto coagulation, microsurgery and other intraocular surgical procedures have revolutionized the treatment of diabetic retinopathy and its complications (Motilal Raichand, 1981).

iii) Diabetes and Sexual Dysfunction

Symptoms arising out of sexual dysfunctions are common and very disturbing in diabetics, particularly in

male patients. Autonomic and peripheral neuropathies and psychogenic abnormalities were the probable causes for sexual dysfunctions observed in these diabetics (Alam et al., 1981).

In men organogenic impotence can arise from diabetic neuropathy and in woman certain forms of vaginitis, and consequent dyspareunia, are diabetic in origin (Arthur Krosnick & Stephen Podolsky, 1981).

iv) Diabetic Nephropathy

This may be caused due to combination of complications resulting from persistant presence of albumen in the urine, puffness of the feet, hypertension, anemia, kidney infection narrowing of kidney arteries thickening of the filtering membrane etc.,

On long-term basis, transplantation has produced higher survival rate better improvement in diabetic retinopathy and neuropathy and better rehabilitation than maintenance hemodialysis.

v) Kidney and Urinary Tract Infections

It is a common observation that the clinical evidence of urinary tract infection and/or bacteruria are

more frequent in diabetics than in nondiabetics. There is a high prevalence or renal parenchymal infection among diabetic patients. Various fungal and yeast infections involving the kidneys, ureters and bladder are common among diabetics (Simha, 1982).

vi) Diabetes and Tuberculosis

Pulmonary tuberculosis is frequently associated with diabetes. It is assumed that hyperglycemia favours the growth and viability of tubercle bacilli and also lowers the resistance to infection (Jain et al., 1980).

vii) Diabetic Neuropathy

Diabetic neuropathy may be caused by an impairment of myoinositol metabolism in the nerves. Myoinositol supplements in diet may improve nerve conduction velocity in diabetic neuropathy. The most serious complication of neuropathy in the elderly diabetic patient is hypoesthesia affecting the lower extremities (Marvin Levin, 1983). Ephedrine markedly reduced neuropathic oedema in insulin-dependent diabetics (Edmonds etal., 1983).

viii) Visceral Neuropathy

This occurs frequently in the organs like, eye, bladder, bottom and joints of the foot, small intestine and many other organs.

ix) Diabetes and Cardio Vascular Disease

The cardiovascular system is invariably involved in diabetes sooner or later, irrespective of the quality of control of the diabetic process (Chhetri, 1981). Myocardial infarction, sudden death, disabling angina pectoris and congestive heart failure apparently occur with a greater severity in diabetics and at an earlier age than in non-diabetics.

Arteriosclerosis affect the large blood vessels particularly the aorta and the major arteries that conduct blood to the hands, legs, lungs, abdomen, heart, kidneys and the nervous system. Atherosclerosis of cerebral vessels and hyperglycaemia are thought to be involved in the premature intellectual deterioration which occurs in some diabetics (Jones et al., 1983). In diabetic patients with defective cardiac innevation, diarrhoea was very common (Chadda et al., 1981).

K. Management of Diabetes Mellitus

1. Primary Prevention

a) Genetic Counselling

Accurate counselling advise is impossible as long as the mode of inheritance of human diabetes mellitus is unknown. The calculated risk for diabetic offsprings of conjugal diabetics is 2.9 times that of those who have only one diabetic parent. The gene is prevalent in the population, and the majority of individuals develop diabetes after the period of child bearing (Krall, 1978).

b) Diet Control

The role of diet in the management of diabetes mellitus has been known since antiquity. In the 20th century prior to discovery of insulin, the treatment of diabetes mellitus included intermittent fasting, undernutrition and carbohydrate restriction (Viswanathan, 1978).

A restricted calorie, high carbohydrate diet, rich in polysaccharides and fibre content is more effective in controlling diabetes than the western type of diet rich in monosaccharides and low fibre content (I.C.M.R., Annual Report, 1980).

Addition of non-digestible fibre such as pectin, whole wheat bran, or guar gum to food improves glucose tolerance in diabetics (Tarun Ray and Kathi Mansell, 1983).

2. Secondary Prevention

a) Case Detection

Although the laboratory diagnosis of diabetes rests on the findings of an elevated blood glucose level, a number of factors must be taken into account in assessing the findings. The best screening test is the fasting glucose level and/or a two hour post prandial blood sample. The urine glucose level is useful only as a preliminary screen to pin point those who may need a blood glucose determination.

By common agreement, the most sensitive diagnostic method is the standard glucose tolerance test (GTT) with the oral glucose tolerance test being as sensitive as the intravenous (Thomas Flood, 1979). The evaluation of the sum of all the values during the GTT however, appears to be the best method for the diagnosis of diabetes mellitus (Mehta et al., 1981).

b) Maintenance of Body Weight

The diabetic should attain and maintain an appropriate body weight. The main measure to be taken is to prevent obesity and to treat obesity when it is already present. The diabetic should maintain his body weight at 5 per cent below his ideal weight (Antia, 1975).

c) Control of Infections

Diabetics are more susceptible to infections than non diabetics. Studies on host defense mechanism in poorly controlled diabetics indicate that the profound defect in these defenses relates primarily to polymorphonuclear leukocytefunction, with depressed chemotaxis and phagocytosis. Cell mediated immunity, particularly in the poorly controlled diabetic, has also been found to be impaired (Purnendu Sen and Donald Louria, 1983).

L. Treatment of Diabetes

The ideal treatment for diabetes would allow the patient to remain not only symptom free but in good health with a normal metabolic state and to escape the long term complications.

1. Dietary Treatment

Optimal control or diabetes mellitus at any stage is possible only with optimal dietary control.

The diet for the unstable diabetic follows conventional lines. It is designed to provide an adequate supply of minerals and vitamins and a sufficient number of caleries, supplied as protein, carbohydrate and fat. New dietary recommendations for diabetics suggest increasing the proportion of dietary carbohydrate toover 50 per cent of food energy, taking this carbohydrate in the form of fibre rich foods to delay glucose absorption and restricting fat, including partly replacing saturated fats with polyunsaturates (Harry Keen, 1982).

2. Exercise

Regular exercise is an absolute necessity and complimentary to medication and diet regime. Exercise is known to facilitate glucose transport in the muscle and other tissues, even in the presence of very low levels of circulating insulin (Siddharthshah and Sheilesh Jain, 1982). Exercise should not be taken at the peak action of the

insulin used, this will precipitate hypoglycemia (Ajgaonkar, 1982). Nor should severe exercise be taken without insulin, it will cause acidosis and even coma. Exercise to be effective needs insulin either endogenous or exogenous.

3. Education

Education has an important role to play in the management of diabetes (Arbind Muller, 1980). The diabetic has to be well informed about his disease and therapy so that he requires the help of a professional physician only rarely.

More over education for a diabetic is a continous process for the simple reason that the disease is life long.

4. Medication

Medication in the case of diabetes treatment falls under two major types namely, oral drugs and insulin injections. The medication not only takes care of the immediate correction and control of the metabolic disorders, it is also aimed at prevention of chronic vascular disorders (Simha, 1980).

a) Insulin Therapy

Insulin has only recently become available in 1922, for the treatment of diabetes mellitus in human patients, as a result of the join investigations in Toronto, Canada of Banting and Co-workers (Young, 1983).

Insulin is prescribed for patients if diet therapy fails, if oral antidiabetic agents are ineffective or if the clinical features indicate probable failure of oral drugs.

Insulin is a protein extracted from the pancreas of animals and is packaged in Crystalline form. Insulin preparations are classified as having short, intermediate and prolonged onset and duration of action (Michael Brownlee, 1979).

b) Newer Insulins

Using physical and biochemical methods both the crystallized beef and pork insulins have been purified and four different types of such insulins are available:

1) Mono Component Insulin
2) Single Component Insulin
3) Single Species Insulin
4) Rare Immunogenic Insulin.

All these insulins have been shown to be the purest form of insulin available and have very little antibody producing capacity in animals and in human (Bansal, 1980).

ii) Dangers of Insulin Therapy

The following are the dangers of insulin therapy: 1) Hypoglycemia (2) Insulin Allergy (3) Insulin Lipo Dystrophy (4) Insulin Presbyopia (5) Insulin Neuropathy and (6) Obesity (Gupta et al., 1981).

b) Oral drug therapy

These are generally prescribed for mild maturity - onset type of diabetes. Oral drugs are not prescribed for Juvenile cases.

Many such oral hypoglycemia drugs are in use in India and a few examples are tolbutamide, glibenclamide, chloropropamide phenformin, metformin and glipizide.

There are reports of untoward hepatic hematologic gastrointestinal, dermetologic and metablic effects of these drugs, but these occur very rarely in carefully monitored patients. Hypoglycemia, the most serious complication, is not frequent (Charles Kilo, 1979).

M. Indigenous drugs in the treatment of Diabetes

In addition to the oral anti-diabetic drugs, indigenous drugs are used in the treatment of diabetes. The advantage of indigenousdrugs is that it is safe, simple, relatively nontoxic and effective. This system also advocates many practices to promote health such as dietary habits, physical exercise and meditation (WHO, 1981).

Since the time of "Charaka" and "Susruta" several remedies have been tried in the treatment of diabetes mellitus is Indian system of medicine. Mostly herbal and mineral in their character they were commonly used in combinations as powder, paste, infusion, decoctions, pills etc., (Mukherjee and Mukherjee, 1966).

Some of the indigenous drugs on which study has been carried out is given below:

1. A study on the effect of Sarkaraikolli on blood sugar level was carried out on diabetic patients (Nageswari, 1978). The mean blood sugar reduction ranged from 2 - 44mg per cent indicating that though sarkaraikolli was not found to decrease the blood sugar level significantly, definitely it has blood sugar lowering effect and antidiabetic property.

2. Onion and garlic are reported to possess hypoglycemic activity. The effect of garlic on blood sugar and serum insulin levels in rats were studied under different experimental conditions by Chang and Johnson (1980). The hypoglycemic effect of garlic seems associated with the increase in insulin level (Kamanna and Chandrasekhar, 1982).

