Biochemical, Histopathological and Therapeutic Studies in Alloxan- and Streptozotocin-induced Diabetes Mellitus in Rabbits

Ph. D. THESIS

By

Sajad Hussain Mir

Dr. R. C. Bhagat
Professor & Head,
Department of Zoology,
University of Kashmir, Sgr.
(Supervisor)

Dr. Mohammed Maqbool Darzi
Head,
Division of Veterinary Pathology,
Shere-Kashmir University of Agricultural Sciences & Technology,
FVSC & AH, Shuhama (Alusteng) Sgr.
(Co-Supervisor)

Parasitology Research Laboratory
Post Graduate Department of Zoology
Faculty of Sciences
UNIVERSITY OF KASHMIR
(NAAC Accredited Grade 'A' University)
Srinagar - 190 006, Kashmir

The University of Kashmir
Post Graduate Department of Zoology
Srinagar - 190 006, Kashmir, India

No: .....

Dated: .....

Certificate

This is to certify that the thesis entitled, **"Biochemical, Histopathological and Therapeutic Studies in Alloxan- and Streptozotocin-induced Diabetes Mellitus in Rabbits",** is the original and bonafide research work of **Mr. Sajad Hussain Mir**, Research Scholar**,** Postgraduate Department of Zoology, University of Kashmir, Srinagar and the work has been submitted for the first time. The work has been carried out under our supervision and guidance.

It is further certified that Mr. Sajad Hussain Mir has put in the required attendance in the department and fulfils all the requirements for the award of the degree of philosophy in Zoology.

Dr. R. C. Bhagat
Professor & Head,
Department of Zoology,
University of Kashmir, Sgr.
(Supervisor)

Dr. Mohammed Maqbool Darzi
Head,
Division of Veterinary Pathology,
Shere-Kashmir University of Agricultural Sciences & Technology,
FVSC & AH, Shuhama (Alusteng) Sgr.
(Co-Supervisor)

Head of the Department

The present experimental study was designed to establish diabetes mellitus in New Zealand white rabbits using diabetogenic drugs so as to investigate/elucidate biochemical, histopathological and behavioural changes/complications. In one group of rabbits diabetes mellitus was induced by intraperitoneal administration of alloxan (@ 80 mg/kg b.w.) and the other group of rabbits was made diabetic using intravenous administration of streptozotocin (@ 65 mg/kg b.w.).Another group of rabbits was kept as control (normal healthy) which received normal saline. The establishment of diabetes mellitus in rabbits was confirmed by periodical elevated levels of fasting blood glucose, blood urea and serum creatinine. The subsequent effect of hyperglycemia on tissue morphology of diabetic rabbits was studied by processing of different organs *viz.*, pancreas, kidneys, liver, lungs, heart, brain and gut of both diabetic and normal rabbits for histological/hiostopathological study using Haematoxylin and Eosin stain and modified Gomori's staining technique.

The drug-induced diabetic rabbits showed a decline in body weight throughout the experimental period. Further, the diabetic rabbits showed a change in behaviour such as dullness, lethargy, decreased physical activity, a tendency to lie down, polyuria and polydipsia. The subsequent effect of hyperglycemia on tissue morphology of diabetic rabbits revealed degenerative changes in most of the organs.

The experimental study was further extended to elucidate the therapeutic efficacy of antidiabetic drugs viz, *Abroma augusta* (@ 2 ml daily), *Syzygium jambolanum* (@ 2 ml daily) and glimepiride (@ 2mg/kg b.w.) on biochemical, behavioural and histomorphological parameters of rabbits. The drug treated diabetic rabbits showed a significant improvement in behaviour in contrast to saline-treated diabetic rabbits. The biochemical changes with regard to fasting blood sugar, blood urea and serum creatinine of all the drug treated diabetic rabbits showed a significant improvement especially in glimepiride treated diabetic rabbits. Furthermore, a significant amelioration of diabetic organs was observed. Further, regeneration of beta cells in all the treated groups of diabetic rabbits was observed.

The entire experiment presents an overview of diabetic pathogenesis, particularly impaired carbohydrate metabolism leading to hyperglycemia. It analyses

how herbal/allopathic medicines and their ingredients correct/manipulate the vitiated homeostasis of carbohydrate metabolism and other related complications particularly biochemical and histomorphological changes.

The study is first of its kind in the University of Kashmir duly approved by the Board of Research Studies vide Notification No: F (BORS-SC. Minutes) Acad/KU Dated: 11-03-2005. All the experimental animals used in the study received human care according to the guidelines outlined in the "Guidelines for the Care and Use of Animals in Scientific Research" prepared by the Indian Science Academy, New Delhi.

ACKNOWLEDGEMENTS

All praises to 'ALLAH', The Almighty, who bestowed me with zeal and enough courage to undertake this study

I am highly indebted to late Dr. Abdul Baqui, Professor, Postgraduate Department of Zoology, University of Kashmir for assigning the present research problem and for his valuable guidance, constant encouragement and inspiring attitude without which the present work would have been impossible.

I am highly elated and feel immense ecstasy to express my sense of gratitude to my supervisor and Head, Department of Zoology, Prof. R. C. Bhagat for providing valuable help in completing the research work successfully.

It gives me immense pleasure to express my deep sense of gratitude to Dr. M. M. Darzi, Head, Division of Pathology, FVSC & AH, Shuhama (Alusteng) Srinagar for his valuable guidance, cheerful company and for providing unrestricted laboratory facilities.

I express my heartfelt gratitude to Dr. Masood Saleem Mir, Assistant Professor, Division of Pathology, FVSC & AH, Shuhama (Alusteng) Srinagar for his sustained interest and assistance in histopathology part of my research work.

I gratefully acknowledge the valuable suggestions of my teachers especially Prof. M. Z. Chishti, Prof. G. M. Shah, Dr. Fayaz Ahmad and Dr. Ulfat Jan.

I would like to express my infinite gratitude to Syed Jalal-ud-Din Aijaz for his blessings that helped me a lot for completion of this work.

Sincere thanks are due to the Mohd Shafi Wadoo, Mohd Shafi Bhat and Ali Mohd, Division of Pathology, FVSC & AH, Shuhama (Alusteng) Srinagar for their assistance in the histopathology part of my research work.

I extend my heartfelt thanks to Gh. Mohi-ud-Din Dar, Assistant controller, University of Kashmir and Gh. Qadir Dar for their cheerful company and encouragement.

I extend my sincere thanks to Manzoor Ahmad Mir (Statistician) for his keen help in statistics.

My sincere thanks are due to my intimate friends and research scholars especially Abdul Majid Bhat, Bilal Ahmad Bhat, Abdul Wahid, Tariq Ahmad, Pervez Ahmad, Zubair Ahmad, Showkat Ahmad, Yasmeena Jan, Maqbool Fatima, Ruqaya Yousuf and Zeenat Nisa for their company during the progress of the present work.

Appreciation is also acknowledged to Mr. Javeed Ahmad Bhat, Universal Computers, Sadrabal-Hazratbal who patiently fabricated and designed this thesis with keen interest.

Last but not the least, I owe greatest debt to my revered parents and other family members especially Attiya Jan and Sumairah Mir for their blessings, immaculate love, affection and constant encouragement that provided me an incentive for completion of this work.

Sajad Hussain Mir

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Diabetes mellitus has plagued man for a very long time, since the writings from the earliest civilizations (Asia Minor, China, Egypt and India) refer to boils and infections, excessive thirst, loss of weight and passing of large quantities of a heavy sweet urine which often drew ants and flies. The earliest known record of diabetes (1552 B.C) is found in third Dynasty Egyptian Papyrus by physician Hesy-ra. He mentions polyuria (frequent urination) as a symptom of diabetes. In the first century A. D., Arateus described diabetes as the meeting down of flesh and limbs into urine. It is noteworthy that during 15^{th}, 16^{th} and 17^{th} centuries doctors had to taste the urine of patients for sweetness in order to detect the disease. Soon there emerged two schools of thought concerning diets. One school, exemplified by the British physician Willis (1675), believed in dietary replacement of the sugar lost in the urine, comprising milk, barley water and bread, while the other believed in restriction of carbohydrate so as to reduce the effects which were attributed to an excess of sugar. In the early 18^{th} century, first chemical tests were developed to indicate and measure the presence of sugar in the urine. French physician, Bouchardat, noticed the disappearance of glycosuria in his diabetes patients during the limited availability of food in Paris while under siege by Germany during the Franco-Prussian war of 1870 to 1871 and formulated the idea of individualized diets for his diabetes patients. In 1869, Paul Langerhan, a German Medical student, announced that the pancreas contains two systems of cells. One set

secretes the normal pancreatic juice; the function of other was unknown. However, several years later, these cells were identified as the "islets of Langerhan's". Later, Lane (1907) gave the idea of a single bihormonal metabolic regulator and reported that certain islet cells contained alcohol-precipitable granules, named them alpha cells and called the others beta cells. In 1922, Banting and Best prepared the first extract capable of reducing hyperglycemia and glycosuria, and mitigating the symptoms of diabetes. The name 'insulin' was given to the active principle of the extract from the islets of Langerhan's (Iswariah and Guruswami, 1979). The crude pancreatic extracts were injected into depancreatized dogs and were successfully treated (Banting and Best, 1922). One year later, Kimball and Murlin (1923) reported that aqueous extracts of pancreas raised blood sugar levels of depancreatized dogs by 200mg/100ml or more and believed that this was due to a glucoregulatory hormone they named "glucagons" meaning "glucose-driving". In 1955, oral drugs were introduced to lower blood glucose levels. In 1959, two major types of diabetes were recognized namely type I (Insulin-dependent diabetes) and type II (non-insulin-dependent diabetes).

Carbohydrates from various dietary sources are the primary exogenous source of glucose. Glucose is the main fuel for energy requirement of the body. Therefore, a continuous supply of glucose is necessary to ensure proper function and survival of all organs. Hence, mammals have evolved sophisticated systems to maintain glucose levels in the blood within tight limits, despite large fluctuations in food intake. Homeostatic mechanisms are in place to maintain blood glucose levels with a very narrow range (of around 5mm), protecting the body against hypoglycemia during periods of fasting and against excessively high levels (hyperglycemia) following the ingestion of a high carbohydrate diet. These goals are met chiefly through the hormonal modulation of the production of glucose by the liver and the peripheral uptake

of glucose by skeletal muscle, heart muscle and fat. When mammals fast, glucose homeostasis is achieved by triggering expression of gluconeogenic genes in response to glucagon, and when they take a carbohydrate-rich diet, the function is taken over by insulin for its uptake and utilization peripherally. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism place an over-exertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances leading primarily to hyperglycemia and subsequently, diabetes mellitus.

Diabetes mellitus is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated (Tiwari and Rao, 2002). In this metabolic disorder there is a defective or deficient insulin secretory response (Lazarus and Volk, 1959) for normal function of many cells of the body resulting in persistent hyperglycemia (Mohan, 2000). In some cases elevated levels of glucagon secreted from the α cells of the islets of Langerhan's of pancreas contribute to the development of persistent hyperglycemia (Unger *et al.*, 1976). Due to inadequate presence of insulin in the body, there are disorders of all kinds of metabolism, commonly with an increase in blood sugar accompanied by glycosuria, polyphagia, polyuria and polydipsia (Frank, 1962; Nelson, 1985). Insulin unavailability may be due to degenerative changes in β cells in the pancreatic islets, reduced effectiveness of the hormone owing to the formation of anti-insulin antibodies or inactive complexes, immune-mediated islet cytotoxity or inappropriate secretion of hormones by neoplasm in other endocrine organs (Botazzo *et al.*, 1976). In this disease glucose accumulates rapidly in the body fluids and as the blood glucose concentration increases beyond a certain point it is excreted by the kidneys. Glycosuria causes a continual waste of this essential nutrient and due to reduced

ability to use glucose produces a depression of the functions of brain, muscles and many other tissues and follows with other serious metabolic consequences (Rastogi *et al.*, 1998).

Insulin is a major anabolic hormone. It promotes the uptake of glucose by cells and the formation of intracellular glycogen from glucose. It stimulates cells to utilize amino acids for protein synthesis rather than for gluconeogenesis and it promotes the uptake of free faty acids by adipose tissue. Lack of insulin, therefore, results in a general catabolic state with loss of weight, hyperglycemia, diminished protein synthesis, increased gluconeogenesis, and hyperlipidaemia due to lipolysis in adipose tissue. Although the renal threshold is usually raised, there is heavy glycosuria that results in an osmotic diuresis, causing dehydration and thirst. In the liver, excess free fatty acids are converted via acetyl-Co A into ketone bodies which, in the absence of available glucose, are metabolized for cellular energy. The ketone bodies dissociate to produce hydrogen ions, with a resulting metabolic acidosis (Ketoacidosis). This complex of metabolic disturbances produces hyperosmolarity, hypovolaemia, acidosis and electrolyte imbalance, which have serious effects on the functions of neurons and result in one form of diabetic coma−keto-acidotic coma. The other major form, hyperosmolar non-ketotic coma, that results from massive dehydration and profound hyperglycemia in the absence of keto-acidosis. Relative or absolute over dosage with insulin causes hypoglycemia effects, including coma, which, unless treated, may prove fatal (Anderson, 1985).

Too much insulin may cause hypoglycemia, which is a serious symptom causing sweating, hunger, incoherence, convulsions, coma and death. On the other hand, glucagon, the hypoglycemic factor, exerts an effect upon blood sugar opposite to that of insulin and has positive inotropic effects on the heart, possibly as a consequence of stimulating cyclic AMP production (Rastogi *et al.*, 1998). The unique biologic opposition of the two hormones endows the alpha-

beta cell unit with the ability to vary glucose flux in a manner physiologically appropriate to the prevailing circumstances while maintaining extra cellular glucose concentrations within remarkably narrow limits, irrespective of those circumstances. Levine (1972) demonstrated that insulin is the hormone of glucose efflux from the extra cellular space. If extra cellular fluid (ECF) glucose concentration is to remain unchanged during wide changes in glucose flux, glucose and influx must at all times remain approximately equal. This critical balance appears to be due to variation in the insulin-glucagon mixture.

For example, during violent exercise, efflux into muscle is markedly increased. Hypoglycemia is prevented by a proportionate increase in glucose influx, partly because of a marked increase in glucagons and adequate glucose delivery to the central nervous system thus, maintained. Conversely, during a meal, when exogenous glucose influx is increased glucose efflux is increased proportionately to prevent hyperglycemia through increased insulin secretion, glucose efflux rates often approaching the rate of influx. The insulin and glycogen thus serve the nutrient needs of the far-flung tissues of the body, directing the storage of nutrient. When these are in abundance and retrieving them as required in time of famine, flight, fight, or injury, always in perfect accord with the needs of the moment. At all times, hyperglycemia, hypoglycemia and unnecessary nitrogen losses are successfully avoided (Unger and Texas, 1976).

Diabetes mellitus is also referred to as "a disease of rich and prosperous". Abnormal food intake causing obesity subjects the islets to constant strain, which results in degeneration of the islet cells (Boyd, 1970). The glycosuria often disappears when weight is reduced to a sufficient degree. When fully expressed, diabetes is characterized by fasting hyperglycemia, but the disease can also be recognized during less overt stages and before fasting hyperglycemia appears, most usually by the presence of glucose intolerance (Kahn and Weir, 1994).

The major symptoms of diabetes include excessive thirst, frequent urination, increased appetite, weakness and fatigue, and weight loss. Other symptoms may include muscle cramps, impaired vision and poor wound healing. These symptoms are correlated with the complications of diabetes. The diabetic complications include retinopathy, neuropathy, nephropathy, and atherosclerotic coronary artery disease, peripheral atherosclerotic vascular disease (Kaczmar, 1998), microangiopathy, ketoacidosis, hypersomolar non-ketotic coma and hypoglycemia (Mohan, 2000). The development of most complications of diabetes has been implicated by two biochemical mechanisms namely non-enzymatic glycosylation and polyol pathway mechanism (Mohan, 2000). In non-enzymatic glycosylation the free amino group of any body proteins binds reversibly to glucose and causes chemical alterations in the involved tissue proteins. Accumulation of glycosylation products on collagen and other tissues of the blood vessel wall form irreversible advanced glycosylation end products, which bind to receptors on different cells and produce variety of biologic and chemical changes. The polyol pathway mechanism is responsible for producing lesions in the aorta, lens of the eye, kidney and peripheral nerves. These tissues have an enzyme, aldose reductase that reacts with glucose to form sorbitol and fructose in the cells of the hyperglycemic patient. Intracellular accumulation of sorbitol and fructose so produced in the cells of the hyperglycemic patient results in entry of water inside the cell and consequent cellular swelling and cell damage. Also, intracellular accumulation of sorbitol causes intracellular deficiency of myoinositol, which promotes injury to schawn cells and retinal pericytes. These polyols results in disturbed processing of normal intermediary metabolites leading to complications of diabetes.

Classification of Diabetes Mellitus

Clinically, there are two major forms of diabetes namely type I and type II (Lacy and Kissane, 1977), and a number of specific diseases in which diabetes occurs as a secondary event (Anderson, 1985).

Type I diabetes, previously termed as juvenile-onset diabetes, or insulin-dependent diabetes mellitus (IDDM) (Mohan, 2000), is caused by absolute deficiency of insulin resulting from an immune-mediated, selective destruction of >90% of insulin-secreting beta cells (Kaczmar, 1998).

These patients, therefore, respond to exogenously administered insulin (Mohan, 2000). There are abnormalities of beta cell function and secretion. The patient is usually under 25 years at presentation, is wasted and may develop keto-acidosis. Currently, the pathogenesis of type I diabetes is explained on the basis of three mutually interlinked mechanisms i.e. genetic susceptibility, autoimmunity, and certain environmental factors (Mohan, 2000; Kumar *et al.*, 2001). Type I diabetes has been found to occur most frequently in persons of Northern European descent indicating genetic susceptibility. The disease is much less common among other racial groups, including blacks, Native Americans and Asians. This disease can run in families and about 6% of children of first-order relatives with type I diabetes develop this disease. Among identical twins, the concordance rate (i.e. both twins affected) is 40%, indicating that both genetic and environmental factors must play an important role. Secondly, a higher frequency (80%) of type I diabetes has been observed in HLA-DR_3 and HAL-DR_4 individuals (Mohan, 2000).

Type I diabetes is believed to be an autoimmune disease (Palmer *et al.*, 1983) that results in specific immunologic destruction of β cells of islets of Langerhan's. Presence of islet cell antibodies in type I diabetes, lymphocytic

infiltration in and around islets (insulitis) (Maclean and Ogilivie, 1959; Gepts, 1965; Bottazo *et al.*, 1985), and association of type I diabetes with other autoimmune diseases supports the evidence of autoimmunity. About 10% of cases of type I diabetes have other organ specific autoimmune diseases such as Graves' disease, Addison's disease or autoimmune thyroiditis (Mohan, 2000). The another presentation of insulin-dependent diabetes mellitus has been recently demonstrated that with immunological testing approximately 10% of patients initially diagnosed of having non-insulin dependent diabetes mellitus (NIDDM) may have a slow onset form of IDDM that has been termed latent autoimmune diabetes in adults (Tuomi *et al.*, 1993).

Epidemiological studies in type I diabetes have revealed involvement of certain environmental factors in its pathogenesis. The factors are certain viruses (coxsakie B virus, cytomegalovirus, mumps, measles and infectious mononucleases), chemicals (alloxan, streptozotocin, pentamidine etc.) and common environmental toxins. Thus, in type I diabetes some "environmental factor" initiates the "autoimmune destruction" of β cells in "genetically susceptible individuals".

Type II diabetes, or maturity onset diabetes, or non-insulin dependent diabetes mellitus (NIDDM), is more common and constitutes 80-90% cases of diabetes (Mohan, 2000). The basic metabolic defect in this type of diabetes is either a delayed insulin secretion relative to glucose load (deranged insulin secretion), or the peripheral tissues are unable to respond to insulin (insulin resistance) (Anderson, 1985). In this type, the patient is usually 40 years of age at presentation and 80% are obese. Ketoacidosis is not a feature but hyperosmolar non-ketotic coma may be a complication. The pancreas in NIDDM is usually of normal size (Rahier *et al.*, 1983; Kloppel *et al.*, 1985) but with a tendency to fatty infiltration (Westermark and Wilander, 1978), most probably due to the obesity present in many of these patients.