3. Gowar (Cyamopsis Tetragonoloba) and its seeds are being used as a folk lore remedy in the treatment of diabetes mellitus in many parts of India. Whole gowar decoction (20g/kg) dose, significantly lowered blood sugar at 1 and 3 hr period and a highly significant reduction was observed at 5 hr period (Pillai et al., 1980).

4. Madhwashara, a compound preparation formulated in honey base is effective in the treatment of maturity onset diabetics (Udupa, 1979).

5. Ficus bengalensis (Banayan tree) is one of the several medicinal plants reputed for its antidiabetic properties. Ethanol extract of the bark showed hypoglycemic activity at a dose 1g/rabbit of about 1.5 kg. weight (Venkanna Babu and Suryanarayana Murthy, 1983).

6. Nimbidin isolated from neem oil is a cream

coloured water insoluble granular powder souble in 10 per cent alcohol in water. Neem oil and nimbidin exert hypoglycenic effect (Pillai et al., 1981).

7. The hypoglycemic effect of ginger was studied on normal albihorats (Giri et al., 1981). Fresh ginger juice extracted from 1g of ginger was administered to rats. In ginger fed rats the reduction in blood glucose ranged from 10 - 24mg/100 ml of blood.

8. The effect of bitter gourd juice on blood glucose levels and absorption of glucose was studied in alloxanised rats (Giri et al., 1982). Bitter gourd juice is found to lower the blood glucose by inhibiting the glucose absorption from the intestine.

9. Inthe 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 and serum triglyceride levels were found to decrease significantly (Giri et al., 1983).

N. Tulasi in the Treatment of Diabetes

Ocimum sanctum (Tulasi) is a well known plant grown all over India and considered sacred by many Indians. It is

a highly sweet scented plant and it is considered to be of great medicinal value.

It is a strongly scented herb, undershrub or shrub about 1-3 feet long, erect, containing opposite leaves, oval and narrowed at lower end, flowers whorled, tips of pedicles re-curved, base woody, root vertical and fibrous. No less than half a dozen varieties are found. The black and the white varieties possess identical qualities. (Sarat Chandra Ghose, 1974).

a) Constituents of Tulsai

Ocimum is an important source of many essential oils and aroma chemicals. Ocimum species with oil rich in camphor, citral, geraniol, linalool, linalyl acetate, methyl chavicol, eugenol, thymol and so on are important and can be harnessed for successful utilization (Atal and Kapur, 1977).

If it is distilled it yields a yellowish green volatile oil, lighter than water, which solidifies in time into a crystalline camphor isometric with that of turpentine.

b) Action and Uses of Tulasi

Several medicinal properties have been attributed to the plant in the traditional system of medicine. Pharmacological studies carried out by various workers during the last few decades indicate the presence of anabolic, hypotensive, cardiac depressant, smooth muscles relaxant and antifertility properties in the plant. This plant has been found to possess adaptogenic (Antistress) properties (Bhargava and Singh, 1981). It is demulcent, expectorant and anti-periodic.

According to Dhar et al., (1968) Tulasi (Ocimum Sanctum Linn) of family labiatae has hypoglycaemic action. But the actual degree of lowering of blood sugar level has not been reported. Hence the present study has been taken up to determine quantitatively the effect of Tulasi on alloxan induced diabetes in rats.

EXPERIMENTAL PROCEDURE

III. EXPERIMENTAL PROCEDURE

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

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 Tulasi Leaf extract
6. Collection of blood and liver samples
7. Biochemical analysis
   a. Estimation of glucose
   b. Estimation of urea
   c. Estimation of cholesterol
   d. Estimation of triglyceride
   e. Isolation and estimation of liver glycogen

B. Experiment - II

An invitro study of the absorption of glucose by the intestine was conducted to study the effect of tulasi leaf extract on glucose absorption.

C. Experiment - III

Estimation of the following minerals in Tulasi:

1. Zinc
2. Copper
3. Manganese
4. Iron

D. Statistical Analysis

A. Experiment - I

1. Formulation of Diet:

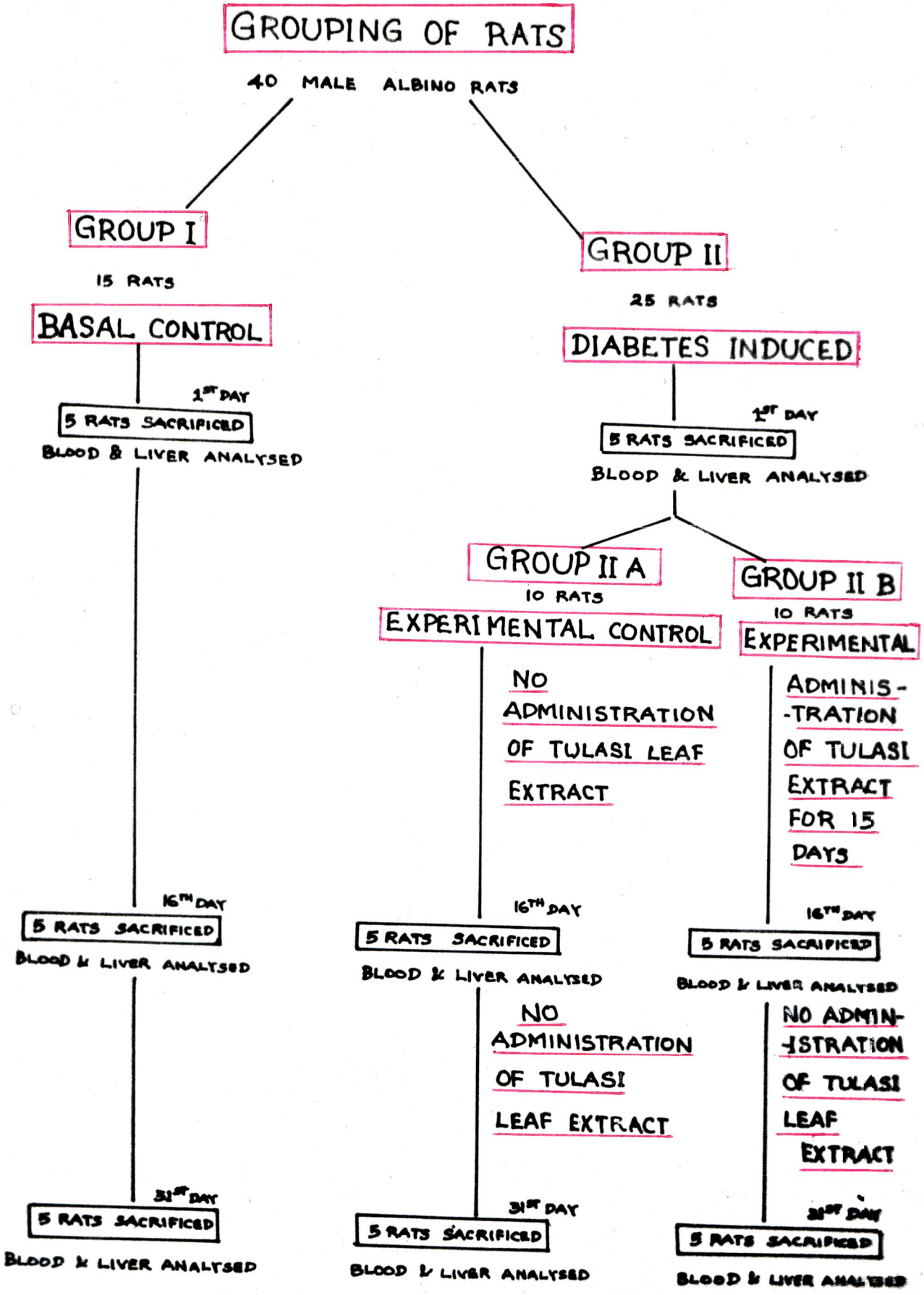
The stock diet was formulated and fed to all the rats. Its composition is given in Table I.

TABLE I

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
Ground nut oil	10

Figure 1.


GROUPING OF RATS
40 MALE ALBINO RATS
GROUP I
15 RATS
BASAL CONTROL
1ST DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
16TH DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
31ST DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
GROUP II
25 RATS
DIABETES INDUCED
1ST DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
GROUP II A
10 RATS
EXPERIMENTAL CONTROL
NO ADMINISTRATION OF TULASI LEAF EXTRACT
16TH DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
NO ADMINISTRATION OF TULASI LEAF EXTRACT
31ST DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
GROUP II B
10 RATS
EXPERIMENTAL
ADMINIS--TRATION OF TULASI EXTRACT FOR 15 DAYS
16TH DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED
NO ADMIN-ISTRATION OF TULASI LEAF EXTRACT
31ST DAY
5 RATS SACRIFICED
BLOOD & LIVER ANALYSED

2. Selection and Grouping of Animals:

Forty male albino rats of wistar strain weighing 150-190g were selected from the stock colony and divided into group I and group II as shown in Figure - I. Both group I and group II rats were fed the stock diet through out the experimental period. Group I consisting of 15 rats were not given either alloxan or tulasi leaf extract and thus formed the basal control group. Group II consisted of 25 rats. This group was given alloxan subcutaneously (140mg/1kg. body weight). Diabetes was found to be induced in two days. But the rats were left for 30 days without any treatment to make sure that the diabetes was permanently induced. After 30 days, the group II rats were divided into sub-groups, group IIA and group IIB each consisting of 10 rats. Group IIA rats were used as experimental control while group IIB rats formed the experimental rats. Group IIB rats were given tulasi leaf extract for 15 days. As for the experimental control (group IIA) rats, they were not given tulasi leaf extract at any stage in the experiment. At three stages in the experiment, described below and indicated in Figure - I, 5 rats from each of the groups were stunned by a blow on the head and immediately blood was collected from the jugular vein ~~by cutting open the jugular~~

vein, by cutting open the jugular region. They were then decapitated quickly and the liver was removed for bio-chemical studies.

The three stages being:

i) 30 days after the induction of diabetes

ii) 15 days after the treatment of tulasi leaf extract and

iii) 15 days after discontinuing the treatment with tulasi leaf extract.

3. Induction of Diabetes:

The rats were fasted for 24 hours and then quickly given a single subcutaneous injection of alloxan (140 mg/kg. body weight dissolved in physiological saline). Diabetes was induced in 2 days, but they were left as such for 30 days to make sure that diabetes was induced permanently.

4. Preparation of diet and feeding:

Food was weighed, mixed with sufficient water and cooked by steaming for 30 minutes. All the rats were fed the same amount of food. The food intake varied between 50 - 90g from the beginning to the end of the experiment. Water was fed at libitum.

FORCE FEEDING OF TULASI LEAF EXTRACT

5. Preparation and feeding of tulasi leaf extract:

Fresh leaves of tulasi (ocimum sanctum) the green variety was taken from the same source and homogenised in a waring blender using distilled water such that a 100 per cent solution was got. This was filtered through a muslin cloth and 2 ml of the tulasi leaf extract was given to the experimental rats (Group II B) by force feeding, daily in the morning, before feeding the stock diet (Plate - I).

6. Collection of blood and liver samples:

The rat was removed from its cage, gently to avoid exciting it, it was stunned by a blow on the head and immediately blood was collected from the jugular vein, by cutting open the jugular region. It was then decapitated quickly and the liver was removed, 0.1g quickly weighed and transferred to 6ml of 30 per cent potassium hydroxide.

7. Bio-chemical analysis:

All the estimations were done in duplicate.

a) Estimation of glucose:

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.

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 triglyceride:

The serum triglyceride level was estimated by Van Handel and Zilversmit, (Varley, 1980). The details of this method are given in Appendix - IV.

e. Isolation of liver and estimation of liver glycogen:

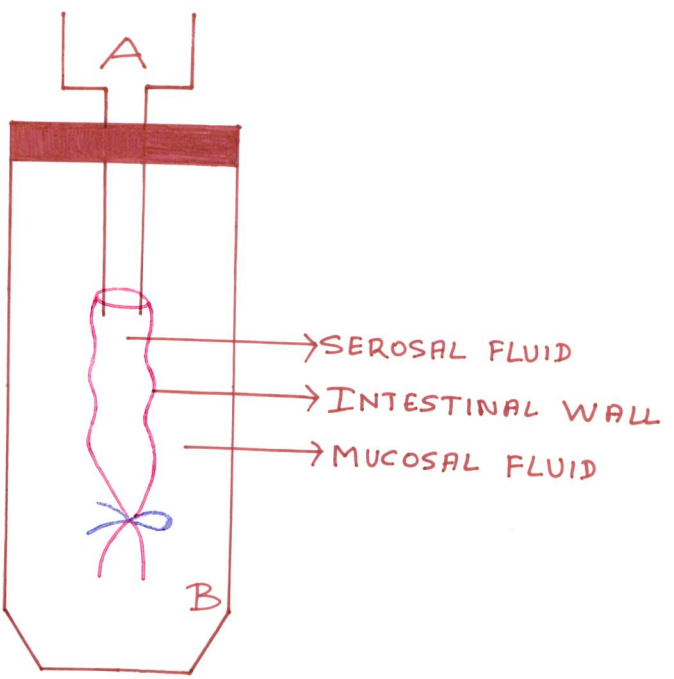
The liver was removed after decapitation and used for the estimation of glycogen. The details of the method are presented in Appendix - V.

B. Experiment - II:

In this experiment an in vitro study of the absorption of glucose by the intestine was conducted to study the effect of tulasi leaf extract on glucose

Figure. 2.

EVERTED SAC METHOD - INTESTINAL ABSORPTION OF GLUCOSE.



absorption. The method followed is that of Wilson (1958) with slight modifications.

Diabetic rats were fasted for 30 hours and then anesthetized with chloroform before the experiment. The intestine was separated from the mesenterum and cut into 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 II. One ml of Ringer solution was (Umbreit et al., 1972) introduced into the intestinal sac through the tube A, avoiding air bubbles, and the intestine was lowered into tube B, which contained 10ml of Krebs Ringer phosphate buffer, containing 0.5 per cent 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 per cent glucose and tulasi at 100 per cent concentration. (Tulasi was homogenized with Ringer solution to produce 100 per cent solution).

C. Experiment - III

In tulasi the minerals copper, zinc, manganese and iron were estimated by atomic absorption spectrophotometry. The details of the method are give in Appendix - VI.

D. 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 the second sample.

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 tulasi leaf extract on blood glucose level in alloxan induced diabetic rats.
2. The effect of tulasi leaf extract on blood urea level in alloxan induced diabetic rats.
3. The effect of tulasi leaf extract on serum cholesterol level in alloxan induced diabetic rats.
4. The effect of tulasi leaf extract on serum triglyceride level in alloxan induced diabetic rats.
5. The effect of tulasi leaf extract on liver glycogen content in alloxan induced diabetic rats.

Experiment - II

The effect of tulasi leaf extract on glucose absorption by the intestine.

Experiment - III

Estimation of copper zinc, iron and Manganese content in tulasi leaf.

Experiment - I

1. The Effect of Tulasi Leaf Extract on Blood Glucose Level in Alloxan Induced Diabetic Rats.

Table II gives the mean glucose level in blood after the induction of diabetes in rats by administration of alloxan.

It can be seen from Table II that the mean blood glucose level of the experimental control and experimental group rats are raised more than three fold when compared the based control group which were not injected alloxan. The increase in blood glucose in each case is statistically significant at 1 per cent level. Figure 3 diagramatically gives the same.

It is clear from the results presented in Table II that the mean blood glucose value of the experimental group after 15 days treatment with tulasi leaf extract was reduced by 43 per cent (from 183.2 ± 43.9 to 103.2 ± 9.926 mg./100ml.), while in the experimental control group the mean blood glucose value has further increased.

The differences in the mean blood glucose values between the basal control and experimental control, basal control and experimental, and between experimental control and experimental group are statistically significant at 1 per cent level.

TABLE II

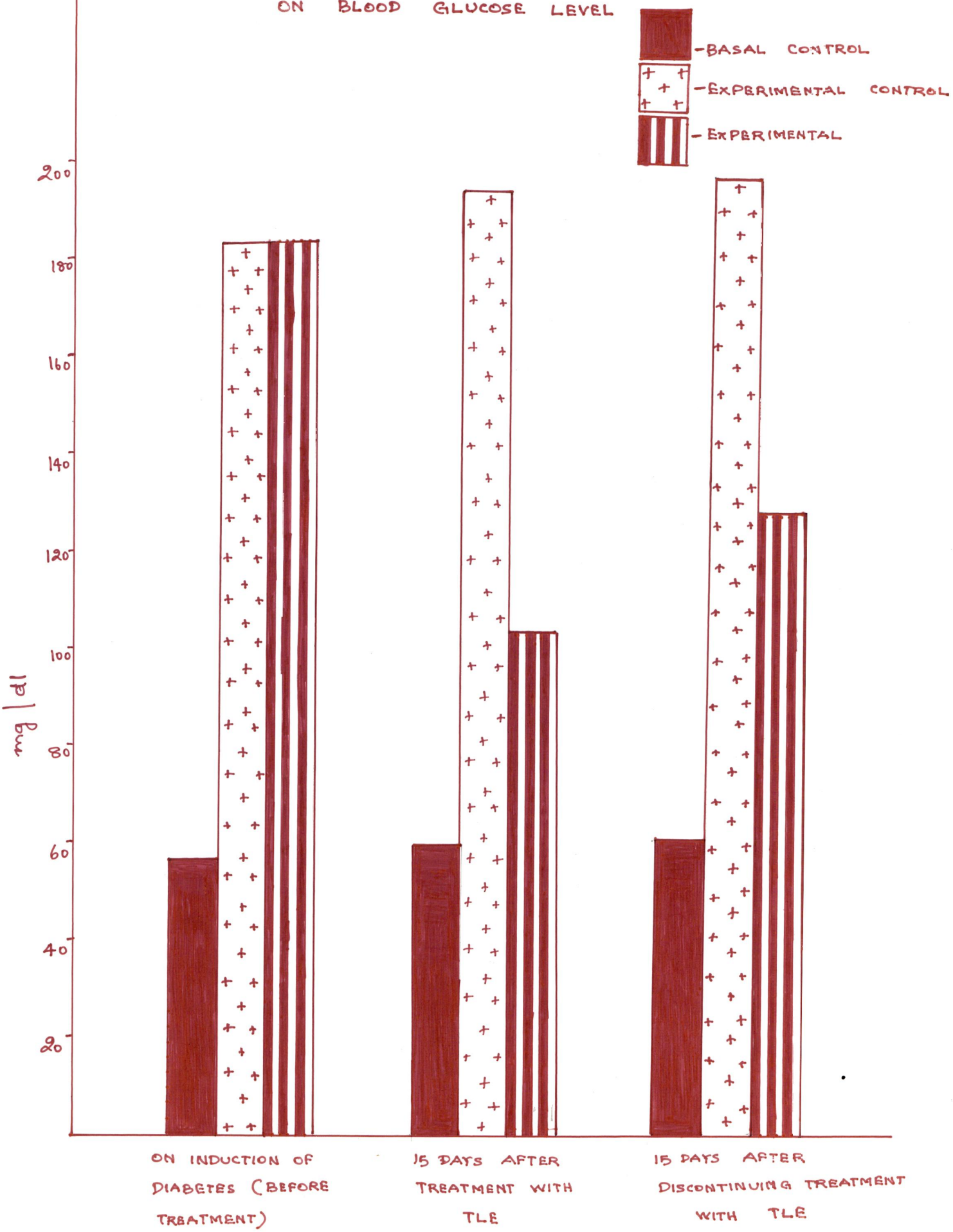
BLOOD GLUCOSE VALUES OF RATS

Different experimental conditions	Basal control Mean ± S.D. mg/dl	Experimental control Mean ± S.D. mg/dl	Experimental mean ± S.D. mg/dl.	Groups compared	Statistical significance
Diabetic condition	56± 5.656 a_1	183.2± 43.9 b_1	183 ± 43.9 c_1	a_1 Vs b_1	6.425**
				a_1 Vs c_1	6.425**
				b_1 Vs c_1	0 NS
				a_1 Vs a_2	1.1181N.S.
				a_2 Vs a_3	0.2795 N.S.
				a_1 Vs a_3	1.1181 N.S.
15 days after treatment with tulasi leaf extract	59.2± 2.993 a_2	194.4± 13.99 b_2	103.2± 9.926 c_3	a_2 Vs b_2	21.129**
				a_2 Vs c_2	9.489**
				b_2 Vs c_2	11.8878**
				b_1 Vs b_2	0.5434NS
				b_2 Vs b_3	0.3879NS
				b_1 Vs b_3	0.70737NS
15 days after discontinuing the feeding of tulasi leaf extract	60± 5.656 a_3	197.6± 12.02 b_3	128± 5.656 c_3	a_3 Vs b_3	23.16**
				a_3 Vs c_3	19.0077**
				b_3 Vs c_3	38.910**
				c_1 Vs c_2	3.974**
				c_2 Vs c_3	4.8536**
				c_3 Vs c_1	2.7883*

** - Significant at 1% level
* - Significant at 5% level
NS - Not significant

Figure. 3.