Type II diabetes is further of two subtypes i.e., obese and non-obese. Obesity is a common finding in type II diabetes. There is impaired insulin sensitivity of peripheral tissues, such as muscle and fat cells to the action of insulin, in obese individuals (insulin resistance). Lack of exercise and obesity are considered major contributors to type II diabetes; roughly 90% of individuals with type II diabetes are obese. These conditions predispose to hyperinsulinemia. Increased insulin resistance results in increased fasting and postprandial beta cell synthesis, which leads to "beta cell burnout" and eventually, diabetes. The condition of insulin resistance may exist for many years before pancreatic beta cell function actually becomes impaired (Kaczmar, 1998). Weight reduction in such obese patients produces improvement in the diabetic state. It has been observed that insulin resistance is a factor not only in obese type II diabetes but also in non-obese type II diabetes. In such individuals, the increased insulin resistance of peripheral tissues is due to either decrease in the number of insulin receptors or there is post receptor defect.

There are some other types of diabetes such as **Gestational diabetes,** which refers to the hyperglycemia temporarily during pregnancy in individuals, having inherited liability to develop this disorder. Although this form usually disappears following delivery, 40% of women with gestational diabetes will go on to develop type II diabetes later in life (American Diabetes Association, 1997). Other types of diabetes may be secondary to pancreatic disease or removal of pancreatic tissue; secondary to endocrine disease such as acromegaly, Cushing's syndrome, pheochromocytoma, glucagonoma, somatostatinoma or primary aldosteronism; secondary to the administration of hormones causing hyperglycemia. **Iatrogenic diabetes** may develop during various forms of therapy by drugs (Antihypertensive drugs, thiazide diuretics, preparations containing estrogen, psychoactive drugs, sympathomimetic agents, etc.) (Harris

and Cahill, 1979). It is also occurring mainly in those patients who are genetically susceptible.

Genetic and Spontaneous Animal Models

In animals different types of diabetes have been identified. The classification of diabetic dogs and cats is modeled after the human classification (Suter, 1989) but especially in the diabetic dogs, many aspects are different while as diabetic cat resembles type II diabetic human patients more closely (Hoenig, 2002). In rabbits diabetes mellitus is essentially similar to the insulin independent diabetes mellitus in humans (Roth and Conaway, 1982). The non-obese diabetic (NOD) mouse and biobreeding (BB) rat are the two most commonly used animals that spontaneously develop diseases with similarities to human type I diabetes (Rees and Alcolado, 2005). Sand rat (Psammomys Obesus) is model of nutritionally-induced type II diabetes mellitus and is prone to developing hyper-insulinemia, hyperglycemia and obesity when transferred to a high-energy diet. However, the potential to become diabetic decreases with age (Ziv *et al.*, 1999). The fatty Zucker {Zucker diabetic fatty (ZDF)} rat has been valued as a model of obesity, as the characteristics of the model are described as hyperglycemia, early hyperinsulinemia, fasting hyperglycemia, abnormal glucose tolerance, hyperlipidemia and mild hypertension (Corsetti *et al.*, 2000). The spontaneously diabetic KK mice are reported to have moderate obesity, polyphagia, polyuria, persistent glycosuria, glucose intolerance, moderate hyperglycemia, hyperlipidemia, insulin resistance of peripheral tissues and hyper insulinemia. The diabetic characteristics of KK mice and the variant KKAy are reverted to normal after 40 weeks of age (Suto *et al.*, 1998). The OLETF (Otsuka-Long-Evans-Tokoshima-Fatty) rat is a spontaneously diabetic rat with characteristic features of late onset hyperglycemia (after 18 weeks of age), a chronic disease state, increased urinary protein excretion, higher glomerular filtration rate, increased kidney weight etc. The clinical and

pathological features of the disease state in OLETF rats resemble those of human renal complications in human type 2 diabetes mellitus (Mori *et al.,* 1996). The Cohen, diabetic rat is an exceptional, genetically derived, diet induced type 2 diabetes mellitus model that expresses genetic susceptibility to a carbohydrate rich diet, a central feature of type 2 diabetes mellitus in humans (Zangen *et al.,* 2001).

Experimentally-induced Models

Diabetes mellitus in animals can be induced by chemical destruction or surgical removal of part of the β cell mass, lesioning the ventromedial hypothalamus, feeding with high fat and high sugar diets, malnutrition in utero, high doses of counter-regulating-hormones particularly glucocorticoids and prolonged cell exposure to hyper-insulinemia (Pickup and Williams, 2003; Keen *et al.,* 1982).

The classification of drug-induced diabetes in the experimental animals varies with quality and quantity of drug. Alloxan diabetes has been commonly utilized as an animal model of insulin dependent diabetes mellitus (IDDM) (Szkudelski, 2001). Streptozotocin is used to induce both insulin dependent (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). The range of the streptozotocin dose is not as narrow as in the case of alloxan. The frequently used single intravenous dose in adult rat to induce IDDM is between 40 and 60 mg/kg between (Ganda *et al.,* 1976). Multiple low doses of streptozotocin treatment is used predominantly in the mouse and the induction of IDDM is mediated by the activation of immune mechanisms (Szkudelski, 2001). NIDDM can easily be induced in rats by intravenous or intraperitoneal treatment with 100 mg/kg between streptozotocin on the day of birth (Portha *et al.,* 1974). High doses of streptozotocin and alloxan induce insulin deficiency and type I diabetes mellitus with ketosis. However, doses calculated to cause a partial

destruction of beta cell mass can be used to produce a mild insulin deficient state of type II diabetes mellitus, without a tendency to cause ketosis (Portha *et al.,* 1989). Streptozotocin is preferred because it has more specific beta cell cytotoxicity, but the sensitivity of this agent varies with species, strain, sex and nutritional state and there are batch differences in activity (Okamato, 1981).

Incidence of Diabetes Mellitus

Diabetes Mellitus is one of the most common metabolic disorders with a worldwide prevalence estimated to be between 1% and 5% (Meral *et al.,* 2004). According to Roberts (2001), diabetes is a deadly diseases affecting an estimated 135 million people worldwide and the numbers are increasing in rural and poor populations throughout the world. According to the report of the International Diabetes Institute and the World Health Organization (WHO) that diabetes mellitus "appears to be epidemic in many regions of world" and the figure will become double or even triple by the year 2010 (Yuan *et al.,* 1993). In India, over 20 million people are affected by diabetes. These numbers are expected to increase to 57 million by 2025 (Arvind *et al.,* 2002). The World Health Organization (WHO) has declared India as the country with the largest number of diabetic subjects in the world. By 2025, approximately 20 percent of the total diabetic patients worldwide would be from India (King *et al.,* 1998). Diabetes mellitus is responsible directly for about 38,000 deaths annually and cardio-renal-vascular complications resulting from diabetes mellitus are responsible for additional deaths annually, making diabetes a leading cause of death in United States (Norris, 1985). As per the report of American Diabetes Association the prevalence of diagnosed diabetes in the US is about 3% of the population (Kumar *et al.,* 2001). An estimated 16 million people in America have diabetes and is the seventh leading cause of death. Approximately 10% of the diabetic population is composed of type I or insulin-dependent diabetes, where as the remainder of diabetes are type II or non-insulin dependent. It is

estimated that a third of the non-insulin dependent diabetics are unaware of their disease. Among diabetic complications about 85% of all diabetics develop retinopathy, 25-50% develop kidney disease and 60 to 70% have mild to severe forms of nerve damage. Diabetes patients are also 2-4 times more likely to develop cardiovascular disease and 2-4 times more likely to suffer a stroke (American Diabetes Association, 1997). The prevalence of diabetes mellitus varies widely around the world and among racial and ethnic groups, probably as a reflection of genetic and environmental factors that have yet to be totally elucidated (Kumar *et al.,* 2001).

Diabetogenic Agents

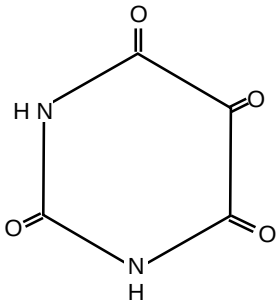
Many pharmacologists and toxicologists have found different chemicals such as alloxan, streptozotocin, cyprohepatidine, vacor (N-3-pyridylmethyl-N′-P-nitrophenyl urea), pentamidine, hexamethylmelamine or crude extracts of anterior pituitary etc. producing diabetes mellitus in animals (Copenhaver *et al.,* 1975; Fischer, 1985). Insulin secreting cells appear to be more sensitive to chemical insult than other hormone secreting cells of the pancreas and the diverse nature of the chemical structures of the substances known to produce deleterious changes indicate that there may be a number of different mechanisms by which β cells are damaged. Until these mechanisms are elucidated there is a great risk of exposure to diabetogenic agents (Fischer, 1985).

Many chemicals have been found to cause diabetes mellitus (diabetogenic agents) in man and animals, which are listed below:

Agent	Compound primarily used as	Species known to be Susceptible to diabetes
Alloxan	Experimental tool to produce diabetes in laboratory animals	Almost all
Streptozotocin	Anti-cancer drug and experimental tool to produce diabetes in animals	Almost all
Cyprohepatidine	Antihistamine – anti 5-HT drug	Rat, Mouse
Vacor (N-3-pyridylmethyl-N′-P-nitrophenyl urea)	Rodenticide	Human
Pentamidine	Antitrypanosomal drug	Human
Hexamethyl-melamine	Anticancer drug	Rat

Alloxan, a simple nitrogenous organic compound, was discovered by Frederick Wohler and Justin J. Liebig while beginning with the synthesis of urea in 1828, then of uric acid and the naming of same 13 derivatives of uric acid, including alloxan. The name "alloxan", given by Wohler and Liebig, is recorded as being derived from a combination of allantoin (a product of uric acid among others excreted by the foetus into the allantoins) and 'oxalsure' (oxaluric acid derived from oxalic acid and urea, found in the urine) (McLetchie, 2002). Alloxan was originally obtained by the action of dilute nitric acid on uric acid. Unlike its parent, uric acid, which presents as a suitable crystalline compound insoluble in water, alloxan presents as brownish-red crystals with great avidity for water and is very unstable with half like of a few minutes in room temperature, less at body temperature (McLetchie, 2002) and is longer at lower temperatures (Lenzen and Munday, 1991). Its chemical name is 2,4,5,6 (1H, 3H) pyrimidinetutrone:- 2,4,5,6 tetra oxo-hexahydropyridine

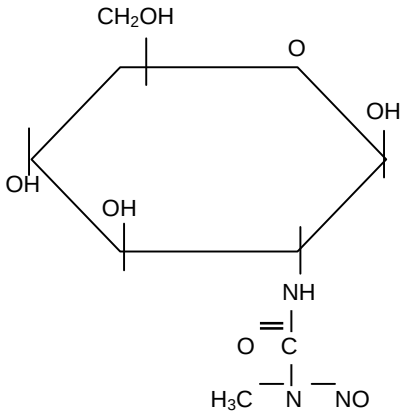
having the molecular formula $C_4H_2N_2O_4$ with molecular weight 142.07 (Rastogi *et al.*, 1998). It is represented diagrammatically as follows:



Chemical structure of alloxan

The remarkable discovery that a single injection of alloxan can produce diabetes mellitus in laboratory animals was made in 1942 by John Shaw Dunn and Norman McLetchie. The action of alloxan in the pancreas is preceeded by its rapid uptake by the beta cells (Weaver *et al.*, 1978; Boquist *et al.*, 1983). Rapid uptake by insulin secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity.

The methylnitrosurea analog, Streptozotocin [STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)] is synthesized by Streptomycetes achromogenes and is used to induce both insulin dependent and non-insulin dependent diabetes mellitus (Szkudelski, 2001). Its chemical structure is shown as:



Chemical structure of Streptozotocin

Streptozotocin has replaced alloxan as the primary compound as it selectively destroys beta cells of islets of Langerhan's (Fischer, 1985; Wang *et al.,* 1994; Shenoy *et al.,* 2002).

The antihistaminic drug, cyprohepatidine, and a number of related chemicals possessing a biphenyl methylpiperadine nucleus produce a reversible loss of pancreatic insulin when given in repeated doses (Fisher *et al.,* 1975). Experiments employing isolated rat pancreatic islets indicate that cyprohepatidine is a selective inhibitor of proinsulin synthesis.

The changes in the rat endocrine pancreas produced by cyprohepatidine are obtained in beta cells but not the glucagon-secreting α-cells or somatostatin-secreting δ-cells. Morphologically, with continued treatment over an 8-day period, there is a progressive loss of insulin secretion granules and a vesiculation of the rough endoplasmic reticulum followed by the formation of large cytoplasmic vacuoles. All of these changes are reversible upon drug withdrawal.

Persons accidentally or intentionally ingesting vacor exhibit insulin-dependent diabetes mellitus if they survive the neurotoxicity of the agent (Johnson *et al.,* 1980). The poison kills rodents by virtue of its toxicity to the peripheral nervous system but does not produce diabetes in laboratory animals including monkeys. Thus, there appears to be a strict species selectivity in the diabetes- producing effects of vacor.

Another agent found to produce diabetes in humans is the trypanocidal drug, pentamidine. An insulin-dependent hyperglycemic state is produced in patients, usually after several weeks of drug treatment. A number of pentamidine-induced diabetics have been reported, and the pattern of blood glucose changes resembles the transient hypoglycemic-permanent hyperglycemic phases characteristic of alloxan and streptozotocin administration to animals (Bouchard *et al.,* 1982).

Another report indicates that the antineoplastic agent, hexamethylmelamine, produces a dose-dependent hyperglycemia in rats chronically treated with the drug (Molello *et al.,* 1984). Its effects were limited to the insulin-secreting β-cells and appeared to be reversible upon withdrawal of the drug.

The management of diabetes by replacement with antidiabetic drugs has revolutionized the concept of disease. Chakravarthy *et al.,* (1980) have reported that a flavonoid fraction extracted from the bark of *Pterocarpus marsupium* Roxb. effectively reversed the alloxan-induced changes in the blood sugar level and the beta cell population in the pancreas. Further, the drug has been reported to possess protective effect when given prior to alloxan administration. Kedar and Chakrabarti (1983) have reported that oral administration of jambolan seed (1g/kg) in casein diet significantly lowered the elevated postmeal values of blood sugar, cholesterol, FFA and triglyceride to levels comparable to phenformin. Santhoshkumarai and Devi (1991) have reported that administration of Ayurveda drugs *Nisakathakathi kashayam*, *Rasnairandadi kashayam* and their mixture to experimental rabbits decreased fasting blood glucose and serum cholesterol and the effect was more significant in the case of the mixture. Maghrani *et al.,* (2003) have suggested that the aqueous extract of *Retama raetam* posses significant hypoglycaemic effect in both normal and streptozotocin-induced diabetic rats. Halim (2003) has reported that combination of water extract of dried powder of root and leaves of *Abroma augusta* and *Azadiracta indica* respectively is helpful in lowering the blood sugar of alloxan-induced diabetic rats. Akhani and his associates (2005) have reported that the fresh as well as dried rhizome of ginger, *Zingiber officinale* possess a potential antidiabetic activity with regard to decrease in serum cholesterol, serum triglyceride, blood pressure, fasting blood sugar and increase in insulin levels in streptozotocin-induced non-insulin dependent diabetic rats. Habib *et al*., (2005) have reported a significant decrease in blood glucose,

eosinophils, monocytes and hemoglobin contents, and no significant change in total erythrocyte count, total leukocyte count and differential leukocyte count in normal rats treated with neem leaf extract, nayantara leaf extract and bitter melon fruit juice with the patent drug gliclazide. Bairy *et al.,* (2005) have reported that the extract of *Syzygium malaccense* with their beneficial effects on blood sugar and hyperlipidemia associated with diabetes could serve as good adjuvant to other oral hypoglycemic agents. Baqui *et al*., (2005) have reported a significant improvement in biochemical and behavioural patterns of alloxan induced diabetic rabbits by the oral application of antidiabetic drugs. Iriadam *et al.,* (2006) have reported that the extract of aerial parts of *Artemisia herba-alba* when administered orally produced a significant hypoglycemic effect in normal and streptozotocin-induced diabetic rabbits. Tedong *et al.,* (2006) have demonstrated the efficiency of hexane extract of *Anacardium occidentale* in reducing the functional and histological alterations in the kidneys of streptozotcin-induced diabetic rats.

The perusal of literature indicates that most of the work on diabetes mellitus has been restricted to some biochemical indicators. Little work has been done to elucidate the other diabetes-related complications. Hence, the present study has been conducted to approach the understanding of the disease in elucidating the biochemical, behavioural and histological alterations. The objective of the study include:

1) In the study, rabbits were selected as experimental animals because of their timid and non-aggressive nature, easy handling and especially their close relation to primates. Being animal models, the results obtained can be extrapolated to other animals including man.

2) To study the biochemical alterations in the blood/serum in alloxan- and streptozotocin-induced diabetes mellitus in rabbits.

3) To study the effect of the induced diabetes on the behavioural patters of the rabbits.

4) To study the subsequent effect of the disease on gross and histomorphological alterations in various organs of the diabetic animals.

5) To study the diabetogenic potential of alloxan and streptozotocin and their comparative effects on the biochemical and histomorphological patterns.

6) To study the efficacy of various herbal/allopathic drugs in combating diabetes and diabetes-related complications with regard to behavioural, biochemical and histomorphological alterations on the rabbits and to harvest their therapeutic efficacy.

Diabetes mellitus, a metabolic disorder, is characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins with an increased risk of vascular disease (Pickup and Williams, 2003). The minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration (Keen and NgTang, 1982; Ziv *et al.*, 1999). It is a complex heterogeneous assemblage of disorders having the common feature of appearance of glucose in the urine. The characteristic symptoms that are recognized clinically include polydipsia, polyuria, pruritus, weight loss, or one or more of the many complications associated with or attributable to the disease. Diabetes mellitus may present as a relatively sudden, potentially lethal metabolic catastrophe or it can be associated with few symptoms or signs and may escape detection for many years. These extremes of clinical manifestations constitute the basis for subdividing diabetes mellitus into the insulin dependent (IDDM) and the non-insulin dependent (NIDDM) types (Norris, 1985).

The major problems associated with diabetes are retinopathy, nephropathy, the cardiovascular system with accelerated arteriosclerosis and neuropathy (Lacy and Kissane, 1977). The pathogenic mechanisms of diabetes mellitus responsible for the diminished available insulin are multiple, but they usually are related either to destruction of islets secondary to severe pancreatic or to selective degeneration of islet cells (Thomson, 1989).

Diabetes mellitus has been studied extensively in different experimental and domestic animals. Diabetes mellitus was reported in rabbits (Roth and Conaway, 1982), dogs (Meier, 1960) and several species of rodents like guinea pigs (Lang *et al.*, 1976), Chinese hamster (Meier and Yerganian, 1959; Like *et al.*, 1974), rats (Schmidt-Nielson *et al.*, 1963) and mice (Boucher and Notkins, 1973).

In case of dogs, diabetes mellitus is a common endocrine disorder with a reported incidence of 1 in 200 (Meier, 1960). Most cases of spontaneous diabetes occur in mature dogs and in females approximately twice as often as in males (Wilkinson, 1960; Kaneko, 1980). It is characterized by disturbances of carbohydrate, lipid and protein metabolism and glucose intolerance (Milne, 1987). There is a relative or absolute deficiency of insulin resulting in hyperglycemia (Nelson, 1985). According to Nelson (1985) the four classic symptoms of a dog affected by diabetes mellitus are polyuria, polydipsia, polyphagia and weight loss.