THE EFFECT OF TULASI LEAF EXTRACT (TLE) ON BLOOD GLUCOSE LEVEL



When the mean blood glucose value of experimental group rats in diabetic condition is compared with the value of the same group after 15 days treatment, the decrease in blood glucose is statistically significant at 1% level. This shows that tulasi leaf extract has hypoglycemic effect.

On discontinuing the administration of tulasi leaf extract to diabetic rats for 15 days, the mean blood glucose level has further increased (from 103.2 ± 9.926 to 128 ± 5.656 mg/100ml in the experimental group.

But the value is still lower than that of the experimental control group. When the mean blood glucose value of experimental group is compared with the corresponding values of basal control and experimental control, the differences noted between the groups are statistically significant at 1 per cent level.

Figure 3 diagrammatically represents the same.

2. <u>The Effect of Tulasi Leaf Extract on Blood Urea Level in Alloxan Induced Diabetic Rats</u>.

Table III gives the mean urea level in blood after the inductionof diabetes in rats by administration of alloxan.

TABLE III

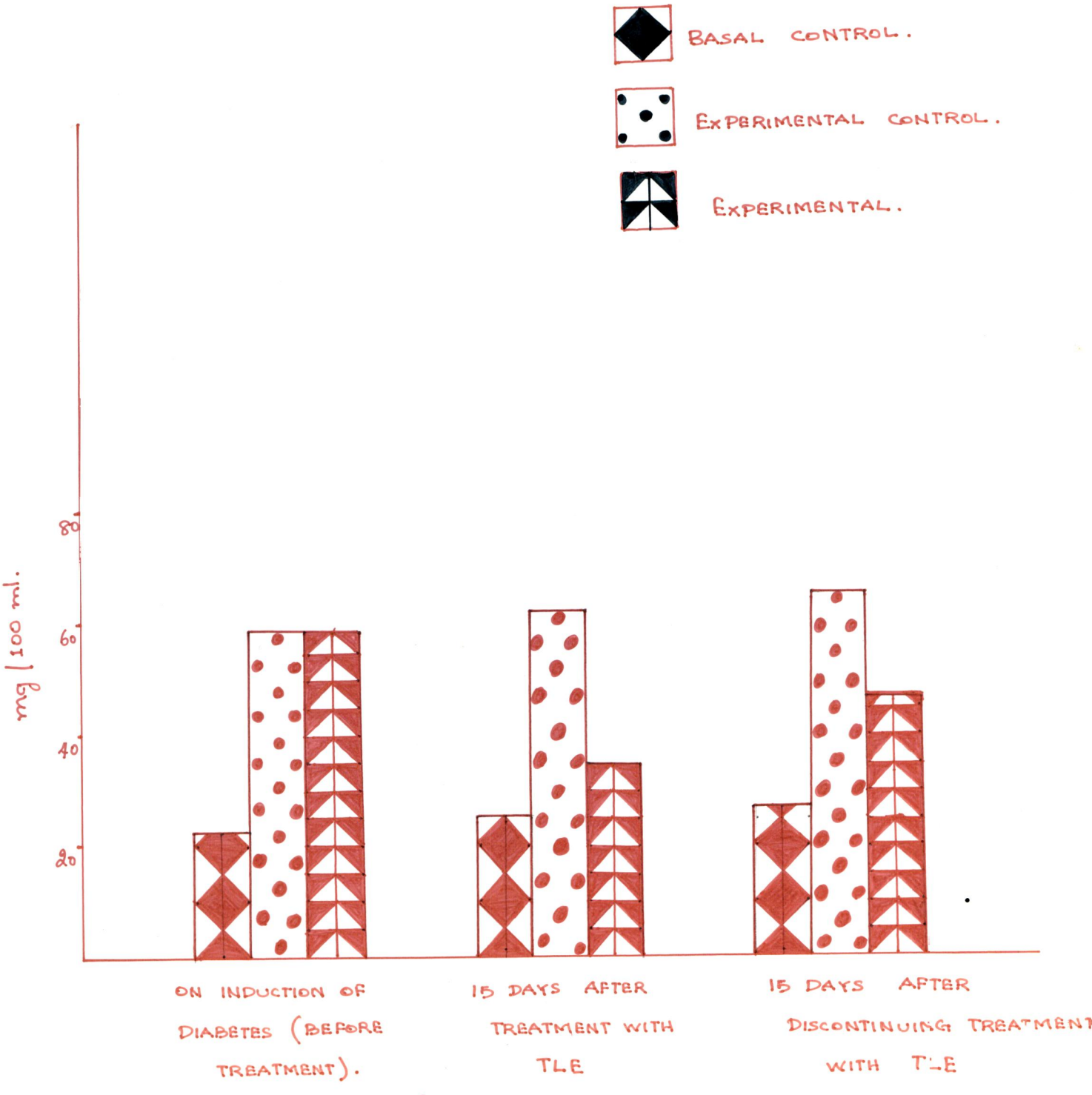
BLOOD UREA VALUES OF RATS

Different experimental conditions	Basal Control Mean ± S.D.	Experimental Control Mean ± S.D. mg/dl.	Experimental Mean ± S.D. mg/dl.	Groups compared	Statistical significance
Diabetic condition	23.2± 4.099	59 ± 19.32	59 ± 19.32	a_1 Vs b_1	4.05288**
				b_1 Vs c_1	) 0 NS
	a_1	b_1	c_1	a_1 Vs c_1	4.05288**
				a_1 Vs a_2	0.8810 NS
				a_2 Vs a_3	0.7504 NS
				a_1 Vs a_3	1.4559NS
15 days after treatment with tulasi leaf extract	25.2± 2.993	63 ± 5.291	35 ± 3.687	a_2 vs b_2	13.9032**
				a_2 Vs c_2	4.6141**
	a_2	b_2	c_2	b_2 Vs c_2	9.7078**
				b_1 Vs b_2	0.44648 NS
				b_1 Vs b_3	0.7417NS
				b_2 Vs b_3	0.6708 NS
15 days after discontinuing the feeding of tulasi leaf extract	26.8± 3.71	66± 8.484	46.8± 5.741	a_3 Vs b_3	9.4653**
				a_3 Vs c_3	6.5425**
	a_3	b_3	c_3	b_3 Vs c_3	4.19068**
				c_1 Vs c_2	2.7282*
				c_2 Vs c_3	3.8668**
				c_1 Vs c_3	1.3534ONS

** Significant at 1% level
* Significant at 5% level
NS Not significant

Figure. 4.

THE EFFECT OF TULASI LEAF EXTRACT (TLE) ON BLOOD UREA LEVEL.



It can be seen that the mean blood urea level of the experimental control and experimental group rats are raised more than 2 fold when compared to the basal control group which were not injected alloxan. The increase in blood urea level in each case is statistically significant at 1 per cent level.

Figure 4 diagrammatically gives the same.

It is clear from Table III that the mean blood urea value of the experimental group after 15 days treatment with tulasi leaf extract is reduced by 40% (from 59$\pm$ 19.32 to 35$\pm$ 3.687 mg/100ml). The basal control and the experimental control groups have shown no statistically significant change. The differences in mean blood urea value between the basal control and experimental control, basal control and experimental, and between experimental control and experimental are statistically significant at 1 per cent level in each case.

When the mean blood urea value of experimental group rats in diabetic condition is compared with the value of the same groups after 15 days treatment, the decrease in blood urea is statistically significant at 5 per cent level. This shows that tulasi leaf extract has hypoglycemic effect.

It is noted from Table III that in the experimental group the mean blood urea level has further increased (from 35± 3.687 to 46.8± 5.741 mg/100ml) on discontinuing the administration of tulasi leaf extract for 15 days. But the value is still lower than that of the experimental control groups. When the blood urea value of experimental group is compared with the corresponding values of basal control and experimental control the difference noted between the groups was statistically significant at 1 per cent level.

Figure 4 diagrammatically indicates the same.

Since the diabetic cannot meet energy requirement from the oxidation of glucose and fat, there is breakdown of tissue protein leading to negative energy balance. Transport and uptake of amino acids in peripheral tissues is also depressed. Causing an elevated circulating level of amino acids, particularly alanine which provide fuel for gluconeogenesis in the liver. The amino acid break down during gluconeogenesis in the liver results in increased production of urea nitrogen. Thus the level of urea in the blood is elevated.

During treatment with tulasi leaf extract due to better utilisation of carbohydrates the breakdown of tissue

proteins is reduced. Thus the concentration of amino acids in the blood is decreased. The deamination of amino acids in the liver is reduced which decreases the blood urea level.

But on discontinuing the treatment, again increased breakdown of tissue protein occurs;. and this probably leads to an increase in amino acid concentration in blood and higher deamination in liver leading to the production of urea which is again elevated in blood.

3. The Effect of Tulasi Leaf Extract on Serum Cholesterol Level in Alloxan Induced Diabetic rats.

Table IV gives the mean cholesterol level in serum of rats after the induction of alloxan diabetes. It can be seen that the mean serum cholesterol level of the experimental control and experimental group rats are raised more than 2 folds when compared to the basal control, group which were not injected alloxan. The increase in serum cholesterol level is statistically significant at 1 % level Figure 5 diagramnatically gives the same.