Sandhu *et al.* (2000) have reported that alloxan-induced diabetes mellitus in dogs is characterized by vomiting, polydipsia, polyuria, inappetence, dehydration, hypothermia, dullness, depression, hindleg weakness and recumbency followed by death. Development of diabetes mellitus in young dogs may be associated with idiopathic atrophy of the pancreas, acute pancreatitis with necrosis and haemorrhage, or aplasia of pancreatic islets. The overall size of the pancreas with idiopathic atrophy is reduced to a third of normal or less, and there is evidence of both endocrine and exocrine deficiency. Aplasia of pancreatic islet has been a cause of diabetes mellitus in dogs from two to three months of age. The islets are absent, but the pancreatic acini and ducts are present and functional (Thomson, 1989). Histopathological examination of alloxan-induced diabetic dogs has revealed vacuolation, necrosis of pancreatic cells along with hyalinzation and degeneration, exfoliation of tubular

epithelium, coagulative necrosis in the kidneys and degenerative changes in the brain, liver, heart and intestines (Sandhu *et al., 2*000).

Cats with diabetes mellitus usually have specific degenerative lesions localized selectively in the islets of Langerhans, whereas the remainder of the pancreas appears to be normal. The selective deposition of amyloid in islets, with degenerative changes in α and β cells, is the most common pancreatic lesion in cats with diabetes. However, scattered amyloid deposits in the pancreatic islet occur in many cats without development of overt diabetes mellitus (Johnson *et al.*, 1970). A common islet lesion in cats with diabetes mellitus is hydropic (vacuolar) degeneration of β and α cells. Vacuolar degeneration with glycogen accumulation in cats appears to develop in β cells as a response to long term over stimulation (exhaustion) because of insulin resistance (Thomson, 1989).

The diabetes mellitus in rabbits is essentially similar to the insulin independent diabetes mellitus in humans (age dependent diabetes) (Roth and Conaway, 1982). Experimental studies of diabetes mellitus in rabbits have shown an increase in biochemical parameters such as increased blood urea and serum creatinine vis-à-vis a decrease in β cell number (Dubey *et al.*, 1994; Rastogi *et al.*, 1998). The histopathological examination of pancreas shows, differently to other species, a hypergranulation of the β cells. Due to this, a malfunction of the insulin secretion is assumed the reason of this disease (Conaway *et al.*, 1981). The naturally occurring diabetes mellitus in rabbits seems to be caused by a genetic predisposition but also other still unknown environmental influences might be the reason for the occurrence of the disease (Conaway *et al.*, 1980; 1981). The disturbances of the insulin secretion (Roth *et al.*, 1980) are assumed to be due to hypergranulation of the β cells, which result in decrease of serum insulin levels. The diabetic symptoms of rabbits as

reported by Roth *et al.,* (1982) include polyphagia, polydipsia, polyuria, glycosuria and the development of cataracts.

In case of rats, several studies regarding diabetes mellitus have been made to understand the diabetic complications such as cataract (Rawal *et al*., 1986), peripheral motor neuropathy (Narama and Kino, 1989) and granular lesions (Tago *et al*., 1991). Aged male rats of WBN/Kob strain with spontaneous diabetes frequently suffer long term hyperglycemia and glycosuria, and it has been suggested that they are useful animal models for type II–non-insulin dependent diabetes mellitus (Nakama *et al*., 1987; Mori *et al*., 1988).

Diabetogenic Activity of Alloxan

Since the discovery of its diabetogenic property, it has been extensively used for induction of diabetes mellitus in experimental animals through different routes (Rerup, 1970; Copenhaver *et al*., 1975; Duckworth *et al*., 1979; Chakravarthy *et al*., 1980; Rawal *et al*., 1986; Agarwal *et al*., 1987; Dubey *et al*., 1994; Rastogi *et al*., 1998) causing hyperglycemia. Alloxan exerts its diabetogenic action when it is administered parenterally, intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse (Eizirik *et al.*, 1994). The most frequently used intravenous dose of this drug in rats is 65mg/kg b.w. (Gruppuso *et al., 1*990; Boylan *et al., 1*992). When alloxan is given intraperitonially or subcutaneously its effective dose must be 2-3 times higher. Fasted animals are more susceptible to alloxan (Szkudelski *et al.,* 1998), whereas increased blood glucose provides partial protection (Bansal *et al.,* 1980; Szkudelski *et al.,* 1998). The action of alloxan in the pancreas is preceded by its uptake by the B cells (Boquist *et al.*, 1983). Rapid uptake by insulin-secreting cells has been proposed to be one of the important

features determining alloxan diabetogenicity. Another aspect is the formation of reactive oxygen species (Heikkila *et al.,* 1976). The formation of reactive oxygen species is preceded by reduction of alloxan. In beta cells of the pancreas its reduction occurs in the presence of different reducing agents. Since, alloxan exhibits a high affinity to the SH-containing cellular compounds, reducing glutathione (GSH), cysteine and protein-bound sulphydryl groups including SH-containing enzymes are very susceptible to its action (Lenzen and Munday, 1991). Alloxan is vulnerable to glucokinase, which is one of the SH-containing compound essential for proper glucose-induced insulin secretion (Lenzen *et al.,* 1987).

Once alloxan is reduced in the body it forms dialuric acid which is then re-oxidized back to alloxan establishing a redox cycle for the generation of superoxide radical (Munday, 1988). The dialuric acid formed undergoes auto-oxidation to yield detectable amounts of hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl free radicals (Fischer, 1985). Superoxide radicals are able to liberate ferric ions from ferritin and reduce them to ferrous ion. Fe^{3+} can also be reduced by alloxan radicals (Sakurai and Ogiso, 1995).

One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in B cells exposed to alloxan (Sakurai and Ogiso, 1995). DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. This effect is, however, suggested to be due to their ability to scavenge free radicals rather than to a restriction of poly ADP-ribosylation initiated by alloxan (Sandler & Swenne, 1983). Further, like beta cells, all cells take up glucose for metabolism but also have special monitoring glucose transporters. A specific surface glucose transporter (GLUT2) has been characterized on the surface of beta cells which is exploited by alloxan and streptozotocin (Wang and Gleichmann, 1998).

Another diabetogenic action of alloxan includes disturbances in intracellular calcium homoeostasis elevating cytosolic free Ca^{2+} concentration in pancreatic B cells (Kim *et al.,* 1994). This effect arises due to alloxan-induced calcium influx from extracellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx may result from the ability of alloxan to depolarize pancreatic B cells (Dean and Mathews, 1972). Depolarization of the cell membrane opens voltage-dependent calcium channels and enhances calcium entry into cells. The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by hydrogen peroxide which itself exerts a similar effect on calcium concentration in B cells (Park *et al.,* 1995).

Diabetogenic Activity of Streptozotocin

The biological action of streptozotocin corresponds closely to alloxan being both labile and hydrophilic. Streptozotocin has thus been used to induce diabetes mellitus in experimental animals (Zysset *et al*., 1987; Jamshid *et al*., 1988; Wang *et al*., 1994; Mulder *et al*., 1997; Hardikar *et al*., 1999; Shenoy *et al*., 2002). The action of streptozotocin in B cells is accompanied by characteristic alterations in blood insulin and thereby glucose concentrations. Two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin. About six hours later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease (West *et al.,* 1996). These changes in blood glucose and insulin concentrations reflect abnormalities in B cell function. Streptozotocin impairs glucose oxidation (Bedoya *et al.*, 1996) and decreases insulin biosynthesis and secretion (Bolaffi *et al.,* 1987; Nukatsuka *et al.,* 1990). It was reported that streptozotocin at first abolishes the B cell response to glucose. Temporary

return of responsiveness then appears which is followed by its permanent loss and cells are damaged (West *et al.,* 1996).

Intracellular action of streptozotocin results in changes of DNA in pancreatic B cells comprising its fragmentation (Morgan *et al.,* 1994). During the decomposition of streptozotocin, highly reactive carbonium ions are formed, which causes alkylation of DNA bases (Doux *et al.,* 1986). Streptozotocin may also damage the B cell membrane and break the DNA strand which leads to the activition of poly (ADP-ribose) synthetase, NAD depletion and further reduction of the ATP content (Heller *et al.,* 1994) and ultimately to cell death (Okamato, 1981; Portha *et al.,* 1989). The concept of unfavourable consequences of augmented poly ADP-ribosylation as a result of streptozotocin action was confirmed by experiments revealing that the inhibition of this process prevents the toxicity of this diabetogenic agent. It was found that 3-aminobenzamide, a strong inhibitor of poly (ADP-ribose) synthetase, protected against the action of streptozotocin in rats, even when this substance was administered 45-60 min after streptozotocin (Masiello *et al.,* 1990). Another inhibitor of poly (ADP-ribose) synthetase is nicotinamide which is also scavenging oxygen free radicals, exerted best protection when it was administered shortly after streptozotocin (Masiello *et al.,* 1990).

Being nitric oxide (NO) donor, streptozotocin has been found to bring about the destruction of pancreatic islet cells and contributing DNA damage (Morgan *et al.,* 1994). The participation of NO in the cytotoxic effect of streptozotocin was confirmed experimentally (Kroncke *et al.,* 1995). Pancreatic B cells exposed to streptozotocin manifested changes characteristic for NO action, i.e., increased activity of guanyl cyclase and enhanced formation of cGMP (Turk *et al.,* 1993). Streptozotocin is, however, not a spontaneous nitric oxide donor but this molecule is liberated when streptozotocin is metabolized inside cells (Kroncke *et al.,* 1995). On the other hand NO scavangers lowered

NO concentration in pancreatic islet cells by inhibitions of the inducible form of nitric oxide synthase and partially counteracted DNA cleavage induced by strepreptozotocin (Kroncke *et al.,* 1995).

Beta cells of islets of Langerhan's posses high activity of an enzyme called xanthine oxidase. Augmented ATP dephosphorylation caused by streptozotocin increases the supply of substrate for xanthine oxidase and enhances the production of uric acid which is the final product of ATP degradation (Nukatsuka *et al.,* 1990a). Xanthine oxidase in turn catalyses reaction in which the superoxide anion is formed (Nukatsuka *et al.,* 1988). As a result of superoxide anion generation hydrogen peroxide and hydroxyl radicals are formed (Nukatsuka *et al.,* 1990a).

Calcium, which may also induce necrosis, does not seem to play a significant role in the necrosis evoked by streptozotocin since calcium channel antagonists do not protect B cells against streptozotocin, as they do in the case of alloxan (Katsumata *et al.,* 1992).

Pathology/Complications of Diabetes Mellitus

The consequences of hyperglycemia are almost in every tissue and organ of the body which undergoes biochemical, functional and structural alterations that occur in pancreas (Gepts, 1965; Kloppel *et al.,* 1985; Haward and Van, 1986), kidneys (Mauer *et al.,* 1979; O'Donnel, 1986), liver (Zysset & Tlach, 1987), bladder (Lincoln *et al.,* 1984; Kolta *et al.,* 1985; Longhurst and Belis, 1986; Uvelius, 1986), fat cell (Chiappe De Cingolani, 1986), cardiovascular system (Vadlamudi *et al.,* 1982; Latifpour and McNeill, 1984; Macleod and McNeill, 1984; McNeil, 1985), eyes (Kinoshita *et al.,* 1965; Rawal and Gandhi, 1986), reproductive system (Balasubramanian *et al.,* 1991) and nerves (Buck *et al.,* 1976; Hosking *et al.,* 1978; Niakan *et al.,* 1986) accounting for the major complications in diabetes. Possibly these complications are related to the severity

of hyperglycemia since control of blood glucose from clinical point of view is associated with minimizing the development of complications (Mohan, 2000). Extensive studies with respect to biochemical alterations in respect of sialic acid, acetylcholinasterase, surface glycoproteins and key glycolytic enzymes of diabetic red cell membrane (IDDM) have been made (Suhail and Rizvi, 1989; Suhail *et al.,* 1992).

Islet Damage

A number of pathological changes have been demonstrated in the islets in association with diabetes. Many of the structural islet lesions reflect important pathologic events in the pancreas (Opie, 1901). Distinct differences exist in the pathologic changes observed in the pancreas of individuals with classic juvenile- and maturity-onset diabetes. In recent-onset insulin-dependent diabetes mellitus, no significant reduction of the pancreatic size is found (Maclean and Ogilivie, 1959; Gepts, 1965) whereas in long standing insulin-dependent diabetes mellitus there is often, but not always, a significant reduction of the pancreatic weight (Maclean *et al.,* 1959) accompanied by interstitial fibrosis of the exocrine tissue (Doniach and Morgan, 1973; Rahier *et al.,* 1983). In type I diabetes mellitus there is often a qualitative reduction in the number of islet per area (Gepts, 1965; Doniach and Morgan, 1973) but in quantitative studies there is considerable overlap with the normal pancreas (Junker *et al.,* 1977). In long-standing insulin dependent diabetes mellitus the islets are small (Rahier *et al.,* 1983) and there is a major reduction of the islet volume (Maclean and Ogilivie, 1959; Gepts, 1965), which depends largely on the almost complete loss of islet β cells (Gepts, 1965). In spite of the pronounced β cell loss, a few β cells are commonly found in many cases of insulin dependent diabetes mellitus of long duration. In contrast to IDDM, the pancreas in non-insulin dependent diabetes mellitus (NIDDM) is usually of normal size (Westermark *et al.,* 1978; Rahier *et al.,* 1983) but with a tendency

to fatty infiltration (Westermark *et al.,* 1978) most probably due to the obesity present in many of these patients. Arteriosclerotic changes are the rule and diffuse or focal fibrosis is common. A pronounced β cell loss as seen in IDDM does not occur in NIDDM. The β cells in NIDDM are rich in granules and not degranulated as in IDDM. There are contradictory results concerning the α cell mass in NIDDM, with both reduced (Saito *et al.,* 1979; Kloppel *et al.,* 1985) and increased mass reported (Rahier *et al.,* 1983). In a majority of individuals, many of the islets show pathologic alterations, particularly amyloid deposits. The β cell volume in obese NIDDM patients has been reported twice as that of non-obese diabetic individuals (Kloppel *et al.,* 1985).

Glycogen is deposited in β cells of the islets when there is persistent hyperglycemia for long period of time. Previously this lesion was called hydropic degeneration of β cells since the cells appeared greatly vacuolated and it was assumed that the vacuoles contained water. The use of special stains demonstrated that the vacuoles contained glycogen. Glycogenesis of β cells occurs in diabetes in man as well as in experimental animals with diabetes (Toreson, 1951). The deposition of glycogen is attributable to a change in the intracellular metabolism of glucose that shifts the metabolism to the deposition of glycogen (Lacy and Kissane, 1977).

Electron microscopic studies have revealed a second pathologic change in β cells of dogs with experimental diabetes induced by the administration of growth hormone and glucose. This lesion is called "ballooning degeneration" since multiple vacuoles are present in the cytoplasm, the vacuoles do not contain glycogen, and the cells appeared to be undergoing degeneration. This degenerative change may represent the initial stages in the destruction of β cells during prolonged hyperglycemia (Lacy and Kissane, 1977).

Diabetic Nephropathy

Diabetic nephropathy is the most important cause of death in type I diabetic patients, of whom, 30-40% eventually develop end-stage renal failure (Giorgino *et al.*, 2004). Studies have shown that good metabolic control is beneficial in slowing the progession of nephropathy in diabetes, and if the duration of diabetes is prolonged before reinstitution of normoglycemia, nephropathy is not easily reversed (Floretto *et al.*, 1998; Renu *et al.*, 2004). In type II diabetic patients, nephropathy is also one of the major complication leading to death (Dubey *et al.*, 1994; Mohan, 2000). The development of diabetic nephropathy is characterized by a progressive increase in albuminuria and a late decline in glomerular filtration rate, leading eventually to end-stage renal failure (Salah *et al.*, 2004). This severity of renal disease in diabetic patients correlates with the levels of blood urea and serum creatinine (Dubey *et al.*, 1994). Diabetic nephropathy accounts for considerable morbidity and mortality even in patients with well controlled blood sugar values (Grenfel, 1991). In diabetic nephropathy different types of lesions have been described namely glomerular lesions, renal vascular lesions, principally arteriosclerosis, pyelonephritis including narcotizing papillitis (Kumar *et al.*, 2001) and tubular lesions or Armanni-Ebstein lesions (Mohan, 2000; Kumar *et al.*, 2001). The most important glomerular lesions are capillary basement membrane thickening, diffuse glomerulosclerosis and nodular glomerulosclerosis.

The most common features of vascular lesions in diabetic patients are renal glomerular degeneration. Previous studies on the long-term effects of diabetes in experimental animals showed that the effects were not only on the peripheral capillaries but they also induced the glomerular nephropathy along with tubular and interstitial abnormalities (Rasch, 1979; Hirose *et al.*, 1982). Glomerular basement membrane thickening, an indicator of diabetic microangiopathy may be seen in patients with a two year history of diabetes,

whereas it increases by 30% after 5 years of diabetes (Qsterby and Gunderson, 1989). The short-term effects of diabetes have been suggested to be the increased number of glomerular mesangial cells and interstitial alterations (Seyer-Hansen *et al.,* 1980) other than the increased basement membrane thickness, which are the indicators of long term effects of diabetes (Qsterby and Gundersen, 1979). In the very early phase of human and experimental diabetes, renal and glomerular growth is a consistent observation alongwith increased glomerular filtration rate (Hosteller *et al.,* 1981; Seyer-Hansen, 1983), and this early diabetic hypertrophy-hyperfunction syndrome may contribute to the later development of overt diabetic kidney disease (Brenner *et al.,* 1981; Mogenson and Christensen, 1984). Glomerular filtration rate (GFR) is found to be elevated on average by 20 to 40% above that of age matched normal subject to both in adults and children with IDDM (Mogenson, 1971) which is a clinically silent phase of variable duration after diagnoses of diabetes. Approximately 25% of patients with IDDM have a GFR exceeding the upper limit of the normal range. Renal plasma flow (RPF) has been reported to be elevated, normal or reduced in IDDM (Mogenson, 1971; Ditzel and Junker, 1972) although more recent work shows an elevation of RPF ranging between 9 and 14% (Christiansen, 1984). The increased GFR and RPF are accompanied by an increase in kidney size of approximately 20%, and a good correlation between GFR and kidney volume has been described in patients with IDDM (Mogensen and Anderson, 1973; Christiansen *et al.,* 1981). Approximately 40% of patients with IDDM have kidneys that are larger than normal. A large kidney is a prerequisite for the occurrence of the GFR above the upper limit of the normal range, but normal GFRs can be found in patients with large kidneys (Wiseman and Viberti, 1983).

Histologically, vacuolations and abundance of mucopolysaccharides have been reported in kidneys of streptozotocin-induced diabetic rats (Tedong *et al.,* 2006). In alloxan-induced diabetic dogs, the kidney sections has been reported

to show exfoliation of epithelial lining and degeneration of glomerular and tubular epithelium (Bansal *et al.,* 1994). Bulut and his associates (2001) have reported that glomerular capillaries entirely fill the renal corpuscle along with mesangial cell proliferation and hypertrophy in alloxan-induced diabetic rabbits. In diabetic dogs, degeneration of glomeruli and tubular epithelium along with the presence of hyaline casts, mildly sclerotic glomerulus and coagulative necrosis of tubular epithelium has been reported (Sandhu *et al.,* 2000). Mir and Baqui (2005) have reported structural alterations in kidney sections of experimentally induced diabetic rabbits.

Bladder Dysfunction

Besides the above complications associating with diabetes mellitus, bladder dysfunction is also a common complication of diabetes mellitus. Ellenberg and Weber (1967) reported that there is an 83% incidence of neurogenic bladder dysfunction in diabetics who have signs of peripheral neuropathy. Histochemical and functional studies have shown that there is a diabetes-induced alteration in the cholinergic innervation and/or change in the response of bladder of smooth muscle to cholinergic agonists in both humans (Buck *et al.,* 1976) and experimental animals (Kolta *et al.,* 1985; Longhurst *et al.,* 1986; Jamshid *et al.,* 1988).