It is seen from the values presented that the mean serum cholesterol value of the experimental group after

TABLE IV
SERUM CHOLESTEROL VALUES OF RATS

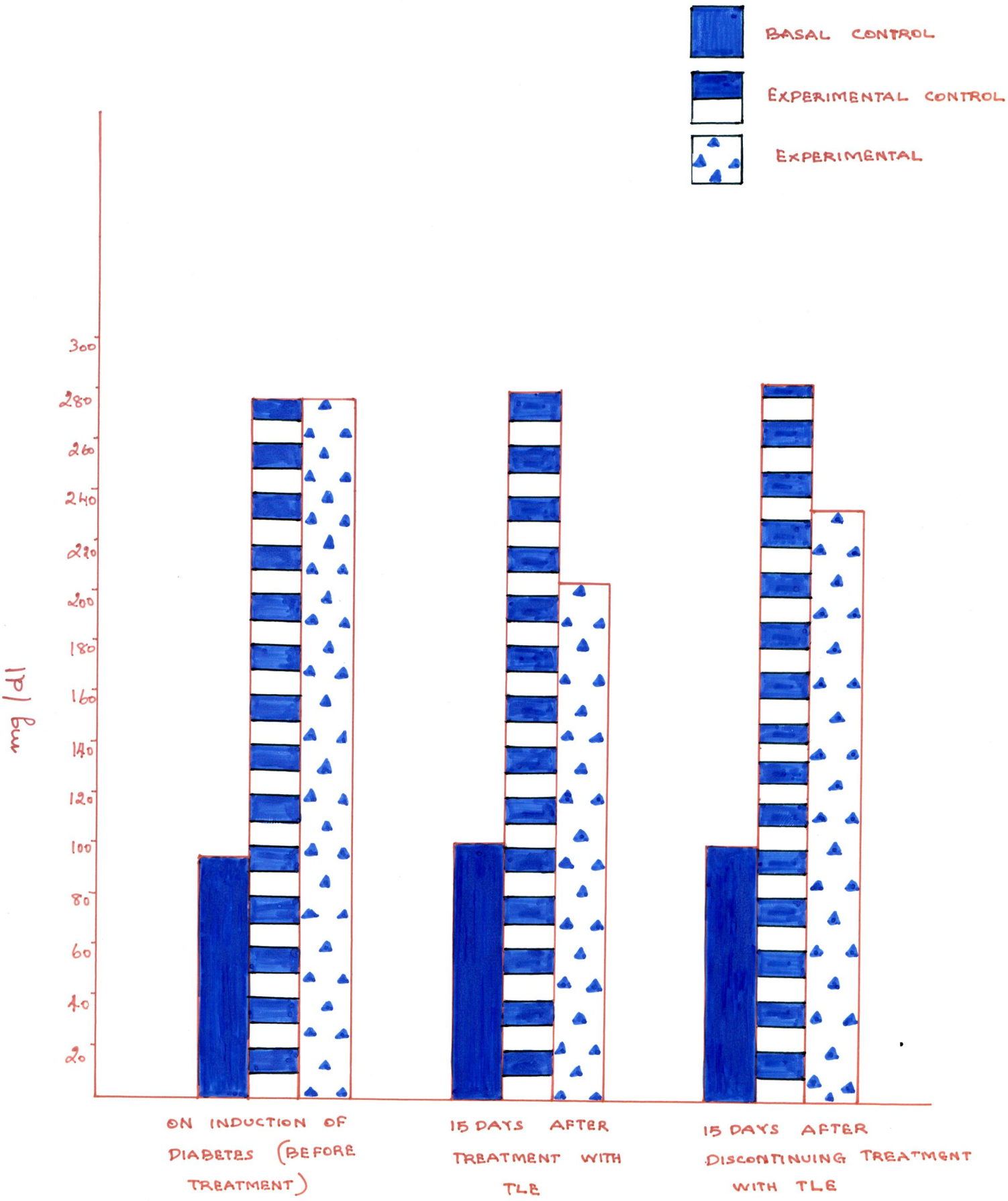
Different Experimental conditions	Basal control Mean ± S.D. mg/dl.	Experimental control Mean ± S.D. mg/dl.	Experimental Mean ± S.D. mg/dl.	Groups compared	Statistical significant
Diabetic condition	94 ± 16.55	276 ± 10.2	276 ± 10.2	a_1 Vs b_1	20.932**
	a_1	b_1	c_1	a_1 Vs c_1	20.932**
				b_1 Vs c_1	0 NS
				a_1 Vs a_2	0.5112 NS
				a_2 Vs a_3	0 NS
				a_1 Vs a_3	0.4427 NS
15 days after treatment with tulasi leaf extract	99 ± 14.29	280 ± 7.071	204 ± 18.81	a_2 Vs b_2	25.3826**
	a_2	b_2	b_3	a_2 Vs c_2	9.9383**
				c_2 Vs b_2	8.456**
				b_1 Vs b_2	0.7206 NS
				b_1 Vs b_3	1.2111 NS
				b_2 Vs b_3	0.6982 NS
15 days after discontinuing the feeding of tulasi leaf extract	99 ± 19.07	284± 10.68	234± 10.68	a_3 Vs b_3	18.9246**
	a_3	b_3	c_3	a_3 Vs c_3	13.8099**
				c_3 Vs b_3	7.4016**
				c_1 Vs c_2	7.5235**
				c_1 Vs c_3	6.3587**
				c_3 Vs c_2	3.1010*

** - Significant at 1 per cent level; * - Significant at 5% level
NS - Not significant

Figure. 5.

THE EFFECT OF TULASI LEAF EXTRACT (TLE)

ON SERUM CHOLESTEROL LEVEL



15 days treatment with tulasi leaf extract, is reduced by 25% (from 276± 10.2 to 204 ± 18.81 mg/100ml) and it is significant at 1 per cent level. The differences in mean serum cholesterol values between the basal control and experimental control, basal control and experimental and between experimental control and experimental groups are statistically significant at 1 per cent level in each case.

This shows that tulasi leaf extract has hypocholesteremic effect.

It is further seen from the results presented in Table IV that on discontinuing the administration of tulasi leaf extract to diabetic rats for 15 days the mean serum cholesterol level has further increased(from 204 ± 18.81 to 234± 10.68 mg/100ml) in the experimental group. But the value in still lower than that of the experimental control group. When the mean serum cholesterol value of experimental group is compared with the corresponding values of basal control and experimental control, the difference noted between the groups is statistically significant at 1 per cent level.

Figure 5 diagrammatically gives the same.

In insulin deficiency conditions as in diabetes, a decrease in acetyl-COA, ATP, NADPH andglycero phosphate in all tissues results in decreased fatty acid and lipid synthesis. Stored lipids are hydrolyzed by increased lipolysis in diabetics and the liberated fatty acids reaching the liver in high concentrations inhibit fatty acid synthesis by a feed back inhibition at the acetyl-C OA carboxylase step. The increased acetyl-COA which no longer can enter either the citric acid pathway or be used for fatty acid synthesis is shunted to the synthesis of cholesterol (Harper, 1981).

Hence during the treatment with tulasi leaf extract probably the liberation of fatty acids are dpressed and the fatty acids reaching the liver is low in concentration. This might result in decreased synthesis of acetyle COA which in turn decreases the synthesis of cholesterol. Thus the serum cholestrol level is lowered.

After discontinuing the treatment with tulasi leaf extract the synthesis of cholesterol takes place again because the increased free fatty acid liberation increases acetyl COA concentration thus increasing cholesterol synthesis.

4. <u>The effect of tulasi leaf extract on serum triglyceride level in alloxan induced diabetic rats</u>:

Table V gives the mean serum triglyceride level after the induction of alloxan diabetes.

It can be seen from Table V that the mean serum triglyceride level of the experimental control and experimental group rats are raised more than four fold when compared to the basal control group which were not injected alloxan. The increase in serum triglyceride level is statistically significant at 1 per cent level.

Figure 6 diagrammatically shows the same.

It is clear from the results presented that the mean serum triglyceride value of the experimental group after 15 days treatment with tulasi leaf extract is reduced by 39 per cent (from 165.25 ± 4.359 to 100 ± 4.031 mg/100ml) the reduction being significant at 1% level. In the experimental control group the mean serum triglyceride value has slightly increased (from 165.25± 4.359 to 169.75± 3.652 mg/100ml) The differences in mean serum triglyceride values between the basal control and experimental control basal control and experimental and between experimental control and experimental groups are statistically significant at one per cent level in each case.