Liver Damage

Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homoeostasis and is severely affected during diabetes (Seifter and England, 1982). Liver participates in the uptake, oxidation and metabloic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes a profound alteration in the concentration and composition of lipid occurs (Sochor *et al.,* 1985). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes

of glucose metabolism in the diabetic liver (Baquer, 1998). Structural alterations in the diabetic liver of experimental animals showed dilatation of sinusoids, disruption, degeneration, congestion and necrosis of hepatic architecture has been reported (Sandhu *et al.,* 2000). Bansal and his associates (1994) have reported fatty changes in liver of diabetic dogs. Further, a reduction in volume of hepatocytes, their nuclei and sinusoids in rat liver by streptozotocin injection have been reported (Noorafshan *et al.,* 2005). A reduction in the mean volume and weight of the liver by 15% and 12% respectively has been reported.

Lesions of the liver are characterized by hyperplasia of the ductules, together with dilatation with granular, brownish material. Hepatomegaly, due to either fatty metamorphosis or cirrhoses often occurs in diabetic dogs (Thomson, 1989). The enlarged liver is friable and soft. Palpation can lead to its disruption, rupture and intra-abdominal haemorrhage. The accumulation of fat in the liver is the result of increased fat mobilization in diabetic patients. In addition, parenchymal cells in the liver are injured when ketonemia is present, resulting in decreased utilization of fats (Thomson, 1989).

Diabetic Retinopathy

Diabetic retinopathy is present in virtually all patients with IDDM with nephropathy (Parving *et al.,* 1988). Visual impairment, sometimes even total blindness, is one of the more feared consequences of long standing diabetes (Kumar *et al.,* 2001). The ocular involvement may take the form of retinopathy, cataract formation (Rawal *et al.,* 1984), or glaucoma, (Mohan 2000, Kumar *et al.,* 2001). The development of lesions (Boyd, 1970) caused due to changing patterns of blood flow through the retina (Rawal *et al.,* 1986) result in ischaemia, microaneurysm in the retinal capillaries, new formation of the retinal capillaries, new formation of the capillaries within of the retina, subsequent haemorrhage into the vitreous, and formation of granulation tissue (Lacy and

Kissane, 1977). The development of these lesions requires many years with a varying degree of severity in individual patients and long periods of remissions with no further impairment of vision.

Alloxan induced cataractous lenses have shown an increase in glutathione reductase (GSH-R) activity and fall in glutathione (GSH) content in blood, aquous humor and lens indicating that alloxan interferes with intracellular oxidation reduction process in the lens and thus lead to cataract formation (Rawal and Gandhi, 1984). Further, in alloxan- induced cataractous lenses, an increase in sodium, calcium, water content and a fall in potassium content and total proteins have been reported (Rawal and Gandhi, 1986). Gabbay (1973) has reported that cataract formation in the diabetic patient is related to the unique sorbitol pathway by which glucose is metabolized in the lens.

Cardiovascular Damage

The prevalence and severity of atherosclerosis is higher among diabetic patients, particularly coronary artery disease (CAD) that is a major contributor to mortality and morbidity among type II diabetic subjects (Pyorala *et al.*, 1987). Diabetic subjects have been shown to have a higher risk for CAD compared to the non-diabetic population (Keen *et al.*, 1999). Further, independent of the cardiovascular risk factors seen among non-diabetic subjects, diabetes specific factors also contribute to the increased CAD risk and also to vulnerability of plaque ruptures (Nesto and Rutter, 2002). The pathophysiological process of atherosclerosis in diabetic subjects is accelerated by several factors such as hyperglycemia, insulin resistance, abnormal lipid profile, oxidative modification of lipoproteins, increased blood pressure, altered rate fibrinolysis (Arvind *et al.*, 2002).

Diabetic Foot

The greatest fears of the diabetic patient are loss of eyesight and amputation. In the Unites States, 50,000 major non-traumatic amputations (above-knee and below-knee) are performed each year, 30,000 of them involving patients with diabetes (Kahn *et al.,* 1994). Diabetes mellitus accelerates the development of arteriosclerosis with a resultant earlier onset of coronary arteriosclerosis and atherosclerosis in general (Lacy and Kissane, 1977). The arteriosclerotic process also involves the vessels to the lower extremity with resultant production of gangrene of the toes and feet. The precipitating causes of gangrene of the lower extremities are usually mechanical, thermal or chemical trauma resulting in ulceration, infection and subsequent gangrene. Tropic disturbances, such as ulceration of the fingers or toes and neuropathic arthropathy may develop as complication of diabetic peripheral neuropathy and susceptibility to infections all tend to promote gangrene of the extremities in diabetics (Anderson, 1985).

Diabetic neuropathy

A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals (Tomlinson *et al.,* 1992; Ozturk *et al.,* 1996). This variety of alterations, generally called diabetic neuropathy, affects the brain, spinal cord and peripheral nerves (Gallego *et al.,* 2003). They were reported as degenerative changes in the autonomic nervous system of diabetic rats, with widespread degeneration of ganglionic tissue, reduction of axonal calibre and demyelinization (Tomlinson and Yusof, 1983; Schmidt and Pulard, 1986; Kniel *et al.,* 1986). In the central nervous system, diabetes reduces brain weight and neocortical volume, which is associated with a reduction of the number of cortical neurons (Jokobsen *et al.,* 1987). These central and peripheral changes indicate decreased neuronal activity.

Biochemical changes in diabetic neuropathy are more widespread than anatomical changes. The plasma and tissue catecholamine levels are increased, decreased or unchanged based on the selection of tissue, severity or duration of diabetes (Fushimi *et al., 1*984; Bitar *et al.*, 1986; Hilsted, 1995). Gallego *et al.* (2003) have reported that diabetes alters the catcholaminergic system in a very specific manner. The dopamine content is reduced only in the dopaminergic nigrostriatal system. Norepinephrine is altered, increased or decreased, in the sympathetic nervous system, but not in the central nervous system, and epinephrine is only altered in the adrenal gland and serum.

Drug Treatment of Diabetes Mellitus

Insulin was first isolated by Banting and Best from dog pancreas in 1921 (Banting and Best, 1922), and the first injection of insulin was given to a patient with diabetes at the Toronto General Hospital on January 12, 1922 (Best, 1956). Insulin promotes the storage of fat as well as glucose within specialized target cells and influences cell growth and tissues (Katzung, 1989). Transient and partial recovery of β cell function occurs in many IDDM patients during the first few months of conventional insulin treatment, and is manifested by increased circulating C-peptide concentrations, symptomatic remission and a decline in insulin requirement (Wallensteen *et al.,* 1988). Type II diabetes mellitus is a progressive disorder, and maintenance of near-normal glycemic control has been demonstrated to reduce the risk of its associated long-term vascular complications, and this treatment goal can be achieved in most patient with use of single oral agents, combination of oral agents, or insulin (Buse, 2000). Oral hypoglycemic agents include the sulphonylureas, the biguanide metformin, the α-glucosidase inhibitor acarbose and in some cases anti-obesity drugs such as D-fenfluramine are useful (Pickup and Williams, 1997). In NIDDM, drugs correct the underlying metabolic disorders, namely, insulin resistance and inadequate insulin secretion, and they should be used to modify

the patients lifestyle, particularly restriction of fat and total energy intake and increased physical exercise (Pickup and Williams, 1997). The UK Prospective Diabetes Study (UKPDS) in 1998 showed that, compared with conventional diet and life style modification, more intensive therapy with metformin, sulphonyluria, or insulin was associated with a 0.9% absolute reduction in $HbA1_c$ value over 10 years, a 25% reduction in microvascular endpoints, and a 16% reduction in microvascular disease.

Sulphonylurea Treatment

Sulphonylurea has represented the backbone of NIDDM therapy for more than 30 years, yet there is still much controversy about its mode of action and specifically whether they lower blood glucose through extra-pancreatic mechanisms other than stimulation of insulin secretion (Groop, 1992). In vitro studies using the perfused rat pancreas and in-vivo studies using the hyperglycemic clamp have demonstrated that sulphonylurea stimulate insulin secretion in a biphasic fashion (Grodsky *et al., 1*977; Groop *et al., 1*987). The insulinotrophic effect of sulphonylurea is augmented by glucose, and they apparently increase β cell sensitivity to glucose and non-glucose stimuli (Pfeifer *et al.,* 1980). Glipizide, glyburide and glibenclamide are potent second generation sulphonylurea drugs that improve glucose tolerance by augmenting insulin secretion and enhancing insulin action (Loubatieres, 1957; Pfeifer *et al.,* 1980; Groop *et al.,* 1985). Glyburide appears to exert greater effect to lower the fasting plasma glucose concentration, whereas glipizide has a greater effect on meal-stimulated insulin secretion (Groop *et al.,* 1985). In addition, glibenclamide seems to suppress hepatic glucose production more effectively than glipizide when examined at identical plasma concentrations (Groop *et al.,* 1987), whereas glipizide results in greater postprandial glucose excursions than glibenclamide (Sonkson *et al.,* 1981; Groop *et al.,* 1985).

Hypoglycemia is the most common and severe side effect of sulphonylurea and in elderly subjects it can lead to permanent neurological damage and death (Ferner and Neil, 1988; Jenning *et al.*, 1989). Glibenclamide has been reported to possess hypoglycemic activity (Mishra *et al.*, 1982). Most cases of severe and fatal hypoglycemia have been reported with the long acting sulphonylureas, chlorpropamide and glibenclamide (Berger, 1985; Ferner and Neil, 1988; Jenning *et al.*, 1989). About 20% of patients treated with sulphonylureas in the UK reported atleast one episode of symptomatic hypoglycemia during the previous six months (Jenning *et al.*, 1989).

Biguanide Treatment

Biguanides have been reported to improve the sensitivity for insulin without stimulating its production (Bailey and Turner, 1996). Synthetic biguanide (Metformin and Phenformin) were introduced in the late 1950s for the treatment of NIDDM (Pickup and Williams, 1997). Phenformin was withdrawn in most countries because of its association with lactic acidosis (Katzung, 1989; Pickup and Williams, 1997), but is only given to type II diabetic who is allergic to sulphonylurea and insulin and who fails diet therapy (Katzung, 1989). Metformin has remained in use throughout Europe and in many other countries and was recently approved by the Food and Drug Administration (FDA) for introduction in the USA (Pickup and Williams, 1997). Metformin is the only biguanide registered, since phenformin causes the serious adverse effect of lactic acidosis (Misbin, 1977; Misbin *et al.*, 1998) and it has been demonstrated that treatment with metformin in obese patients with type II diabetes is accompanied by a significant decrease in glycosylated haemoglobin. A number of studies have confirmed that metformin is as effective as sulphonylureas in reducing fasting plasma glucose concentrations (Clarke and Duncan, 1968; Herman, 1979). Dunn and Peters (1995) have studied that the increase in body weight did not take place during metformin

therapy as is normally observed when glycemic control is improved. This makes metformin pre-eminently suitable as primary drug for obese patients with type II diabetes with insufficient glycemic control in spite of diet (Stades *et al.,* 2000). Various studies have reported a 20 to 25% increase in peripheral glucose uptake during treatment with metformin (Widen *et al.,* 1994), mostly due to an increase in non-oxidative glucose metabolism. DeFronzo *et al*. (1991) have found that peripheral glucose uptake increased only under hyperglycemia conditions. Bailey and Turner (1996) and, Pickup and Williams (1997) have reported that hypoglycemia during metformin monotherapy is rare and has been therefore, considered as an antihyperglycemic rather than a hypoglycemic agent. Buse (2000) has reported that metformin restores glycemic control in highly insulin resistant obese patients without increasing peripheral insulin levels, largely by suppressing hepatic glucose output. One recent study (Aviles-Santa *et al.,* 1999) showed a marked additive impact of metformin with insulin therapy after 6 months of treatment among type II diabetes patients with poor glycemic control. In this trial, treatment with insulin plus metformin resulted in a lower $HbA1_c$ level than insulin plus placebo (6.7% Vs 7.7%) at a lower dose of insulin (96 U/day Vs 129 U/day) (Aviles-Santa *et al.,* 1999).

Treatment of Drug-induced Diabetes Mellitus

In experimental diabetes, the use of herbal medicine is widespread. More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only small members of these have received scientific and medical evaluation to assess their efficiency (Bailey and Day 1989; Satyavati *et al.,* 1987). There are various medicinal plants in the world, which are the potential sources of the drugs and most of the herbs are reported to posses some degree of antidiabetic activity (Marles and Farnsworth, 1996).

Gymnema: *Gymnema sylvestre* has a long history of use in India for controlling diabetes and is commonly named as gurmar meaning "sugar-destroying"

because of the plants antisacharogenic property (Suppresses the taste of sugar). Chewing the leaves actually deadens the sense of sweat tastes and also the bitterness of bitter substances (Nadkarni, 1976). In diabetic rabbit model, administration of G. sylvestre was shown not only bringing about blood glucose homoeostasis, but also increasing the activities of enzymes involved in glucose utilization (Shanmugasundaram, *et al.*, 1983). Additionally, the investigators reported that glycogen depletion in the liver and lipid accumulation in the diabetic animals was reversed as a result of *Gymnema sylvestre* therapy.

Garlic and Onions: Garlic and onions contain sulphur compounds, which are believed to be responsible for many of the plants reported health benefits, including antidiabetic properties. S-allyl cysteine sulphoxide (SACS), a compound present in garlic, was reported to decrease fasting blood glucose and lower serum cholesterol levels in diabetic rats in a manner similar to the effects of glibenclamide and insulin (Sheela and Augusti, 1992). Jelodar *et al.* (2005) have reported that garlic was able to reduce blood sugar level in alloxan-induced diabetic rats.

Fenugreek: Fenugreek seeds (*Trigonella foenum graccum*) have been demonstrated to posses hypoglycemic properties in both animal and human studies, thus, lending support to its traditional use (Ribes, 1986; Sharma, 1986). Research further suggests that fenugreek has a lowering effect on plasma cholesterol and triglyceride levels (Bordia *et al.*, 1997).

Bitter Gourd: Bitter gourd *(Momordica charantia)* also known as balsam pear, is a tropical vegetable widely cultivated in parts of Asia, Africa and South America, which has been extensively used in folk medicine as a remedy for diabetes (Welihinda *et al.,* 1982). The antidiabetic action of the fresh juice or extract of the unripe fruit has been established in both animal and human studies (Karunanayake, *et al.,* 1990; Ali *et al.,* 1993; Welihinda *et al.,* 1986).

Syzygium malaccense: The astringent bark of Malay apple i.e., *Syzgium malaccense* an indigenous plant is recommended as a local remedy for a variety of disorders like cough, constipation, headache, diabetes, antibacterial activity, diuretic, abortifacient etc. Further, it has been reported that the extracts of *Syzygium malaccense* with their beneficial effects on blood sugar and hyperlipidemia associated with diabetes could serve as good adjuvant to other oral hypoglycemic agents (Bairy *et al.,* 2005).

Azadirachta indica (Neem): In India neem is widely used as a medicinal plant for thousand of years. Extracts of ripe leaves, tender leaves, fruits and flowers of *Azadirachta indica* have been reported to possess antidiabetic activity (Bhattacharji *et al.,* 1953). The hypoglycemic effects of neem has been well studied (Murty *et al.,* 1978). Recently, it has been reported in diabetic rats that *A. indica* leaves and *Abroma augusta* roots when given together as water extract posses hypoglycemic action and had better effect than given alone (Halim, 2003).

Cassia auriculata: *Casia auriculata* Lin., commonly known as Tanners senna, is a common highly branched shrub with large bright yellow flowers distributed widely in dry regions of the central provinces and western peninsula of India (Kirtikar and Basu, 1981). The plant as a whole has been used as antidiabetic, antidysentric, antimicrobial and for various skin diseases from ancient times (Chaterjee, 1997). In Ayurveda, its seeds are used to treat various gastrointestinal disorders (Chaterjee, 1997). The flowers of the plant are used as folk remedy for the treatment of diabetes mellitus in Southern parts of India (Nadkarni and Nadkarni, 1982). In streptozotocin-induced diabetic rats its flower extract has been reported to suppress the elevated blood glucose and lipid levels and a dose of 0.45g/kg has been found to be comparable to glibenclamide (Pari and Latha, 2002). Recently the ethanol extract of *Cassia auriculata* flowers has been reported to possess antidiabetic activity which is

attributed to the presence of sterols, triterpenoids, flavonoids and tannins (Hatapakki *et al.*, 2005).

Aralia cachemirica Decne: It is known as Khoree in Kashmiri and is found distributed in the temperate himalayas from Kashmir to Sikkim at 2100 to 4000m (Asolkar *et al.*, 1992). The alcoholic extract of *Aralia cachemirica Decne* roots have been reported to show anti-hyperglycemic activity and an enhanced glucose tolerance activity (Bhat *et al.*, 2005).

Nigella sativa*: N. sativa* L. is a spice plant containing black seeds which possess more than 30% of a fixed oil and 0.40-0.45% w/w of a volatile oil (Aqel and Shaheen, 1996). The volatile oil has been shown to contain 18.4 – 24% thymoquinone and a total of 46% of many monoterpenes (El-Tahir *et al*., 1993), which have diuretic and hypotensive activity (Zaoui *et al*., 2000). The aqueous extract of *N. sativa* L. seeds has been reported to decrease the diabetes-induced disturbances of heart rate and some haematological parameters of alloxan-induced diabetic rabbits (Meral *et al*., 2004).

Hedera helix: *Hedra helix* L. is an evergreen woody climber widely distributed in India, Nepal, China and Pakistan (Stewart, 1972; Bahijri *et al*. 1984) and is locally claimed to possess hypoglycemic properties. Zafar *et al*. (2002) have reported that oral administration of ethanolic extract of *Hedera helix* has a beneficial effect on the alloxan-induced diabetic rabbits by lowering the blood glucose level through extra-pancreatic actions rather than by stimulated insulin release.

Rao *et al*. (1998) have reported that for clinical management of alloxan-induced diabetic dogs, oral antidiabetic drug glibenclamide @ 5mg daily alongwith dietetic adjustment consisting of 40 gm each of Rajmah and Bengal gram Dal are recommended. Shenoy *et al*. (2002) have reported that treatment with perindopril prevented streptozotocin-induced hyperglycemia and

decreased the elevated blood pressure in both Wistar diabetic and spontaneously hypersensitive diabetic rats. Babu *et al*. (2002) have reported antihyperglycemic activity of *Cassia kleinii* leaf (alcohol extract) in both glucose-fed hyperglycemic and alloxan-induced diabetic rats. Kakuda *et al*., (1996) reported the hypoglycemic effects of *Lagerstroemia speciosa* known by the Tagalog name of *banaba* in the Phillipines in a study using hereditary diabetic mice (type II, KK-A^y). Dubey *et al.* (1994) have reported that D-400, a herbomineral preparation, showed favourable response against alloxan-induced renal damage and hyperglycemia. Schauberger *et al*. (1977) have reported that pretreatment with n-butanol protects mice from alloxan-induced diabetes by the indirect mechanism of producing hyperglycemia at the time of alloxan administration.

The effective way of clinical management of diabetes mellitus includes exercise and diet control apart from anti-diabetic drugs (Giri *et al*., 1986). Diet has been recognized as a corner stone in the management of diabetes mellitus (Sharma and Raghuram, 1990). Dietary restrictions are similar for both maturity-onset diabetes (MOD) and juvenile-onset diabetic patients although a reduction in total caloric intake is necessary for overweight MOD patients. Specific diets must be determined individually for every patient. Carbohydrates are still essential to the diabetic diet but need to be in the form of polysaccharides (e.g. starch). Mono- and disaccharides are usually avoided because of rapid uptake and resultant hyperglycemia soon after ingestion. The ingestion of simple sugars is less a problem for the MOD patient as the pancreas can respond with some insulin release (Norris, 1985).

The present chapter includes various techniques/methods for induction of diabetes mellitus in the rabbits by alloxan and streptozotocin using different standardized doses. The route of administration for diabetogenic agents included either intraperitoneal (i.p.) or intravenous (i.v.). when diabetes mellitus was well established which was confirmed by behavioural and biochemical changes, some of the rabbits were sacrificed to observe the deleterious subsequent effects of diabetes mellitus on different organs like pancreas, kidneys, liver, lungs, heart, brain and alimentary canal. The behavioural, biochemical and histopathological changes were noted. Apart from above, this chapter covers treatment of diabetic rabbits by different oral antidiabetic herbal drugs. The treatment was assessed by improvement of behavioural, biochemical and histopathological changes along with blood sugar (F), blood urea and serum creatinine levels.