TABLE V

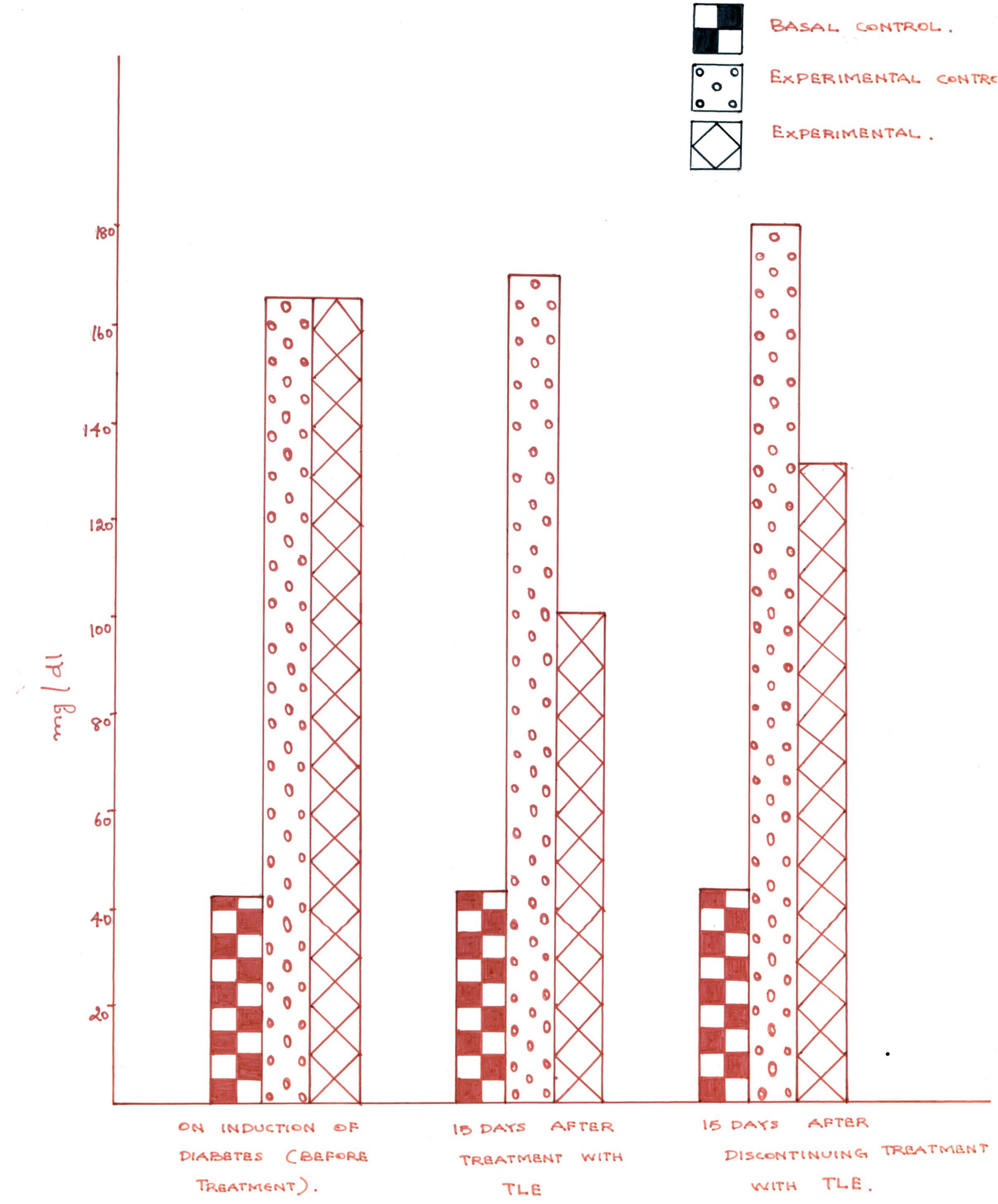
SERUM TRIGLYCERIDE VELUES OF RATS

Different experimental conditions	Basal Control Mean ± S.D. mg/dl.	Experimental Control Mean ± S.D. mg/dl	Experimental Mean ± S.D. mg/dl.	Groups Compared	Statistical significance
Diabetic condition	41.75±2.318 a_1	165.25±4.359 b_1	165.25±4.359 c_1	a_1 Vs b_1	55.9321**
				a_1 Vs c_1	55.9321**
				b_1 Vs c_1	0 NS
				a_1 Vs a_2	0.6762 NS
				a_2 Vs a_3	0.33568NS
				a_1 Vs a_3	0.7862NS
15 days after treatment with tulasi leaf extract	42.75±2.358 a_2	169.75±3.652 b_2	100±4.031 c_2	a_2 Vs b_2	65.322**
				a_2 Vs c_2	27.40968**
				b_2 Vs c_2	28.6713**
				b_1 Vs b_2	1.7693 NS
				b_1 Vs b_3	5.3725**
				b_2 Vs b_3	4.0357**
15 days after discontinuing the feeding of tulasi leaf extract	43.5±4.404 a_3	179.8±4.203 b_3	131.2±4.445 c_3	a_3 Vs b_3	50.0604**
				a_3 Vs c_3	31.3734**
				b_3 Vs c_3	17.7266**
				c_1 Vs c_2	24.5724**
				c_1 Vs c_3	12.1927**
				c_2 Vs c_3	11.6628**

** - Significant at 1% level; * - Significant at 5% level; N.S. - Not significant

Figure. 6.

THE EFFECT OF TULASI LEAF EXTRACT (TLE) ON SERUM TRIGLYCERIDE LEVEL.



This shows that tulasi leaf extract lowers triglyceride level in serum. Table V also shows that in the experimental group the mean serum triglyceride level has further increased (from 100± 4.031 to 131.3± 4.445 mg/100ml) on discontinuingthe administration and tulasi leaf extract to diabetic rats for 15 days. But the value is still lower than that of the experimental control group. In the experimental control group the mean serum triglyceride value has further increased, the increase being statistically significant at 1 per cent level. When the mean serum triglyceride value of experimental group is compared with the corresponding values of basal control and experimental control, the differences noted between the groups is statistically significant at 1 per cent level in all cases.

When glucose cannot be utilised for energy production fats are mobilised from fat depote to the plasma as triglycerides, thus increasing their level in the serum. Severe uncontrolled diabetes is almost always associated with excessive plasma level of triglyceride. During treatment with tulasi leaf extract, the oxidation of glucose is enhanced for energy production and this decreases the mobilisation of fats from fat depots so

there is a decrease in the level oftriglyceride in serum. But on discontinuing the treatment with tulasi leaf extract, again, the fats are mobilised as triglycerides and their level in serum is increased.

5. The Effect of Tulasi Leaf Extract on Liver Glycogen in alloxan induced Diabetic Rats.

Table VI gives the mean liver glycogen content after the induction of alloxan diabetes.

It can be seen that the mean liver glycogen of the experimental control and experimental group rats are lower than that of the basal control which was not injected alloxan. The fall in the liver glycogen content in each case is statistically significant at one per cent level.

It is clear from Table VI that the mean liver glycogen content of the experimental group after 15 days treatment with tulasi leaf extract is further decreased (from 1.3± 0.1095 to 1.0± 0.08943 mg/100mg). The differences in the mean liver glycogen content between the basal control and the experimental control, and between the basal control and experimental group are statistically significant at one per cent level in each case.

TABLE VI

LIVER GLYCOGEN CONTENT OF RATS

Different experimental conditions	Basal control Mean ± S.D. mg/dl.	Experimental control Mean ± S.D.	Experimental Mean ± S.D. mg/dl	Groups compared	Statistical significance
Diabetic condition	2.3± 0.3347 a_1	1.3± 0.1095 b_1	1.3 ± 0.1095 c_1	a_1 vs b_1	6.3519**
				a_1 Vs c_1	6.3519**
				b_1 Vs c_1	0 NS
				a_1 Vs a_2	0.4396NS
				a_1 Vs a_3	0.4396NS
				a_2 Vs a_3	0 NS
15 days after treatment with tulasi leaf extract	2.38± 0.2315 a_2	1.34± 0.1020 b_2	1.0± 0.08943 c_2	a_2 Vs b_2	9.190**
				a_2 Vs c_2	6.678**
				b_2 Vs c_2	1.742NS
				b_1 Vs b_2	0.5982 NS
				b_2 Vs b_3	0.1242 NS
				b_1 Vs b_3	0.6063 NS
15 days after discontinuing the feeding of tulasi leaf extract	2.38± 0.2315 a_3	1.35± 0.1484 b_3	1.25± 0.1415 c_3	a_3 Vs c_3	9.3145**
				a_3 Vs b_3	8.3766**
				b_3 Vs c_3	1.0910NS
				c_1 Vs c_2	1.5499NS
				c_1 Vs c_3	0.62519 N.S.
				c_2 Vs c_3	1.3179NS

** - Significant at one per cent level; * - Significant at 5 per cent level
N.S. - Not significant

It is also seen from the values presented in Table VI that in the experimental group the mean liver glycogen content shows an upward trend (from 1.0 ± 0.08943 to 1.25 ± 0.1415 mg/100mg) on discontinuing the administration of tulasi leaf extract for 15 days. But the value is still lower than that of the experimental control group.

After inducing diabetes glycogen synthesis is depressed as a result of decreased glycogen synthetase activity. But on treatment with tulasi leaf extract it seems to be further reduced. On discontinuing tulasi leaf extract treatment, the liver glycogen values increases, but this is not significant.

Treatment with tulasi leaf extract reduces the blood glucose level, but this reduction does not seem to be due to conversion of glucose to liver glycogen.

This needs further investigation. According to Giri, et al., (1982) when diabetic rats were treated with bitter gourd juice there was reduction in blood glucose level and simultaneously depletion of liver glycogen stores. The explanation given by them was that absorption of glucose in the intestine was reduced leading to lower blood glucose level and consequent reduced synthesis of liver glucogen. It may be that

by similar mechanism the blood glucose and liver glycogen values are reduced on treatment with tulasi leaf extract. However to test this Experiment - II was done.

Experiment - II:

The Effect of Tulasi Leaf Extract on Glucose Absorption in the Intestine:

Table VII gives the intestinal absorption of glucose in diabetic rats in presence and in absence of tulasi leaf extract (100 per cent solution) at various time intervals. Figure 7 gives the diagrammatic representation of the same.

A comparison of absorbed glucose values of A and B at different time intervals indicate a lowering of the glucose value (absorbed) when tulasi leaf extract is added to glucose in the mucosal fluid. In the present study the percentage of absorbed glucose varie from 2.8 to 15 per cent in the absence of tulasi leaf extract and 1.8 to 8.0 per cent in the presence of tulasi leaf extract. The above findings indicate that tulasi leaf extract has an inhibitory action on glucose absorption.

Figure. 7.

THE EFFECT OF TULASI LEAF EXTRACT ON THE INTESTINAL ABSORPTION OF GLUCOSE.

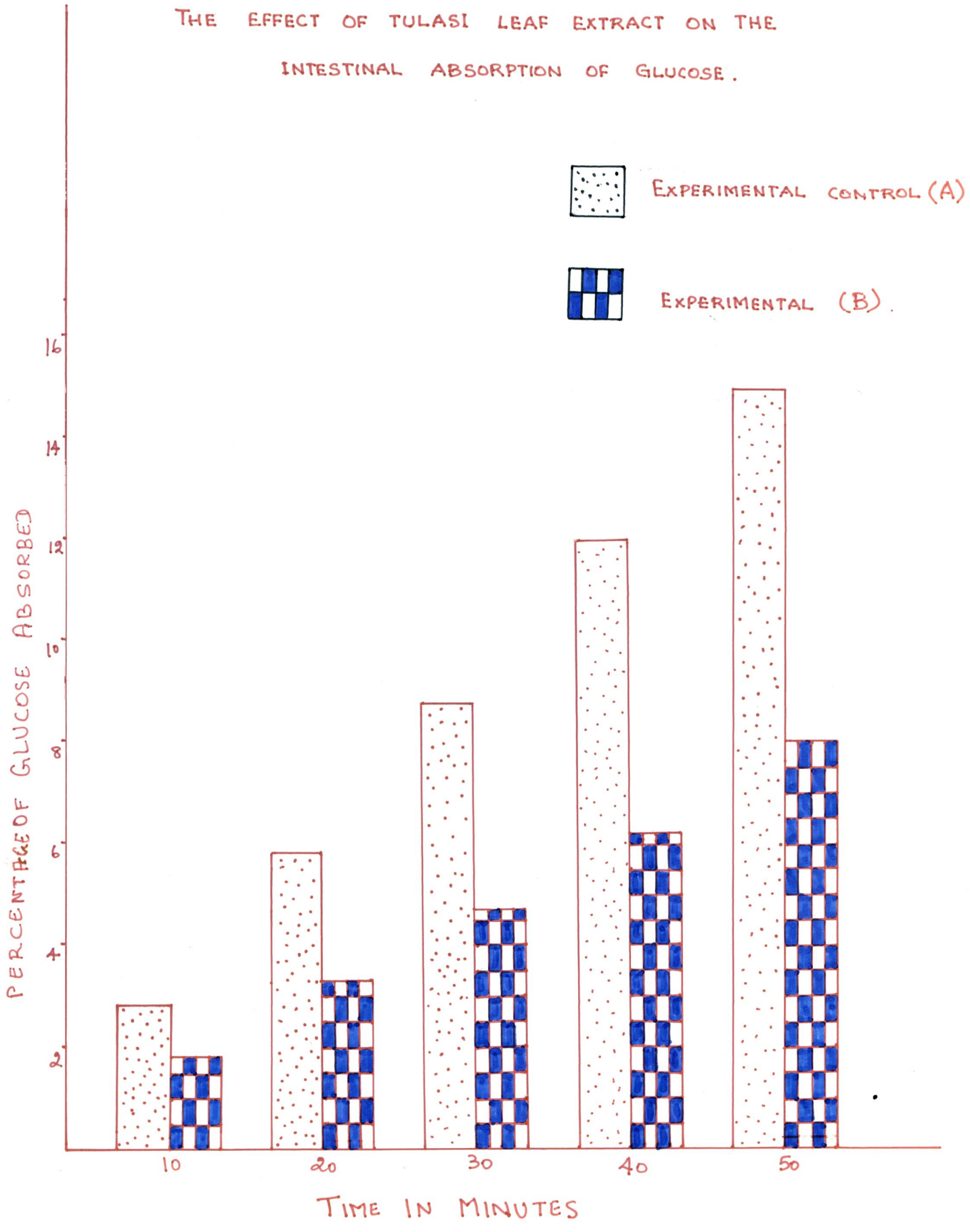


TABLE VII

GLUCOSE VALUES IN SEROSAL FLUID WITH AND WITHOUT TULASI LEAF EXTRACT

Time in minutes	Amount of glucose absorbed in mg in presence of		Percentage of Glucose absorbed in presence of	
	0.5% Glucose A	0.5% glucose + Tulasi Leaf 100% Extract B	Glucose	Glucose + Tulasi Leaf extract
10	140	90	2.8	1.8
20	290	165	5.8	3.3
30	440	235	8.8	4.7
40	600	310	12.0	6.2
50	750	400	15.0	8.0

Experiment III

Estimation of Copper, Zinc, Iron and Manganese Content of Tulasi Leaf:

TABLE VIII

MINERALS IN TULASI (ppm)

	Zinc	Copper	Manganese	Iron
Sample	75	10	25	82

Table VIII gives the zinc, copper, manganese and iron content of Tulasi Leaf. Zinc is present to an extent of 75 ppm, copper 10ppm, manganese to an extent of 25 ppm and iron 82 ppm.

Ample but not excess levels for zinc commonly fall in the range 25 to 100 ppm, for copper the range in most plants is between 5 and 20 ppm and for manganese the range in most plants falls between 20 to 500 ppm. For Iron the values vary greatly from plant to plant (chapman, H.D. 1981). Zinc has been shown to enhance the action of insulin in promoting the uptake of glucose by tissues. Glucose tolerance is lowered in zinc deficient rats. Hence it could be presumed that on administration of tulasi leaf extract to diabetic rats, the zinc present in the extract enhances the action of insulin in promoting the uptake of glucose by tissues. Thus it could lower blood sugar level.

It is not known if the other minerals play any role in bringing down the blood glucose level.

Hence further work needs to be carried out to bring to light more information regarding minerals and their effect on diabetes.

SUMMARY AND CONCLUSION

V. SUMMARY AND CONCLUSION

The study consisted of three experiments, I, II, and III.

In a study by Dhar et al., (1968) Tulasi has been reported to have hypoglycemic effect. However, no quantitative study has been done so far. Hence in the present study an attempt has been mde to determine quantitatively the effect of tulasi on blood sugar level and on metabolites such as urea, cholesterol triglyceridr and liver glycogen in diabetes, and its possible use as a substitute for oral hypoglycemic drugs.

Experiment - I

Forty male albino rats of wistar strain, weighing 150 - 190g. were selected and divided into group I and II (Figure I). Group I consisting of 15 rats formed the basal control group. Group II consisted of 25 rats. This group was given alloxan subcutaneously 140mg/kg. body weight in physiological saline. Diabetes was found to be induced in two days, but the rats were left for 30 days without any treatment to ensure permanent induction of diabetes. After 30 days the group II rats were divided into group IIA (Experimental Control) and group II B (Experimental). Group IIA consisted of 10 rats and group IIB consisted of 10 rats. Group IIB rats

were given tulasi leaf extract for 15 days. Hundred present Tulasi leaf extract was prepared by homogenising tulasi leaves in distilled water and filtering through a muslin cloth. Two ml. of this extract was fed daily for 15 days to the experimental rats (Group IIB) in the morning, before feeding the stock diet. After 15 days the treatment with tulasi was discontinued. The experimental control and the basal control rats were not fed tulasi leaf extract at any stage in the experiment. At each stage blood and liver were collected from the rats and analysed for gulucose, urea, cholesterol, triglyceride and liver glycogen.

On induction of diabetes in the experimental and experimental control rats, the mean blood glucose level rose three fold to 183.2± 43.9 mg/100ml, as compared to that of the Basal control rats which were not induced diabetes (56± 5.656 mg/100ml) the increase being statistically significant at 1 per cent level. After 15 days treatment with tulasi leaf extract, the raised blood glucose level of the experimental rats fell to 103.2± 9.926mg/100ml. The decrease in mean blood glucose level was statistically significant at 1 per cent level. On discontinuing the treatment with tulasi leaf extract, the mean blood sugar level increased from 103.2± 9.926

to 128± 5.656 mg/100ml. in the experimental rats, the increase being statistically significant at one per cent level. On induction of diabetes in the experimental and experimental control rats, the mean blood urea level rose to 59±19.32mg/100ml as compared to that of the basal control rats, which were not induced diabetes (23.2 ± 4.099 mg/100ml) the increase being statistically significant at one per cent level. After 15 days treatment with tulasi leaf extract, the raised blood urea level of the experimental rats was reduced to 35± 3.687 mg/100ml. The decrease in mean blood urea level was statistically significant at 5 per cent level On discontinuing the treatment with tulasi leaf extract the mean blood urea level was increased from 35± 3.687 to 46.8 ± 5.741 mg/100ml in the experimental rats. The increase being statistically significant at 1 per cent level.

On induction of diabetes, in the experimental and experimental control rats, themean serum cholesterol level rose to 276± 10.2 mg/100ml, as compared to that of the basal control rats which were not induced diabetes (94± 16.55 mg./100ml) this being statistically significant at 1 per cent level. After 15 days treatment with tulasi leaf extract, the raised serum cholesterol level of the

experimental rats was reduced to 204 ± 18.81 mg/100ml. The decrease in mean serum cholesterol level was statistically significant at one per cent level. On discontinuing the treatment with tulasi leaf extract, the mean serum cholesterol level was increased from 204± 18.81 to 234 ± 10.68 mg/100ml. in the experimental rats, the increase being statistically significant at 5 per cent level.

On induction of diabetes in the experimental and experimental control rats the mean serum triglyceride bvel rose to 165.25± 4.359 mg/100ml., as compared to that of the basal control rats, which were not induced diabetes (41.75± 2.318 mg./100ml) this being statistically significant at 1 per cent level. After 15 days treatment with tulasi leaf extract, the raised serum triglyceride level of the experimental rats was reduced to 100± 4.031 mg/100ml. The decrease in mean serum trighlyceride level h was statistically significant at 1 per cent level. On discontinuing the treatment with tulasileaf extract, the mean serum triglyceride level was raised from 100± 4.031 to 131.3 ± 4.445 mg/100ml in the experimental rats, the increase being statistically significant at 1 per cent level.

Regarding changes in liver glycogen, on induction of diabetes is the experimental and experimental control rats, the mean liver glycogen content was reduced to 1.3± 0.1095mg/100ml as compared to that of the basal control rats, which were not induced diabetes (2.3± 0.3347 mg/100mg.). This reduction being statistically significant at 1 per cent level. After 15 days treatment with tulasi leaf extract, there was a further decrease in liver glyeogen content (from 1.30± 0.1095 to 1.0± 0.08943 mg/100mg) in experimental rats but this is not statistically significant. On discontinuing the treatment with tulasi leaf extract, the mean liver glycogen content increased in experimental rats, from 1.0 ± 0.08943 to 1.25 ± 0.1415 mg/100mg. However the rise was not statistically significant.

The present study reveals that tulasi leaf extract has antihyperglycemic qualities. It is not clear why during treatment with tulasi leaf extract liver glycogen level is reduced. This needs further investigation.

Experiment - II

In this experiment, the effect of tulasi leaf extract on intestinal absorption of glucose was studied.

Diabetic rats were fasted for 30 hours and then anesthetized with chloroform, the intestine was separated from the mesenterum and cut into 5cm., long sections. The intestine was everted with a round tipped glass rod, and washed with Kreb's Ringer Phosphate buffer. By means of this everted intestine the absorption of glucose in presence and absence of tulasi leaf extract was determined.