Animals with Drug Induced Diabetes

The various experimental studies regarding the induction of diabetes mellitus in rabbits by alloxan and streptozotocin was done and the subsequent effects on behviour, biochemistry and histopathology were studied thoroughly.

Sixteen New Zealand white male rabbits of 8-12 months old were used. The rabbits were purchased either from Faculty of Veterinary Sciences and Animal Husbandry (FVSC & AH) Shuhama, Alusteng, Srinagar or Institute of Animal Health and Biological Products (IAH and BP), Zakura, Srinagar or Rabbit Breeding Farm, Wusan Pattan. All the rabbits received human care according to the guidelines outlined in the "Guidelines for the Care and Use of Animals in Scientific Research" prepared by the Indian Science Academy, New Delhi.

Management of Animals

The rabbits were housed in rooms where sufficient space was available so that cages were easily maneuvered and operations such as cage changing, sanitation and investigation by the responsible personnel were easily performed. Cages were large enough to allow the rabbit to freely move about, stretch out when lying down and to facilitate feeding and waste removal. Environmental conditions such as illumination, ventilation and noise free housing was maintained.

The rabbits were fed on green vegetables and commercial pelleted diet so as to maintain their health and weight. Feed was offered to rabbits in a J-feeder attached to the cage floor. Fresh, clean and potable water was provided to the rabbits by means of water bottles attached to cages or bowls placed in the cage. The feeders were regularly cleaned to remove feed dust, which might decrease palatability of the food. Further, proper sanitation was maintained. All grossly visible debris was removed by brushing and rinsing with water followed by application of a chemical disinfectant.

Experimental Design

After acclimatizing to standard laboratory conditions for fifteen days, the rabbits were weighed and a mean body weight of 1.33 ± 0.20 kg was recorded. Prior to the alloxan and streptozotocin administration biochemical parameters such as blood sugar (F), blood urea and serum creatinine levels of these rabbits were recorded following twelve hours fasting.

Blood Collection

For biochemical estimation, the following steps were used for percutaneous blood sampling.

1) The hair around the site (ear vein) was shaved and swabbed with an antiseptic such as rubbing alcohol to minimize the chance of introducing skin-associated bacteria into the blood stream.

2) The location of the blood vessel of the ear was identified. The inner side of the ear was grasped between the thumb and index finger.

3) The plunger of the syringe was slightly pulled back before use, thereby breaking the air lock and allowing blood to flow more easily.

4) Using a 25 guage needle with the beveled edge up at a slight angle into the vessel, the venipuncture was made at a site immediately proximal to the thumb and the plunger of the syringe was gently and slowly pulled back as blood fills the shaft of the syringe. Care was taken not to pull the plunger too aggressively which might collapse the vessel and thereby cease blood flow.

5) Gentle manipulation such as slight changes in the orientation of the needle improved collection of blood. Alternatively, haematoma formation in the tissues around the withdrawal site or the presence of clotted blood in the needle might necessitate changing withdrawal site or replacement of the needle, respectively.

6) Another method was used by making a cut in the marginal vein or the central ear artery near its tip.

7) Once the blood sample was obtained, firm pressure with a gauze pad or little cotton wool was maintained at the sampling site for several minutes until bleeding ceased.

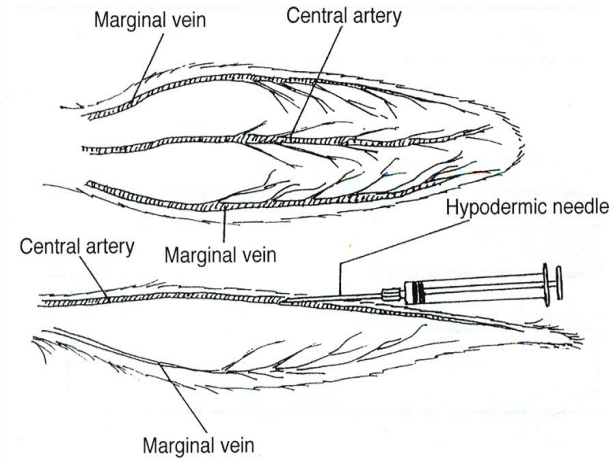


Fig. Location of ear vessels from a dorsal view (top) and from a lateral view, indicating relative orientation of the needle during sampling of blood from the central artery (bottom)

Volume of Sample

4ml of blood was collected for biochemical estimations of blood sugar, blood urea and serum creatinine. Sampling sites were changed each time when blood was withdrawn.

Handling of Sample

Blood samples for harvesting of serum were collected in vials containing no anticoagulants. Clotted blood samples were centrifuged at 3000 rpm for 10-15 minutes for obtaining serum.

Estimation of Blood Glucose

Blood glucose estimations after 12 hours fasting were done using either **Ames Glucometer Gx** (Bayer Diagnostic India Ltd.) or through enzyme kit method.

Procedure for Blood Sugar Estimations by Glucometer

Glucometer Gx is a battery operated instrument which enables convenient and accurate measure of blood glucose with the help of glucostix reagent strips. The test takes less than one minute to perform. To run blood glucose test following procedure is involved:

Material Required:

Glucometer Gx	:	Blood glucose meter
Glucolet	:	Automatic lancing device with endcap and lancet
Glucostix	:	Reagent strips

Whatmann's filter paper.

Test Procedure

1) Whatmann's filter paper was folded in quarters (for blotting) and placed on a clean dry surface.

2) The glucolet lancing device was loaded with a lancet and endcap was replaced or a disposable syringe with 25 gauge needle was kept ready for blood collection.

3) Spirit was applied to the marginal ear vein of rabbit with a cotton swab.

Test Proper

It was followed as per the instructions given in the manual which are as follows:

1) The button is pressed to turn the meter on. All the digits and legends appear in the display to show that they are working properly.

2) 1 to 3 seconds later a programme number appears in the display.

3) The button is pressed again. The legend programme appears above the programme number. The last programme number entered into memory is shown. Pressing of the button is continued to select the proper programme number. Pressing the button repeatedly scrolls the programme number from 1 to 9 and then back from 9 to 1. The programme number must match the programme number printed on the label on the bottle of glucostix reagent strips being used.

4) Moving it to the left opens the test slide. Three bars (- - -) appear in the display, and remain there until the next step is started.

5) Now, a drop of blood collected from the marginal ear vein of rabbit is kept ready and next step is followed quickly.

6) The button is pressed again, and a short beep sound is heard. A 50 appears in the display along with the long beep.

7) Immediately after the beep sound, the drop of blood is applied to the glucose test pads. Both reagent pads are completely covered

with blood. The reagent strip level is kept properly so as to avoid spilling blood off of the test pads.

8) Two short warning beeps which sound at 22 and 21 seconds alert for blotting. At 20 seconds a large beep sounds, the strip is blotted immediately at this time. To ensure a good blot, the reacted strip is placed with pad side up on the folded tissue. The tissue is folded over the test pads and firmly and quickly the tissue is pressed against the pads. It is repeated immediately on a clean area of the tissue. Blotting should be done always on a firm surface.

9) After blotting, the reagent strip is immediately inserted fully into the test slot, making sure the test pads are facing the display.

10) Now, the test sides are closed. The test slide must be closed before the countdown reaches 1 second.

11) After the countdown reaches 1 second, three bars appear briefly, followed by the test result and a long beep. The button is pressed to turn the meter off. The result is automatically stored in the memory.

In order to cross check the results displayed by glucometer Gx, the blood sugar level was also estimated spectrophotometrically using enzymatic kit method. The procedure of the method is given below:

Enzymatic Kit Method for Blood Sugar Estimation

For the analysis of blood sugar enzymatic kit method as described by Tietz (1976) was employed. In this single reagent system, glucose oxide converts glucose to gluconic acid and hydrogen peroxide. The peroxide in the presence of horseradish peroxidase forms a coloured complex of hydroxibenzoate and 4-aminophenazene. The intensity of colour formed is proportional to the glucose content present in the sample. 0.1 ml plasma was

used according to the manual instructions. The reagents supplied in the kit include:

Reagent 1 – Glucose enzyme reagent

Reagent 2 – Glucose standard 100 mg%

Reagent 3 – Phenol reagent.

Preparation of Working Glucose Reagent

6 × 100 ml pack working glucose reagent was prepared by transferring the contents of one vial of glucose reagent-1 to black plastic bottle, after reconstituting it to 100 ml with distilled water, 5 ml of phenol reagent (reagent 3) was added and these were mixed well and stored at cool dry place at 2 to 8°C. This working solution remains stable for 45 days at 2 to 8°C.

Protocol For Spectrophotometry

Reagent	**Blank (B)**	**Standard (S)**	**Test (T)**
Serum/Plasma	-	-	0.2 ml
Reagent 2: Glucose standard 100 mg%.	-	0.2 ml	-
Working glucose reagent	1.5 ml	1.5 ml	1.5 ml
Distilled water	1.5 ml	1.5 ml	1.5 ml

The contents of tubes were mixed well and incubated at 37°C for minutes. The colour intensity was measured in a spectrophotometer at 510 nm against distilled water.

Calculations

$$\text{Serum / plasma glucose mg/100ml} = \frac{\text{Optical Density of Test} - \text{Optical Density of Blank}}{\text{Optical Density of Standard} - \text{Optical Density of Blank}} \times 100$$

After cross checking Glucometer Gx and enzyme kit method for blood sugar estimation, the results were found similar and accurate.

Estimation of Blood Urea

Blood urea estimation was done by Berthelot method (Trinder, 1969). In this method, urea in presence of urease enzyme is converted into ammonia and carbon dioxide. Ammonia reacts with hypochlorite and salicylate in presence of sodium nitroprusside and produces a green colour which is measured at 578nm spectrophotometrically within 60 minutes against reagent blank.

$$\text{Urea} \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2$$

$$\downarrow$$

$$\text{Ammonia} + \text{Hypochlorite} + \text{Salicylate} \xrightarrow{\text{Sodium Nitroprusside}} \text{Green Colour}$$

Reagents supplied in Kit

For kit 2 × 50ml, contents were as:

2 vials of urease and sodium salicylate (R_1)

1 vial of 50ml alkaline Hypochlorite solution (R_2)

1 vial of urea standard (40mg/dl)

All reagents were stored at 2-8°C protected from light and contamination.

Reagent Preparation

25ml of distilled water was added to 1 vial of R_1 and the contents were dissolved slowly without frothing.

Materials required

Normal Saline/Distilled water

Micropipettes upto 1000µl ranges

Test tubes and test tube racks

Incubator

Spectrophotometer.

Sample

Serum, Plasma

Assay Procedure

Dispose	Blank	Standard	Sample
R_1	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl
Mix, incubate for 5 minutes at 37^0C, then add			
R_2	1000 µl	1000 µl	1000 µl

Mix, incubate for 5 minutes at 37°C.

Calculation

Optical density was measured at 578 nm against reagent blank. Concentration of urea was measured in sample in mg/dl.

$$\frac{\text{Optical Density of sample}}{\text{Optical Density of standard}} \times \text{conc. of standard (i.e., 40)}$$

Programming Guidelines

Reaction	Endpoint (Increasing)
Filter	578 nm
Temperature	37°C
Incubation time	5+5 minutes
Sample volume	10 µl
Reagent volume	1000 µl + 1000 µl
Standard concentration	40 mg/dl
Units	Mg/dl
Linearity	400 mg/dl
Blank	Reagent Blank

Estimation of Serum creatinine

The serum creatinine was estimated at weekly intervals throughout the period of experimental study.

Creatinine is an anhydride of creatine and is formed by a spontaneous and irreversible reaction. Free creatine is not reutilized in the process of metabolism. For the measurement of creatinine "Alkaline picrate method" of Baum (1975) was employed. The creatinine reacts with alkaline picrate and produces red coloured complex, which is measured at 520 nm spectrophotometrically.

Working standard solution was prepared by diluting 0.1 ml of stock standard to 10 ml, with diluted water. Firstly, the serum was deproteinized as under:

Serum	:	1 ml
Distilled water	:	1 ml
Creatinine reagent A	:	6.0 ml

These were mixed in a test tube and kept in boiling water for one minute. After cooling the tube was centrifuged till the supernatant became clear.

Test Procedure

The three borosil test tubes were selected and marked as blank, standard and test, respectively.

Reagent	**Blank (B)**	**Standard (S)**	**Test (T)**
Supernatant step I	-	-	4 ml
Working standard	-	1 ml	-
Distilled water	1 ml	-	-
Creatinine reagent A	3 ml	3 ml	-
Alkaline reagent	1 ml	1 ml	1 ml

The tubes were mixed well at room temperature for 20 minutes and the optical density measured against distilled water at 520 nm in a spectro-photometer.

Calculation

$$\text{Serum creatinine in mg/dl} = \frac{\text{Optical Density of Test} - \text{Optical Density of Blank}}{\text{Optical Density of Standard} - \text{Optical Density of Blank}} \times 30$$

Induction of Diabetes Mellitus

Before administration of diabetogenic agents i.e., alloxan and streptozotocin, the rabbits were divided into four groups of four each *viz*., Group I, Group II, Group III and Group IV. Group II and Group IV received alloxan and streptozotocin respectively while as Group I and Group III received normal saline and served as healthy control.

Alloxan Administration

For intraperitoneal administration four doses of alloxan (Loba Chemie) dissolved in 1ml of sterile water was administered @ 80mg/kg b.w. at weekly intervals following the earlier methods (Rastogi et al., 1998; Baqui et al., 2005).

Streptozotocin Administration

Diabetes mellitus was induced in New Zealand white rabbits by single iv dose of streptozotocin (Sisco Laboratories Ltd., Mumbai) @ 65mg/kg b.w. dissolved in 1ml of freshly prepared citrate buffer, pH 4.6 as practized earlier (Kedar and Chakrabarti, 1983; Tawfeeg and Sherif, 2001).

Composition of citrate buffer (pH 4.6)

Stock A: 0.1M disodium citrate (MW 210.0).
2.1gm citric acid dissolved in $20cm^3$ of normal sodium hydroxide and made upto $100cm^3$ with distilled water.

Stock B: 0.1M Hcl (MW 36.45).
$0.8cm^3$ hydrochloric acid in $100cm^3$ of distilled water.

Mix $76.6cm^3$ of stock A with $23.4cm^3$ of stock B to get citrate buffer with pH 4.6.

Technique for Intraperitoneal Drug Administration

The site for intraperitoneal administration of drug is lower (caudal) right abdominal quadrant and is given as:

1. Normally a long needle (1 inch or greater) was used.
2. The rabbit placed on its back was restrained by securing its head and front quarters with legs and extending the lower portion of the rabbits body with one hand (Fig.)

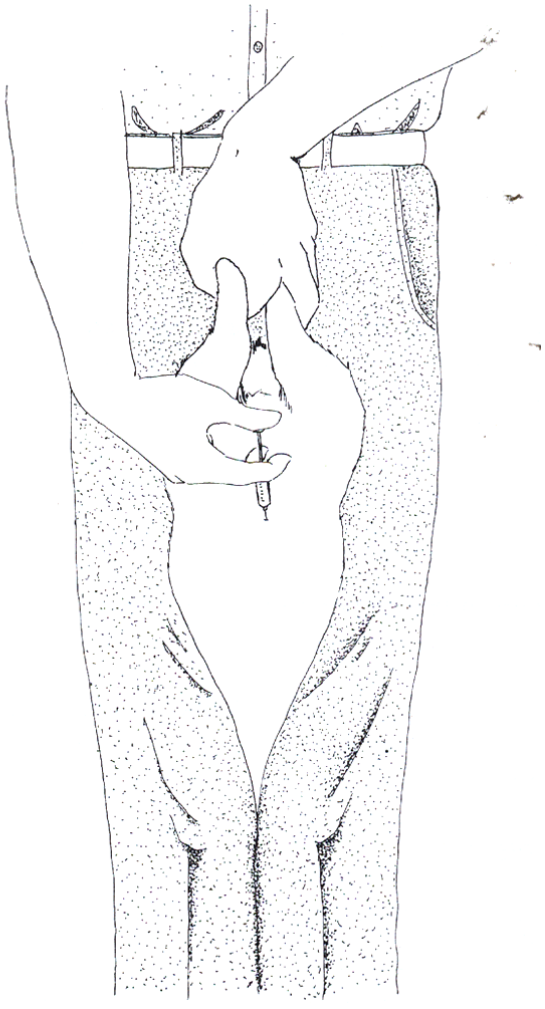


Fig. Restraint and intraperitoneal injection in the rabbit.

3) The rabbits hindquarters were held at a 30° to 45° angle to the horizontal.

4) The needle was inserted into the lower right abdominal quadrant just lateral to the midline and directed at an approximately 45° angle to the body wall.

5) The syringe plunger was gently pulled prior to injection to ensure that neither viscera nor blood vessels have been penetrated. For example, aspiration of yellow fluid implies that the needle has penetrated urinary bladder, while green fluid suggests that the intestinal tract has been penetrated. Unexpected contamination by such materials necessitates discarding the compound to be given and obtaining a fresh sample.

Intravascular Drug Administration

Intravascular administration was done slowly to avoid consequences of an unexpected adverse reaction. The common sites for intravascular administration included marginal ear vein or jugular vein. Before injection of the drug, the syringe plunger was pulled back slightly to confirm the presence of needle within the vessel which was checked by the presence of blood in the hub of the needle and the tip of the syringe shaft. Care was taken not to form a bleb or blister within the skin around the vessel which indicates that the needle is not within the blood vessel.

Behavioural Study

All the groups of rabbits were observed closely throughout the study period for their behavioural patterns, food intake, water intake and urination.

Body Weight Profile

Body weight profiles of all the groups of rabbits were measured prior to the start of experiment and extended to the entire experimental study. The changes in the body weight were recorded at weekly intervals.

Histological Procedure

In order to observe the deleterious effects of diabetes mellitus, induced by alloxan and streptozotocin, on different organs of the rabbits, 50% of all groups of rabbits were sacrificed for histological/histopathological study. The different organs *viz*., pancreas, kidneys, liver, lungs, heart, brain and gut of both diabetic and normal rabbits were processed for histological/hiostopathological study.

The rabbits were killed by injecting a blow of air, using a 5ml syringe, into the central ear vein which resulted in the sudden death of animals. The skins of the animals were incised along the ventral midline with the scalped blade beginning at the lower jaw and continuing along the midline caudally to the pubis. Using the scalpel, the skin was then gently reflected laterally and the subcutaneous tissues and underlying musculature was examined. The abdominal wall was then incised and the abdominal cavity exposed using the dissecting scissors.

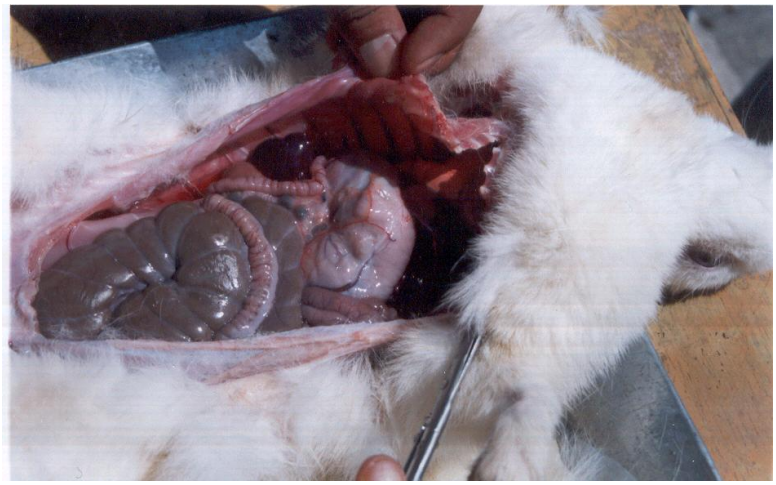


Fig. Incised abdomen with exposed viscera.

The organs and peritoneal surfaces were examined for abnormal colouration, presence of masses, traumatic damage or any other abnormal appearance.

The abdomen was then thoroughly explored so as to find the retroperitoneal location of pancreas. The pancreas is relatively inaccessible as its retroperitoneal location in the upper abdomen means that it is almost completely hidden by the stomach, transverse colon and mesocolon deriving its blood supply from numerous branches arising from major branches of the coeliac and superior mesenteric arteries and further the anatomical relationship of pancreas is summarized as, "the pancreas cuddles the left kidney, tickles the spleen, hugs the duodenum, cradles the aorta, opposes the inferior vena cava, dallies with the right renal pedicle, hides behind the posterior parietal peritoneum of the lesser sac and wraps itself around the superior mesenteric vessels" (Moossa, 1982). However, the pancreas was mobilized out of the retroperitoneum. It was then extracted and placed in normal saline.

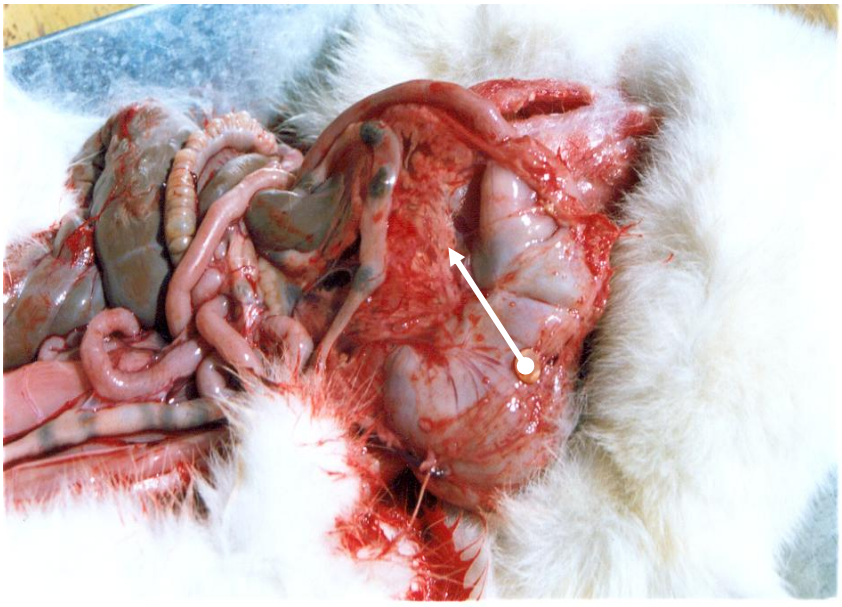


Fig. Photograph showing location of pancreas in situ

The other organs *viz*., kidneys liver and different parts of gut were removed and placed in normal saline so as to remove the adherent blood. The thoracic cavity was then exposed by cutting the diaphragm and then clipping

the ribs using the bone cutting forceps. The clipped portion of the rib cage was then lifted off and removed or reflected laterally. The lungs, heart and pleural surfaces were examined for abnormalities as for the abdominal cavity. The organs were removed by cutting the trachea and all attachments of trachea, lungs, and heart caudally to the diaphragm.

For removal of brain, the skin over the skull was reflected forward and was held firmly in position with one hand. With the tips of heavy jawed bone shares or strong scissors, a nip or cut was made just trough the bone to the cranial cavity on both sides of the head, beginning at the occipital foramen and proceeding forward laterally to the mid point at the anterior edge of the cranial cavity. The cut portion of the brain was lift off and the entire brain was exposed. The nerves and the attachments were cut carefully while the head was tipped upside down to collect the loosen brain. After collection, the brain was divided longitudinally into two halves with sharp scalpel.

After removing the organs from the animals, these were cut into desirable sizes with the help of sharp scalpel and were processed for histological/histopathological study using routine histological methods (Weesner, 1968; Luna, 1968). The different processing steps include:

Fixation

The tissues were immediately placed in Bouin's fluid or 10% neutral buffered formalin keeping the volume of fixatives 30 to 50 times the volume of the tissue. The procedure for preparation of these fixatives is as follows:

Bouin's Fluid

It is composed of:

Picric acid, saturated aqueous solution	750.0 ml
37–40 % formalin	250.0 ml
Glacial acetic acid	50.0 ml

The fixation was done for 4-12 hours or several days. After fixing the blocks/tissues in Bouin's solution, these were washed in several changes of 50% alcohol for 4-6 hours, agitating constantly, to ensure proper removal of the picric acid, and then stored in 70% alcohol.

10% Neutral Buffered Formalin

It is composed of:

Sodium dihydrogen phosphate, monohydrate ($Na_2H_2PO_4$-H_2O)	4.0 g
Sodium monohydrogen phosphate anhydrous (Na_2HPO_4)	6.5 g

After fixing the material in 10% neutral buffered formalin, it is washed in water or alcohol. A convenient stopping place is to bring the tissues, after washing in water, through 50% alcohol and then to 70% or 80% alcohol. At these alcohol concentrations tissues can be stored for several weeks or months without harming the tissue.

Post Fixation Treatment

After fixation, the excess fixative was washed out as recommended for the particular fixative used so as to prevent interference with subsequent processing of the tissue. As paraffin and water are not miscible, the tissues were first dehydrated and cleared, then infiltrated and embedded in paraffin.

Dehydration of the Tissues

Here the tissues were passed through different types of graded alcohol series (80%, 90%, 100% alcohol) keeping the volume of the alcohol 10 times the size of the tissue. The time/duration in each alcoholic concentration is given below in the flow chart:

Flow Chart for Dehydration

Step	Timing	Solution
1.	Holding point	8o% alcohol
2.	Two hours (1st change)	95% alcohol
3.	One hour (2nd change)	95% alcohol
4.	One hour (1st change)	100% alcohol
5.	One hour (2nd change)	100% alcohol
6.	One hour (3rd change)	100% alcohol

Clearing/Dealcoholization

The tissues after dehydration were cleared in xylene keeping the volume of the clearing agents 10 times the size of the tissues. The process of clearing with duration is as follows:

Step	Timing	Solution
7.	One hour (1st change)	Xylene
8.	One hour (2nd change)	Xylene

Infiltration or Impregnation

The tissues were then removed from the clearing agent, drained and placed in the first paraffin beaker in the paraffin for a period of 2 hours. The temperature of the paraffin oven was maintained at 60°C and the volume of the paraffin wax was kept 15 to 20 times the volume of the tissue. The tissue were then transferred to the second and third paraffin beakers for two hours and 1½ hours, respectively.

Step	Timing	Solution
9.	2 hours (1st change)	Paraffin
10.	2 hours (2nd change)	Paraffin
11.	1½ hours (3rd change)	Paraffin

Embedding or Casting or Blocking

Upon completion of infiltration, the tissues were removed from the tissue capsule and were transferred to a small container of freshly melted paraffin with warm forceps. Care was taken that the tips of forcep, while heating, might not get overheated which might result in the cooking of the tissue. The small container of paraffin was placed on the same paraffin oven with maintained temperature. Embedding paper boats or block holders were filled with molten paraffin. With the help of warm forceps, the tissues were picked up gently and placed into mold keeping the tissue towards the bottom of the mold and centered, leaving a margin of several millimeters around the tissue. Manipulation of the tissue in the mold with proper orientation was quick, so that paraffin did not begin to harden. The paraffin block was allowed to harden and then immersed into shallow, cool (10^{o}C) water bath for 10 to 15 minutes to hasten solidification of the paraffin. When the paraffin was completely hardened, it was then removed from mold and labelled with a code number.

Trimming of the Block

The paraffin block was carefully trimmed before attaching to the microtome peg with the help of a scalpel with a clean even edge, the excess paraffin was removed so as to maintain proper size of the block. The block now appeared to be transparent.

Mounting the Tissue Block on the Block Holder or Microtome Peg

The paraffin tissue block was attached to a base or peg for clamping into the microtome. The mounting was done by holding the block holder on the left

hand and a little wax was placed on the upper surface of block holder to which tissue was to be mounted. Spatula or backside of scalpel was heated and the wax placed on the block holder was evenly distributed. It was repeated till the surface coating of the block holder was about 1 mm thick. With the help of heated spatula, a central cavity was melted into the surface of the block holder and the trimmed tissue block was immediately placed into the cavity so that the front of the block (the face along which sections were to be to cut) is upper most, the tissue block was to be gently but firmly pressed down against the peg with the help of forefinger placed on top of the block. The tissue block was kept exactly perpendicular to the surface of the block holder. While setting the base, the block holder containing the tissue block was immersed in cold water in a beaker for complete setting.

A label with code number was attached to the block holder/peg by encircling it with a piece of cellophane tape.

Microtomy or Sectioning

The solid paraffin block containing the tissue was sliced into thin desired sections of 5 micron on a rotary microtome (WESWOX).

Before sectioning the paraffin block containing the tissue block was mounted on the microtome. Proper orientation of the block in the microtome was done. The thickness of the sections was achieved by setting the thickness adjustment scale at desired microns (5 microns). After checking all the parts of microtome, the drive wheel of the microtome was rotated and the subsequent sections of tissue in the form of smooth straight ribbons were picked up with the help of two clean 'camels hair' brushes.

Affixing or Attaching Sections to Slides

Before affixation, the slides were cleaned scrupulously. The sections of tissues were attached to these slides using Mayer's affixative whose composition is:

Mayer's Affixative

Egg albumin	:	50.0 ml
Glycerin	:	50.0 ml
Sodium salicylate	:	1 gm

A small drop of Mayer's egg albumin was smeared over the surface of the slide with the finger and the excess rubbed off. The tissue sections in the form of ribbons were placed in tissue floatation bath whose temperature was maintained according to the melting point of the paraffin wax and were picked on the center of the slides. The slides were dried on a slide-warming place. Using a diamond point pencil each slide was clearly labelled. After the desired number of sections were taken all blocks were sealed with paraffin wax so as to prevent drying and other damages of the exposed tissue/material.

Staining Procedure

Routine Haematoxylin and Eosin stain was used for histological/ histopathological study.

Specimen Preparation: 5 micron thick sections.

Reagents Required

1) Harris Haematoxylin

Haematoxylin crystals	- 5.0gm
Ethanol	- 50.0ml
Ammonium or Potassium Alum	- 100.0gm
Distilled water	- 1000.0ml
Mercuric oxide (red)	- 2.5gm

The haematoxylin in the alcohol was dissolved and the alum in the water by the aid of heat. The two solutions were mixed and brought to a boil as rapidly as possible. The heat was limited to less than one minute and stirred often. The solution was removed from heat and the mercuric oxide was added

slowly. It was reheated to a simmer until dark purple. The vessel was plunged into a basin of cold water until cool. 2-4ml of glacial acetic acid per 100 ml of solution was added to increase the precision of the nuclear stain and was filtered before use.

2) Eosin Solution

1% stock alcoholic eosin

Eosin y, water soluble	- 1.0gm
Distilled water	- 20.0ml

Dissolve and add:

Alcohol, 95%	- 80.0ml

Working Eosin Solution

Eosin stock Solution	- 1 part
Alcohol, 80%	- 3 parts

Just before use, 0.5ml of glacial acetic acid was added to each 100 ml of stain and stirred.

3) 1% Acid Alcohol

Acid Alcohol	- 1 ml
90% alcohol	- 100ml

4) 0.1% Ammonium Water

Ammonia	- 0.1ml
Distilled water	- 100ml

Routine Harris' Haematoxylin and Eosin Staining Method

1. Deparaffinization

Xylene I	- 15min.
Xylene II	- 15min.
Xylene III	- 15min.

2. Hydration

Absolute Alcohol I	- 5min.
Absolute Alcohol II	- 5min.
90% Alcohol	- 5min.

80% Alcohol	- 5min.
70% Alcohol	- 5min.
50% Alcohol	- 5min.
Distilled water	- 15min

3. Staining

Harris' Haematoxylin	- 7min.
Distilled water	- Rinse
Acid Alcohol (1%)	- one dip
Distilled water	- Rinse
Ammonia water (0.1%)	- Till optimum blue
Tap water	- wash
Distilled water	- Rinse
Eosin stain (Alcoholic)	- 4 minutes

4. Dehydration

95% alcohol	- 30 sec.
Absolute Alcohol I	- 30 sec.
Absolute Alcohol II	- 30 sec.
Absolute Alcohol III	- 30 sec.

5. Clearing

Xylene I	- 30min.
Xylene II	- 30min.
Xylene III	- 60min.

6. Mounting

Mounted in DPX mountant

In orders to demonstrate the different types of cells in the islets especially the beta cells, certain special stains were employed *viz*., "Rapid staining of Beta cell Granules in Pancreatic Islets" (Scott, 1952) and Gomoris Modified Stain (Halmi, 1952). The staining methods of these stains are given as:

Rapid staining of Beta cell Granules in Pancreatic Islets (Scott, 1952)

A modification of Gomori's staining technique (Gomori, 1950) was developed by Scott (1952) to demonstrate the pancreatic islets of the mouse. The method is more rapid and precise than Gomori's staining method.

Fixative: - Bouin's fluid

Reagents required

1) 0.5% Potassium Permanganate

Potassium permanganate	- 0.5gm
Distilled water	- 100.0ml

0.5% Sulphuric Acid

Sulphuric acid	- 0.5ml
Distilled water	- 100.0ml

Both the solutions were mixed in equal proportions.

2) 2% Sodium Bisulphite

Sodium bisulphite	- 1.0gm
Distilled water	- 100ml

3) Gomoris Aldehyde Fuchsin

Basic Fuchsin	- 1.0gm
Paraldehyde	- 2.0ml
Conc. Hcl	-1.0ml
Ethanol	- 60ml.
Distilled water	- 40ml.

The basic fuchsin was dissolved in the alcoholic distilled water. The hydrochloric acid and the paraldehyde were added and the solution was allowed to ripen for 2-7 days at room temperature, then filtered and stored at 4°C.

4) 0.5% Phloxine

Phloxine	- 0.5 gm
Distilled water	- 100 ml

5) 5% Phosphotungstic Acid

Phosphotungstic Acid	- 5 gm
Distilled water	- 100 ml

6) 0.2% Fast Green FCF

Fast Green FCF	- 0.2 gm
Distilled water	- 100 ml

Method

1) Bring deparaffinized sections to water.
2) Oxidize in a mixture of equal parts of 0.5% potassium permanganate and 0.5% sulphuric acid for 2 minutes.
3) Rinse in distilled water.
4) Decolourize in 2% sodium bisulphite
5) Wash under tap for 2 minutes
6) Stain in Gomori's aldehyde-fuchsin for 2 minutes.
7) Rinse in 3 changes of 95% alcohol.
8) Run sections down to water.
9) Stain in 0.5% phloxine for 2 minutes
10) Rinse in distilled water.
11) Place in 5% Phosphotungstic acid for 1 minute.
12) Wash under tap for 2 to 5 minutes.
13) Stain in 0.2% fast green FCF for 30 seconds.
14) Rinse in 95% alcohol for 15 seconds.
15) Place in absolute alcohol for 30 seconds.
16) Xylene (2 changes) and mount.

Result

After counterstaining by this method, the cytoplasmic background of the beta cells is stained light green in strong contrast to the deep purple granulation. The cytoplasm of the other cells in the islet and of any duct cells present is also stained light green, while nuclei and erythrocytes exhibit the light red colouration of the phloxine.

Modified Aldehyde Fuchsin Stain (Halmi, 1952)

Halmi in 1952 modified Gomoris aldehyde fuchsin stain and is summarized as:

Specimen Preparation

5 μ thick paraffin sections were cut from tissues fixed in Bouins fluid or 10% neutral buffered formalin.

Reagents Required

1) Lugol's Iodine

Iodine Crystals	- 1.0g
Potassium Iodide	- 2.0g

Made upto 100 ml with distilled water

2) Sodium Thiosulphate

Sodium thiosulphate	- 5.0gm
Distilled water	- 100.0ml

3) Aldehyde Fuchsin

Pararosaniline (CI 42500)	- 0.5gm
70% ethanol	- 100.0ml
Paraldehyde	- 1ml
Conc. Hcl	- 1ml

Pararosaniline in ethanol was dissolved. The paraldehyde and Hcl were added. The stain was allowed to ripen at room temperature for 3 to 5 days and then stored at 4°C.

4) Light Green/Orange G

Light Green SF yellowish (CI 42095)	- 0.2gm
Orange G (CI 16230)	- 1.0gm
Phosphotungestic Acid	- 0.5gm
Distilled water	- 100.0ml
Glacial Acetic Acid	- 1.0ml

5) Celestine Blue

Celestine blue B	- 2.5gm
Ferric ammonium sulphate	- 25.0gm
Glycerin	- 70cm^3
Distilled water	- 500cm^3

The ferric ammonium sulphate in cold distilled water were dissolved and stir well. The celestine blue to this solution was added, then the mixture was boiled for a few minutes. After cooling, the stain was filtered and the glycerin was added.

6) 0.2% Acetic Acid

Acetic Acid	- 2.0ml
Distilled water	- 100.0ml

Method

1) Dewax and rehydrate sections.
2) Place sections in Lugol's Iodine for 10 minutes.
3) Wash in water.
4) Decolourize with sodium thiosulphate for 2 minutes
5) Wash in water.
6) Rinse in 70% alcohol
7) Immerse sections in aldehyde Fuchsin staining solution for 15 – 30 minutes. Check staining microscopically.
8) Rinse in 95% ethanol.
9) Wash in water.
10) Stain nuclei with celestine blue or alum haematoxylin, differentiate and blue.
11) Rinse in distilled water.
12) Counterstain with light Green/Orange G for 45 seconds.
13) Rinse briefly in 0.2% acetic acid, then in 95% ethanol.
14) Dehydrate, clear and mount.

Result

Nuclei	-	Blue/Black
B-cell granules	-	Purple

A-cell granules	-	Yellow
D-cell granules	-	Green
Collagen	-	Green

The results obtained by this method were not satisfactory, as the stain did not differentiate different types of cells in islets of Langerhan's. So, a modification of the stain was done by substituting the lugol's iodine with equal parts of 0.5% $KMNO_4$ and 0.5 sulphuric acid, and sodium thiosulphate with 2% sodium bisulphite respectively. Further, the duration of treatment of sections was reduced from 45 seconds to 5 seconds in the counter satin (Light Green / Orange G). The protocol for staining is as:

1)	Xylene I	- 15min.
2)	Xylene II	- 15 min.
3)	Xylene III	- 15 min.
4)	100% alcohol I	- 5min.
5)	100% alcohol II	- 5min.
6)	90% alcohol	- 5min.
7)	80% alcohol	- 5min.
8)	70% alcohol	- 5min.
9)	50% alcohol	- 5min.
10)	Distilled water	- 15 min.
11)	0.5% $KMNO_4$ + 0.5%H_2SO_4	- 2min.
12)	Distilled water	- Rinse
13)	2% Sodium Bisulphite	- Decolorize
14)	Tape water	- 2min.
15)	70% alcohol	- Rinse
16)	Aldehyde Fuchsin	- 15-30min.
17)	95% alcohol	- Rinse
18)	Tap water	- wash
19)	Alum Haematoxylin	- 10min.
20)	Acetic Acid	- Rinse
21)	1% ammonium water	- Till optimum blue
22)	Tap water	- wash

23) Light Green / Orange G - 5 seconds
24) 0.2% Acetic acid - Rinse
25) 95% ethanol - Rinse
26) 100% ethanol - 5 minutes
27) Xylene I - 15 min
28) Xylene II - 30 min.
29) Xylene III - 30 min.
30) Mount in paramount/DPX mountant.

This method gave satisfactory results and the alpha and beta cells were qualitatively and quantitatively checked.

Photomicrography

The microscopic study of the stained tissue sections for histopathological study was done with the help trinocular microscope using different lens combinations. Photomicrographs were taken using PM-6.

Animals with Therapeutic Study on Experimentally Induced Diabetic Rabbits

During the course of present investigation the diabetic rabbits were given water extracts of *Abroma augusta* and *Syzygium jambolanum* orally so as to study the improvement with regard to biochemical, behavioural and histological changes. An Allopathic drug, glimepiride was also tested in this experimental study.

New Zealand white male healthy rabbits of 8-12 months old were selected for the study. The rabbits were purchased from Institute of Animal Health and Biological Products, Zakura Srinagar. Before the start of the experiment the animals were acclimatized to standard laboratory conditions for a period of 15 days. Fiber rich vegetables and commercially available pelleted diet was provided to rabbits in three divided doses daily.