In presence of tulasi leaf extract there was a fall in the absorbed glucose value. The percentage of glucose absorbed varied from 2.8 to 15 per cent in the absence of tulasi leaf extract, and 1.8 to 8.0 per cent in the presence of tulasi leaf extract at different time intervals. Hence it shows that tulasi leaf extract has an inhibitory action on glucose absorption which decreases the blood sugar level.

The present study reveals that tulasi has anti-hyperglycermic quality. But it does not promote inner glycogen formation. However it will be definitely effective to keep in check the levels of sugar in blood.

However, further research needs to be done to elicit information on the following:-

1. The best time of intake (whether) before a meal, after meal or along with food).

2. The advisable dosage to human beings.

3. The efficiency of tulasi in other forms such as, dried powdered leaves, decoction, and so on as compared to the fresh water extract.

4. The exact mechanism of the extract in preventing intestinal absorption of glucose.

5. Isolation of the antihyperglycemic factor present in the extract.

Experiment - III

In this experiment 1g. of the dried powdered tulasi leaf from the same source was weighed in duplicate and it was digested with 12ml. of triple acid. The digested residue was made upto 50ml. with deionised water, and this was used to estimate the mineral content of tulasi by the Atomic absorption spectrophotometer. Zinc is present to an extent of 75 ppm, copper to an extent of 82 ppm. It is not known if these mineral play any role in bringing down the blood sugar level. Zinc has been known to enhance the action of insulin in

promoting the uptake of glucose by tissues. It has been observed that diabetics excrete increased amounts of zinc in urine and that their plasma, leukocyte and crythrocyte levels are reduced. Glucose tolerance is lowered in zinc-deficient rats. Hence it could be presumed that on treatment of tulasi leaf extract to diabetic rats, the zinc present in the extract enhances the action of insulin in promoting the uptake of glucose by tissues. Thus it could lower blood sugar level.

However, further research needs to be done to elicit more information with regard to minerals and their effect on diabetes.

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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. The cuprousoxide formed was treated with an acid molydate, when a blue coloured solution was got. This colour was compared with that of the standard in a colorimeter at 660 nM.

REAGENTS:

1. 10% Sodium Tungstate

2. Phosphomolybdic Acid Reagent

Dissolved 35g. of molybdic acid and 5g. of sodium tungstate in 100ml of10% sodium hydroxide, and 200ml of water and boiled to remove the ammonia present in molybdic acid. This usually takes 30-40 minutes. Cooled, transferred the washings to a one litre flask, diluted to 750ml. Then added 125ml and phosphoric acid and made up to one litre with water.

3. Alkaline Copper Reagent:

Dissolved 40g. of 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 upto a litre.

4. Stock Standard Glucose Solution:

Dissolved 0.2g. of glucose in 100ml of water and made upto the mark with the same.

5. Working Standard Glucose Solution:

10.0ml of the stock solution was diluted to 100ml with water.

PROCEDURE:

Took 3.5ml of water, added 0.1ml of blood and 0.2ml of 2/3 N sulphuric acid. Then added 0.2ml of 10% sodium tungstate solution. Kept for 10 minutes and the centrifuged. Pipetted out 2.0 ml of supernatant into a Folin-Wu tube.

0.2 to 1.0ml of the standard glucose solution was taken in a series of Folin-Wu tubes and added 2.0ml of the alkaline copper reagent. Heated the tubes for about 8 minutes in a boiling water bath, cooled, and then added 2ml of phosphomolybdic acid reagent. Mixed and then made upto 12.5ml with water. The blue colour developed was read in a colorimeter using a red filter. (660 nM).

APPENDIX - II

ESTIMATION OF UREA BY DAM-TSC METHOD (Varley, 1976)

PRINCIPLE:

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

REAGENTS

1. Acid reagent

Water - 100ml, concentrated a sulphuric acid - 8.0ml Phosphoric acid - 20ml, 5% Ferric Chloride 1.0ml.

2. Colour reagent:

Acid reagent - 30ml, water - 20ml, 2.5% Diacetyle monoxime - 1.0ml, 2.5% Thio semicarbazide - 0.25ml. Colour reagent should be prepared just before use, since the solution is not stable for more than an hour.

3. Stock standard solution:

Disseolved 100mg of urea in 100ml of water and made upto the mark with the same.

4. Working standard solution:

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

PROCEDURE:

Took 1.8ml of 3% TCA and 0.2ml of blood, after 10 minutes centrifuged. 0.5ml of the supernatant was pipetted out into a test tube. Into a series of test tubes took 0.5ml to 2.5 ml of standard urea solution The values correspond to 10 to 50.

The volumes of all the tube were made upto 3.0ml with water. Added 5.0ml of colour reagent. Mixed well. Corked and heated in a vigorously boiling water bath for 20 minutes. Along with this a blank was also conducted. Remove the tubes and cooled. Readings were taken against a reagent blank at 540 mu.

APPENDIX - III

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD (Varley,1976)

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 mu in a spectrocolorimeter.

REAGENTS

1. Stock Ferric Chloride Reagent:

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

2. Ferric Chloride Precipitating Reagent:

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

3. Ferric Chloride Diluting Reagent:

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

4. Stock Standard Cholesterol Solution:

100mg of pure cholesterol was placed in a clean dried 100ml standard flask and dissolved in glacial acetic

acid. Then made upto the mark with pure glacial acetic acid.

5. Working Standard Solution:

10.0ml of the stock standard was placed in a 100ml standard flask containing 0.85ml of ferric chloride stock reagent and made upto the mark with pure glacial acetic acid. 1.0ml of this solution contains 100 ug of cholesterol.

PROCEDURE:

0.5ml to 2.5 ml of working standard solution were pipetted out into a series of clean dry test tubes. The total volume of each tube was made upto 5.0ml with ferric chloride diluting reagent. To 0.1ml of the serum added 4.9ml of ferric chloride 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 2.5 ml 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 dropy by drop. The solutions were mixed well. The tubes are 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 540mu against a reagent blank.

APPENDIX IV

ESTIMATION OF SERUM TRIGLYCERIDE BY VAN HANDEL AND ZILVERSMIT (Varley, 1980)

PRINCIPLE

Triglyceride is measured after hydrolysis by estimating its glycerol content. The commonest procedure irvolves 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. Zeolite may be used for this purpose.

REAGENTS:

1. Chloroform: Redistilled grade

2. Zeolite:

Activated by heating for 4 hours at 124°C. Stored in a tightly stoppered bottle. Reactivated with IN hydrochloric acid.

3. Alcoholic Potassium Hydroxide:

Dissolved reagent grade potassium hydroxide (about ¼ the of a pellet. in 2.0ml of redistilled 95% ethyl alcohol. Diluted 0.5ml stock potassium hydroxide solution to 2.5 ml with 95% ethanol. (This should be freshly prepared before use.)

4. 0.2N Sulphuric Acid:

2.0ml of concentrated sulphuric acid in 500ml of water.

5. Sodium arsenite (0.5M)

Dissolved 2.25 g. of sodium hydroxide and 5g. reagent grade arsenious trioxide in distilled water and diluted to 100ml with distilled water.

6. Sodium metaperiodate (0.05M)

Dissolved 1.07 g. of sodium metaperiodate in 100ml of distilled water.

7. Chromotropic acid:

1.12g. of sodium salt of chromotropic acid in 100ml of water. Added 300ml of concentrated sulphuric acid to 150ml distilled water. Cooled in ice. When cooled added this to chromotropic acid.

8. Stock standard solution:

50mg olive oil (or corn oil) dissolved in 100ml of chloroform in a volumetric flask. Standard must be checked often.

9. Working Standard Solution:

Diluted 1 in 100 with chloroform or 0.1ml to 10ml (1ml = 0.05mg).

PROCEDURE:

Added 2.0g of zeolite in a glass stoppered tube and 10.0ml chloroform. Added 0.5ml plasma. Shake well Filter through a filter paper and pipetted out 0.5 ml portions of the filterate in two test tubes.

Pipetted out 0.5ml of standard into two test tubes Evaporated both tests and standards at 60° C to 70°C.

To one set of tubes (Test and Standard added 0.5ml of alcoholic potassium hydroxide and to the other, 0.5ml of alcohol. Evaporated at 60°C to 70° C. To all the test tubes added 0.5ml of 0.2N sulphuric acid. Boiled for 10 minutes in a gently boiling water bath. Cooled all the tubes, added 0.1ml of sodium metaperiodate solution, kept for 10 minutes. Then added 0.1 ml of sodium arsenite. An yellow colour appeared andvanished in a few minutes. Added 5.0ml of chromotropic acid reagent to each tube. Mixed well and heated in a boiling. Water bath for 10 minutes. After cooling determined the optial density at 570 nM.

APPENDIX V

ISOLATION (OSER, 1965) AND ESTIMATION OF LIVER GLYGOGEN (REXMONTGONERY,1959)

ISOLATION:

Remove a rat from its cage, gently to avoid exciting it, stun it by a blow on the head and decapitate it quickly. Immediately remove the liver, weight quickly 0.1g. mince the liver portion immediately and transfer samples of the minced liver to a centrifuge tube containing 6.0 ml. of 30% potassium hydroxide. Heat the tubes for 15 to 20 minutes agitating the solution occasionally to ensure thorough disintegration.

Add 7.0 ml of 95% alcohol to each tube mix by tapping and immerse in the water bath until boiling just begins. Allow the tubes to cool at room temperature for 2 hours, centrifuge, decant the supernatant fluid, drain and wash the precipitate with 5.0 ml portion of 60% alcohol by centrifuging the and draining as before. Expel 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 in distilled water and made upto 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 some 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 the water from which 0.5ml was taken for the experiment.

In a series of test tubes aliquotes of the standard 0.5 to 2.5ml corresponding to 10 to 50 ug. of glycogen were taken. To all the tubes added distilled water to makeup the volume to 2.9 ml. Then added 0.1 ml of 80% phenol and finally added 5.0 ml of concentrated analar sulphuric acid. Shook and allowed 30 minutes for the pink colour to develop. Took the readings at 490nM against a reagent blank.

APPENDIX VI

(BY M.C. JACKSON, 1978)

To 1g. of dried sample was added 12ml of triple acid and digested. The residue was then made upto 50ml with deionised water. This solution was used for analysing zinc, copper manganese and iron, using atomic absorption spectro photo meter.