Induction of Diabetes Mellitus

After acclimatization to standard laboratory conditions, the animals were made diabetic by intravenous administration of alloxan (Wasan *et al*., 1998).

For intravenous administration a single dose of alloxan @ 100mg/kg b.w. dissolved in 1 ml of sterile water was given after twelve hours of fasting. The fasting glucose levels above 250mg/dl of rabbits were considered for therapeutic studies. For this set of therapeutic experiments, the methods/ techniques (biochemical and histopathological), were same as elaborated in previous pages of this chapter.

Drug Treatment

1) ***Abroma augusta*:** The water extract of dried powder of leaves of *Abroma augusta* was purchased commercially from Dr. Wellman's Homeopathic Laboratory Pvt. Ltd. Wazirpur India. It was given orally to the diabetic animals @ 2 ml daily for a period of fifteen days.

2) ***Syzygium jambolanum:*** The water extract of *syzygium jambolanum* was purchased commercially from Dr. Wellman's Homeopathic Laboratory Pvt. Ltd. Wazirpur India. The rabbits received an oral dosage of *Syzygium jambolanum* @ 2 ml daily for a period of 15 days.

3) ***Glimepiride:*** One group of diabetic rabbits received allopathic drug, glimepiride @ 2mg/kg b.w. daily for a period of 15 days. The drug was dissolved in 5ml of sterilized water and the homogenous fluid was given.

Method of Oral Administration of Drugs

The oral administration of antidiabetic drugs included either oral gavage or administration by syringe.

Technique for Oral Gavage

1) The total length of the tube to be inserted was estimated as the length from the mouth to the last rib and was marked on the tube before insertion begun.

2) A speculum was placed in the rabbits mouth to prevent chewing of the tube. A small block of wood with a hole drilled in the middle allow passage of the tube was sufficient.

3) The tube (usually an infant feeding tube) was lightly lubricated with petroleum jelly.

4) The tube was passed through the speculum and back to the pharynx. When the rabbit demonstrated the gag reflex, the tube was advanced into the esophagus and on into the stomach.

5) The location of the tube in the stomach was confirmed by examining for a lack of air passage through the tube as the rabbit breath so as to avoid accidental administration of the drug to the respiratory tract.

6) Drugs were administered slowly by a syringe attached to the stomach tube. A small volume of water was subsequently administered to rinse any residual drug into the stomach.

7) After administration, the tube was kinked, to prevent flow of residual material in the tube into the respiratory tree as it passes through the pharynx, and the tube was slowly withdrawn.

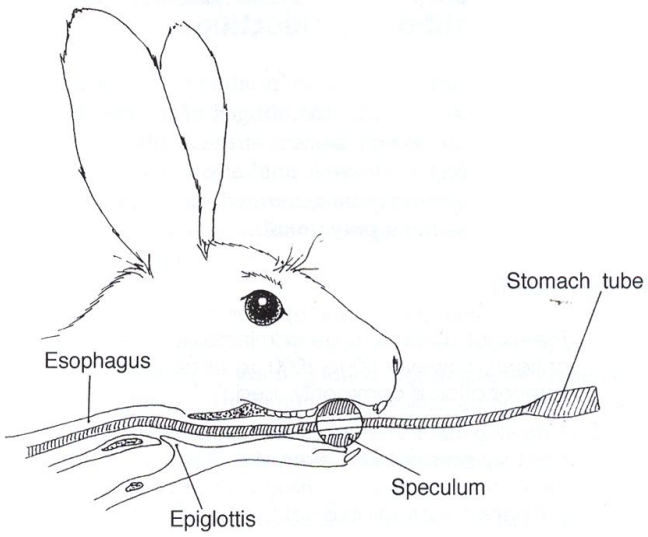


Fig. Orientation of the stomach tube for oral gavage in the rabbit.

Administration by Syringe

By this method the tip of the syringe was placed at the corner of the rabbits mouth and the material was slowly injected.

Grouping of Animals

Five groups of rabbits were selected for part II of the experiments. Group I comprised four normal healthy rabbits. Group II, III, IV and V comprised alloxanized diabetic rabbits of four animals each

- Group I served as normal control and received normal saline orally.
- Group II were untreated alloxanized diabetic rabbits and received normal saline orally.
- Group III alloxanized diabetic rabbits received water extract of *Abroma augusta* @ 2 ml daily.
- Group IV alloxanized diabetic rabbits received aqueous extract of *Syzygium jambolanum* @ 2 ml daily.
- Group V alloxanized diabetic rabbits received glimepiride @ 2mg/kg b.w.

Assessment of Treatment

The efficacy of drugs was based on the improvement of behavioural, biochemical and histopathological changes. Biochemical estimation was restricted to blood sugar (F), blood urea and serum creatinine. Biochemical values were estimated on day 7^{th}, day 15^{th} and day 21^{st}. After 21 days of treatment 50% of all groups of rabbits were sacrificed. Histological study was carried to the pancreas, kidneys, liver, heart, lungs, gut and brain so as to check the efficiency of drugs up to the tissue level.

Quantitative Assessment of Beta Cells

In all the groups of rabbits *viz*, control (saline treated healthy rabbits), diabetic-untreated (alloxan-induced and streptozotocin-induced diabetic rabbits) and diabetic-treated rabbits, the percentage of beta cells were counted. Cells of

approximately four islets on each tissue and forty islets of each group were counted under a light microscope at a magnification of ×100.

Statistical Analysis

Student's 't' test was used for statistical analysis of the data and value P was calculated for evaluating statistical significance. The value of 't' was calculated (Prasad, 2000) according to the following equation:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{SE_D}$$

Where, $\overline{X}_1$ = Mean of one variable (Diabetic)

$\overline{X}_2$ = Mean of second variable (Saline Control)

SE_D = Standard error of difference between two means

The standard error was obtained by using following formula:

$$SE_D = \sqrt{SE\overline{X}_1^2 + SE\overline{X}_2^2}$$

where, $SE\,\overline{X}_1$ = Standard error of the first mean

$SE\,\overline{X}_2$ = Standard error of the second mean

SE_M was obtained with the help of following formula:

$$SE_M = \frac{\sigma}{\sqrt{N-1}}$$

'P' value was obtained from the distribution of 't' probability chart.

The diabetes mellitus, induced in rabbits by administration of alloxan and streptozotocin, was confirmed by blood biochemical levels and behavioural changes in the animals. The biochemical changes in blood sugar (F) level, blood urea and serum creatinine were observed in both alloxan-induced and streptozotocin-induced groups of rabbits and compared with the values obtained from normal rabbits. Histomorphological alterations were observed in the different organs like pancreas, liver, alimentary canal, kidney, heart, lungs and brain.

Alloxan-Induced Diabetes Mellitus

The induction of diabetes mellitus in Group II rabbits, by intraperitoneal administration of four doses of alloxan @ 80 mg/kg. b.w., was observed after first week by increased values of blood sugar (F), blood urea and serum creatinine. These rabbits also exhibited changes in behaviour and pathoanatomical features.

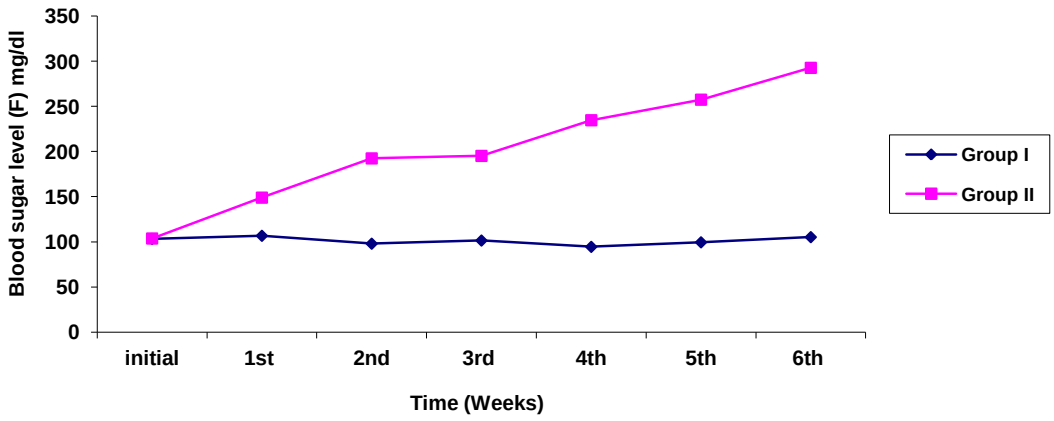
Behavioural Alterations

The behavioural changes included dullness, lethargy, decreased physical activity, polyuria, polydipsia and a tendency to lie down.

Biochemical Alterations

The biochemical levels showed a significant change in alloxan-induced diabetic rabbits (Group II) in comparison to control rabbits (Group I). The blood sugar (F) levels increased steadily upto sixth week reaching 292.75 ± 8.87 mg/dl compared to the values of Group I rabbits which remained almost constant at 105.25 ± 4.65 mg/dl till the end of the experiment (Fig.1). In the seventh week the blood sugar level was comparable to that of the sixth week but started to show fluctuations with a decreasing tendency thereafter.

Fig 1: Effect of Alloxan Administration on the Blood Glucose Level (F) of Rabbits.



The alterations in other biochemical parameters viz., blood urea and serum creatinine were also observed in the Group II rabbits which increased in parallel with blood sugar level. A significant increase of blood urea and serum creatinine from 21.08 ± 1.27 mg/dl to 53 ± 1.54 mg/dl and 1.52 ± 0.25 mg/dl to 3.32 ± 0.16 mg/dl respectively was observed in Group II rabbits in contrast to Group I rabbits which showed almost a consistency of 21.03 ± 1.00 mg/dl and 0.99 ± 0.09 mg/dl in blood urea and serum creatinine respectively up to the sixth week [Fig. 2 and 3]. Blood urea and serum creatinine levels were highest in 6^{th} week as with blood sugar level. Thereafter fluctuations were recorded.

Fig 2: Effect of Alloxan Administration on the Blood Urea of Rabbits.

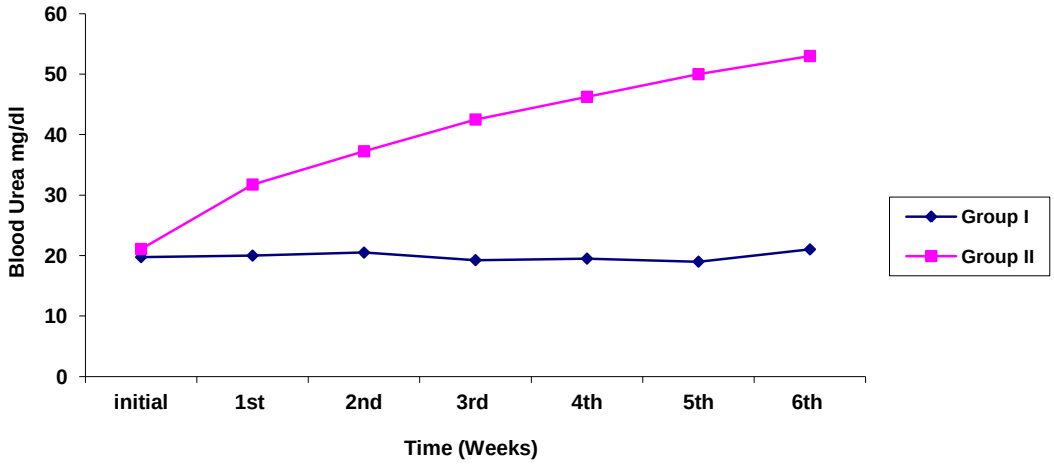
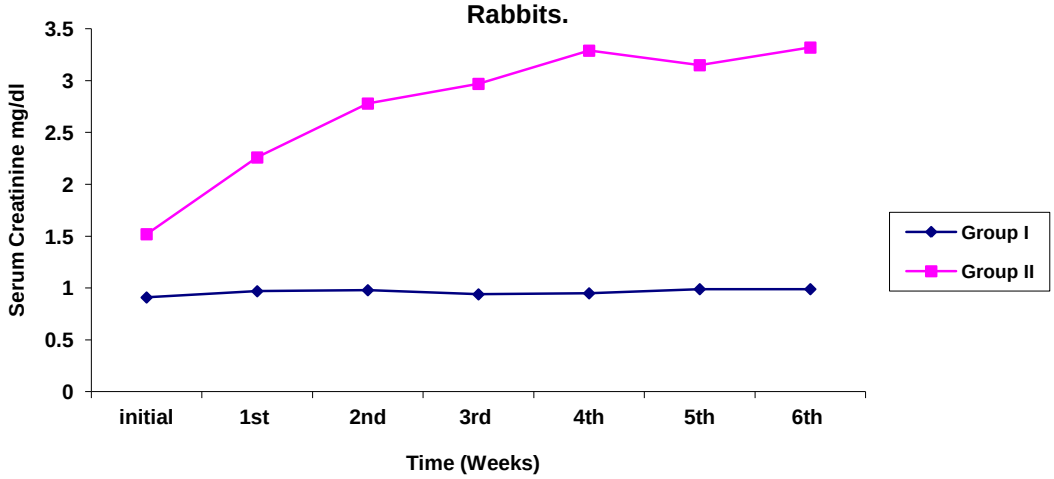


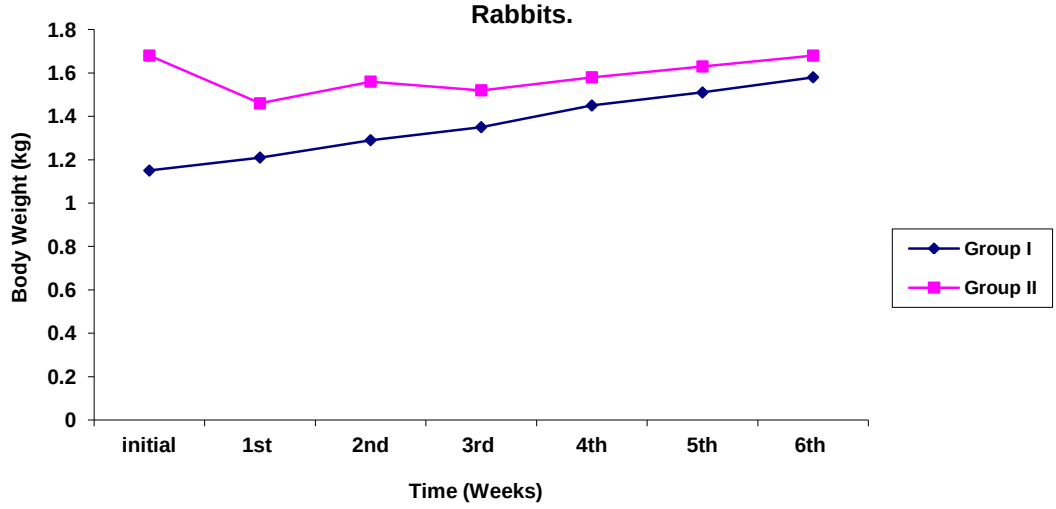
Fig 3: Effect of Alloxan Administration on the Serum Creatinine of Rabbits.



Body Weight Profile

The body weight of Group II rabbits decreased significantly in comparison to Group I rabbits which showed an increased body weight throughout the experimental period (Fig. 4). In Group II rabbits the rapid decrease in body weight was recorded after the first week of alloxan administration from 1.68 ± 0.11 kg to 1.46 ± 0.02 kg. Later on there was a fluctuations and on the sixth week it was 1.65 ± 0.02 kg. However, in Group I rabbits, there was a steady and significant increase in body weight from 1.15 ± 0.09 kg to 1.58 ± 0.05 kg. The statistical evaluation of the data showed a level of significance ($p<0.50$).

Fig 4: Effect of Alloxan Administration on the Body Weight of Rabbits.



A significant increase of blood sugar (F), blood urea and serum creatinine levels starting from one week that peaked in sixth week (Table I). The comparative changes of Group I and Group II rabbits showed a level of significance ($p < 0.001$) for blood sugar, blood urea and serum creatinine where as ($p < 0.50$) for body weight.

Histomorphological Changes on Alloxan-Induced Diabetic Rabbits

The histological study of different organs viz., pancreas, kidneys, liver, alimentary canal, lungs, heart and brain of Group II rabbits showed pathoanatomical features in contrast to Group I rabbits which showed normal histomorphological features.

Histomorphological Changes of Pancreas

Pancreatic sections stained with Haematoxylin and Eosin showed that alloxan caused severe necrotic changes of pancreatic islets, vacuolation, increased eosinophilia, islet congestion and pancreatic congestion. However, chronic pancreatitis, haemorrhage and proliferation of fibroblasts in some pancreatic lobules and disorganization of pancreatic acini were observed in pancreatic sections of five month old Group II rabbits. Using modified Gomori's aldehyde fuchsin staining techniques (Halmi, 1952 and Scott, 1952)

for pancreatic sections, nuclear changes, karyolysis, disappearing of nucleus and rarefaction of nuclear contents were visible. The reduction in the number of β cells of diabetic rabbits was obvious (Fig. 84, p. 95). These histomorphological changes [Fig. 5 to 13, pp. 99-103] of Group II rabbits were significant in comparison to Group I rabbits which showed normal histomorphology [Fig. 14 to 16, pp. 103-104].

Histomorphological Changes of Kidneys

Histological examination of the sections of kidneys of Group II rabbits showed degenerative changes. Nephrosis, occlusion of tubules, lower nephron nephrosis and degeneration in cortex, subcapsular region, collecting tubules and tubular epithelium. However, in five months old Group II rabbits, the kidney sections showed chronic nephritis, interstitial nephritis, tubular nephrosis and chronic changes in medullary sites. The histomorphologic changes [Fig. 17 to 23, pp.105-108] in Group II rabbits were significant in contrast to Group I rabbits which showed normal morphology of tissues [Fig. 24, p.108 and 25, p.109].

Histomorphological Changes of Liver

The liver sections of group II rabbits showed degenerative changes in comparison to Group I rabbits with normal morphological features. Biliary hyperplasia and hepatosis (degeneration of hepatocytes) was observed in alloxan-induced diabetic rabbits [Fig. 26, p.109 and 27, p.110]. However, in five month old Group II rabbits the liver sections showed changes of chronic hepatitis [Fig. 28, p.110]. The changes were significant in contrast to control rabbits (Fig. 29, p.111).

Histomorphological Changes of Heart

Heart sections stained with H&E in Group II rabbits showed edema and histiocyte proliferation at certain places [Fig. 30, p.111] in contrast to Group I rabbits with normal histological features [Fig. 32, p.112]. However, in five

months old group II rabbits, the heart sections showed myocarditis or inflammation of cells [Fig. 31, p.112].

Histomorphological Changes of Brain

Haematoxylin and Eosin stained brain sections of Group II rabbits showed degenerative changes in neurons and purkinji cells in cerebellum, and brain edema [Fig. 34, p.113 and 35, p.114] in contrast to normal histologic sections [Fig. 36, p.114] of Group I rabbits. Moreover, cerebellum showed edema in brain sections of 5 months old Group II rabbits [Fig. 33, p.113].

Histomorphological Changes of Lungs

The lung sections of Group II rabbits stained with H&E showed edema, haemorrahage and bronchial hyperplasia [Fig. 37 to 39, pp.115-116] in contrast to Group I rabbits (Fig. 41, p.117). Further, emphysema (breakdown of alveoli) of lungs was observed in five months old Group II rabbits [Fig. 40, p.116].

Histomorphological Changes of Alimentary Canal

Haematoxylin and Eosin stained sections taken from different parts of alimentary canal of Group II rabbits showed intestinal congestion and mild gastritis [Fig. 42, p.117 and 43, p.118] in contrast to normal histomorphology of Group I rabbits [Fig. 44, p.118].

Streptozotocin-Induced Diabetes Mellitus

The induction of diabetes mellitus in Group IV rabbits by intravenous administration of streptozotocin @ 65 mg/kg b.w. was confirmed biochemically on day 2nd of the experiment. Further, streptozotocin-induced diabetic rabbits showed a change in behaviour in comparison to saline treated normal rabbits. The histomorphological changes were also observed in different organs of the diabetic rabbits.

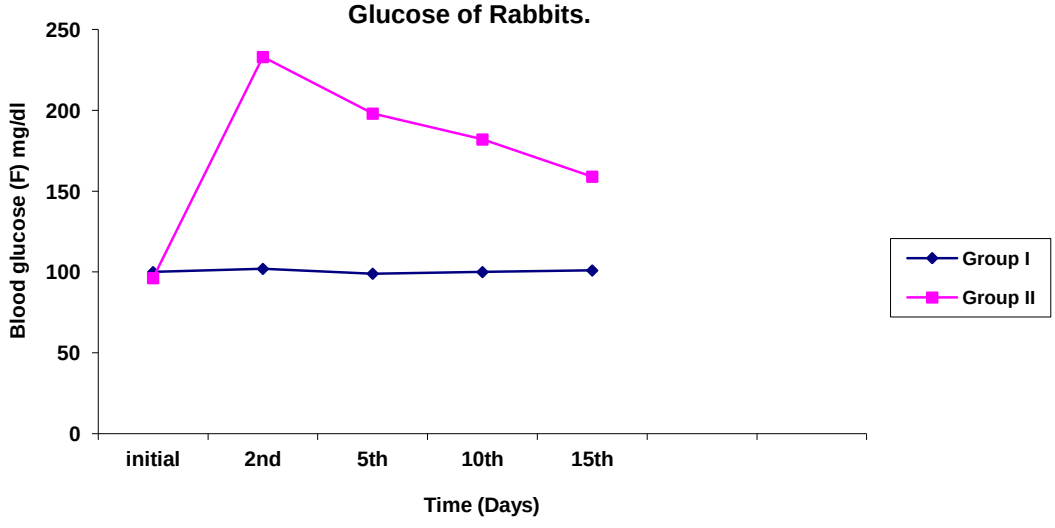
Behavioural Changes

The streptozotocin-induced diabetic rabbits (Group IV) exhibited excessive thirst, frequent urination and decreased physical activity in comparison to Group III rabbits, which showed usual behavioural patterns.

Biochemical Changes

The streptozotocin-induced diabetic rabbits (Group IV) showed alterations in biochemical parameters viz., blood sugar (F), blood urea and serum creatinine in comparison to saline treated (Group III) rabbits. The blood sugar (F) level was highest on day 2nd in Group IV after streptozotocin administration with a mean value of 233.25 ± 9.17 mg/dl followed by a decreasing trend (Fig. 45). However, the values of blood sugar level on day 5th, day 10th and day 15th were significant in Group IV rabbits in comparison to Group III rabbits.

Fig 45: Effect of Streptozotocin Administration on the Blood Glucose of Rabbits.



Blood urea and serum creatinine level of Group IV rabbits showed alterations with highest values on day 2nd recorded to be 39.75 ± 0.84 mg/dl and 2.25 ± 0.32 mg/dl respectively in contrast to Group III rabbits which showed normal values during the entire experimental period. However, these values

later on started to show decline and were consistent with blood sugar level [Fig. 46 and 47].

Fig 46: Effect of Streptozotocin Administration on the Blood Urea of Rabbits.

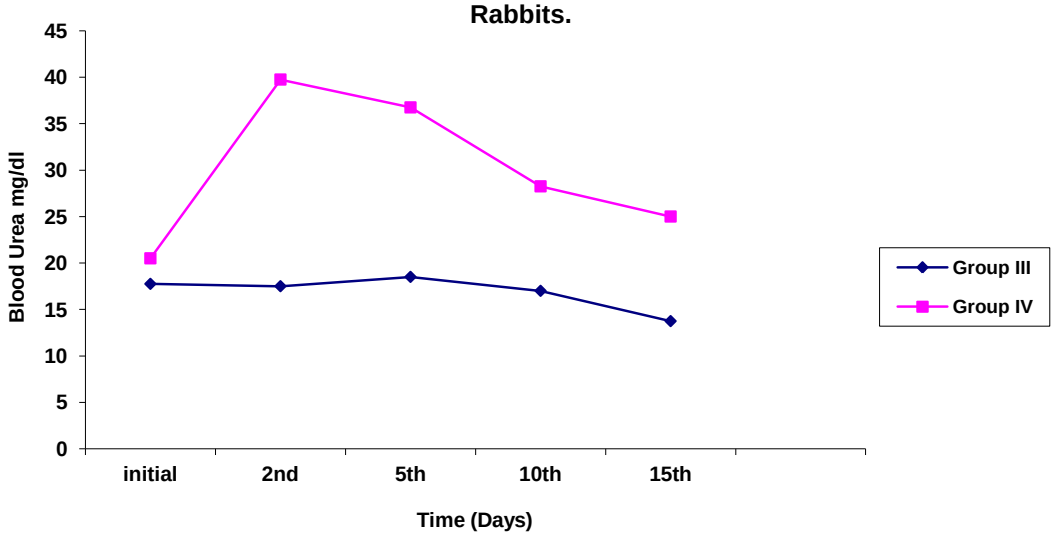
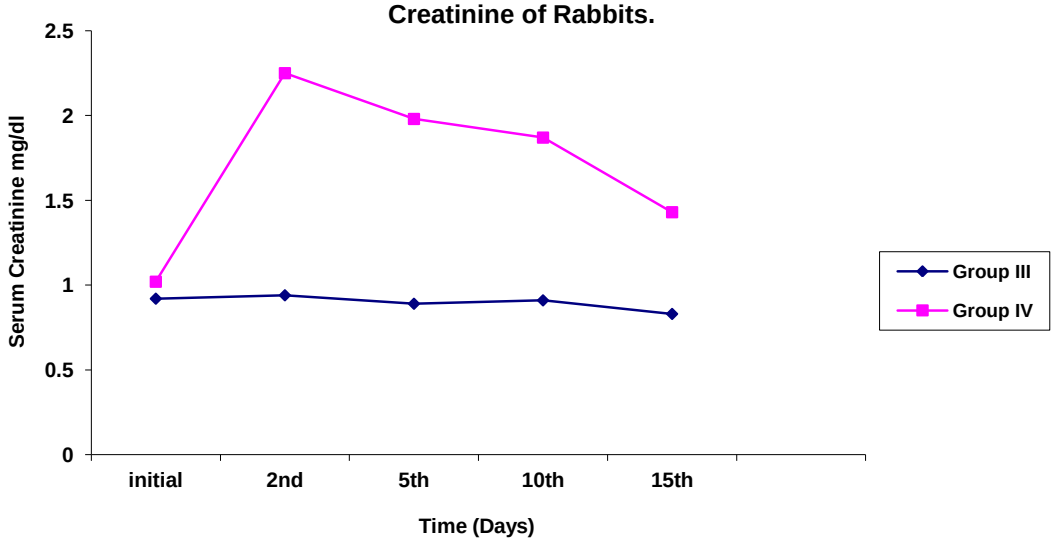


Fig 47: Effect of Streptozotocin Administration on the Serum Creatinine of Rabbits.

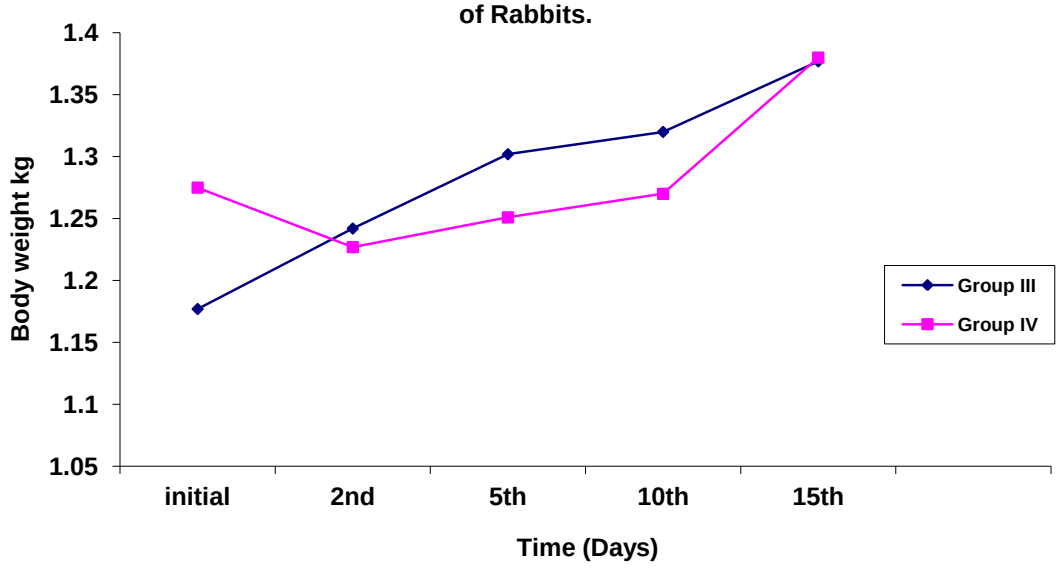


A significant value of blood sugar (F), blood urea and serum creatinine starting after streptozotocin administration showed a level of significance ($p< 0.01$) for blood sugar and ($p< 0.10$) for blood urea and serum creatinine in comparison to saline treated (Group III) healthy rabbits (Table II).

Body Weight Profile

The body weight of Group IV rabbits started to fall on day 2nd which was recorded to be 1.227 ± 0.10 kg from an initial value of 1.275 ± 0.14 kg in comparison to Group III rabbits which showed an increased trend throughout the experimental period. Later on, there was an increase in the body weight of Group IV rabbits and in the day 15th it was recorded to be 1.380 ± 0.06 kg almost similar to 1.377 ± 0.06 kg for Group III rabbits (Fig. 48). However, statistical evaluation of the data did not show any significance.

Fig 48: Effect of Streptozotocin Administration on the Body weight of Rabbits.



Histomorphological Changes of Streptozotocin-induced Diabetic Organs

Haematoxylin and Eosin stained sections of different organs of streptozotocin-induced diabetic rabbits (Group IV) showed histopathological features in contrast to saline-treated (Group III) healthy rabbits.

Pancreatic sections of group IV rabbits showed slight congestion and mild degenerative changes in the acini. The acinar epithelium was swollen. The islets of Langerhan's revealed decreased cellularity and in some islets the cells

appeared to be fusiform. However, using modified Gomori's aldehyde fuchsin stains (Halmi, 1952) for quantitative analysis of beta cells, their number was found to be reduced in Group IV rabbits in contrast to Group III rabbits (Fig. 84, p.95). The histopathological changes in Group IV rabbits [Fig. 49 to 51, pp.119-120] were found to be significant in comparison to Group III rabbits.

The lung sections of Group IV showed congestion and haemorrhage in alveoli and bronchioles [Fig. 52, p.120 and 53, p.121], congestion in kidneys [Fig. 54, p.121], degeneration and congestion in liver [Fig. 55, p.122], haemorrhages and myopathy in heart [Fig. 56, p.122] and mild neuronal damage was observed in the brain [Fig. 57, p.123] sections of Group IV rabbits. However, H&E stained sections of alimentary canal revealed no pathological features but the stomach stained sections showed proliferation of yeasts [Fig. 58, p.123].

Effect of *Syzygium jambolanum* on Diabetic Rabbits

The extract of *Syzygium jambolanum* given by oral administration was effective in improving behavioural, biochemical and histopathological alterations in alloxan induced diabetic rabbits.

Effect of *Syzygium jambolanum* on Behavioural Patterns

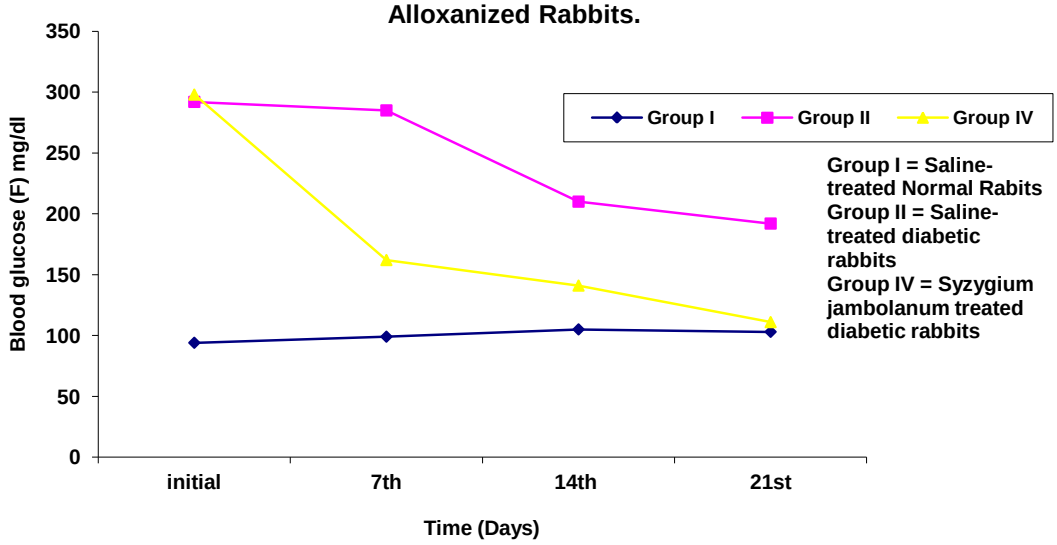
The *Syzygium jambolanum* treated diabetic rabbits (Group IV) showed a significant improvement in behaviour in contrast to alloxan-induced diabetic (Group II) which exhibited polyuria, polydipsia, lethargy, dullness and a tendency to lie down. Further, Group IV rabbits showed comparatively active behaviour.

Effect of *Syzygium jambolanum* on Biochemical Patterns

The biochemical levels showed a significant improvement in Group IV rabbits in comparison to Group II rabbits. The blood sugar of Group IV rabbits decreased steadily upto 21st day reaching 111 ± 4.43 mg/dl in contrast Group II

rabbits with a level of 192 ± 9.41 mg/dl. A decrease of blood sugar from 298 ± 7.90 mg/dl to 111 ± 4.43 mg/dl upto 21st day was observed during the experimental period. The blood sugar level of Group IV rabbits was almost comparable to Group I rabbits (Fig. 59) on day 21st.

Fig 59: Effect of Syzygium jambolanum on the Blood Glucose of Alloxanized Rabbits.



Blood urea and serum creatinine levels decreased consistently in Group IV rabbits. A significant decrease of blood urea and serum creatinine from 49.75 ± 1.31 mg/dl and 3.10 ± 0.10 mg/dl to 22 ± 0.91 mg/dl and 1.75 ± 0.08 mg/dl respectively was observed upto 21st day in Group IV rabbits in contrast to Group II rabbits which showed a fluctuation during the entire period and in the 21st day the values were 40.1 ± 1.16 mg/dl and 2.80 ± 0.14mg/dl respectively. The improvement in blood urea and serum creatinine levels in group IV rabbits was almost comparable to Group I rabbits [Fig. 60 and 61].

Fig 60: Effect of Syzygium jambolanum on the Blood Urea of Alloxanized Rabbits.

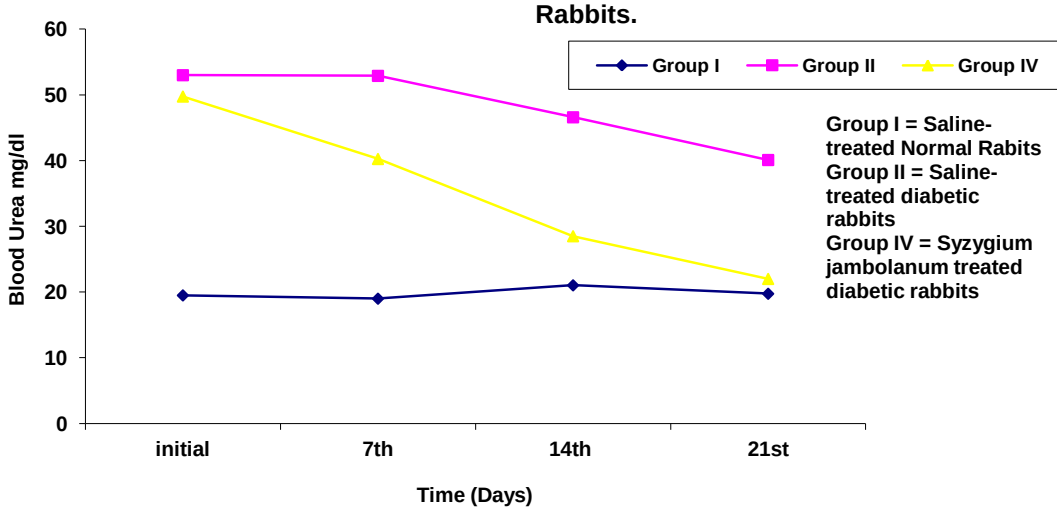
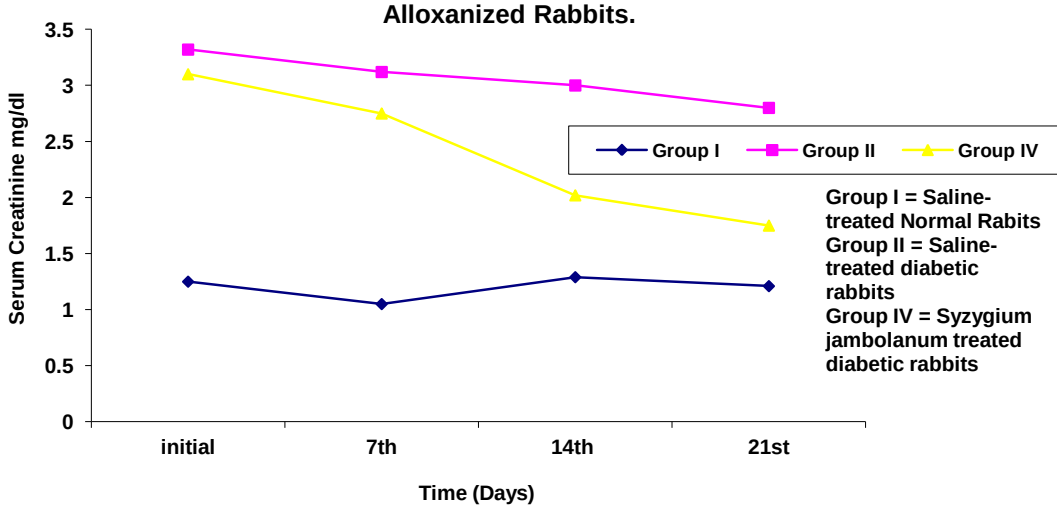


Fig 61: Effect of Syzygium jambolanum on the Serum Creatinine of Alloxanized Rabbits.



An improvement in blood sugar, blood urea and serum creatinine in Group IV rabbits was comparable to Group I rabbits and was significant in contrast to Group II rabbits (Table III).

Effect of *Syzygium jambolanum* on Histomorphological changes of Alloxan-induced Diabetic rabbits

Syzygium jambolanum treated alloxanized diabetic rabbits showed amelioration of histomorphological changes in contrast to Group III rabbits. Haematoxylin and Eosin stained sections of pancreas of Group IV rabbits showed almost normal islets. Using modified Gomori's aldehyde fuchsin stain (Halmi, 1952) the less number of beta cells was observed in Group IV rabbits compared to Group I rabbits. However, the number of beta cells in Group IV rabbits was significant in contrast to Group II rabbits (Fig. 62, p.124 and 84, p.95). The kidney sections of Group IV rabbits showed normal tubular epithelium, normal collecting tubules but slight degenerative changes in tubules [Fig. 63, p.124 and 64, p.125] in comparison to Group I rabbits. Congestion and degenerative changes [Fig. 65, p.125] in liver and slight congestion [Fig. 66, p.126] in lungs of Group IV rabbits were observed. However, H&E stained sections of heart, brain and alimentary canal of Group IV rabbits were normal comparable to Group I rabbits. The slight changes in pancreas, kidneys, liver and lungs were significant in contrast to Group II rabbits.

Effect of *Abroma augusta* on Diabetic Rabbits

The extract of *Abroma augusta* given orally to alloxan-induced diabetic rabbits showed an improvement in behavioural, biochemical and histomorphological alterations.

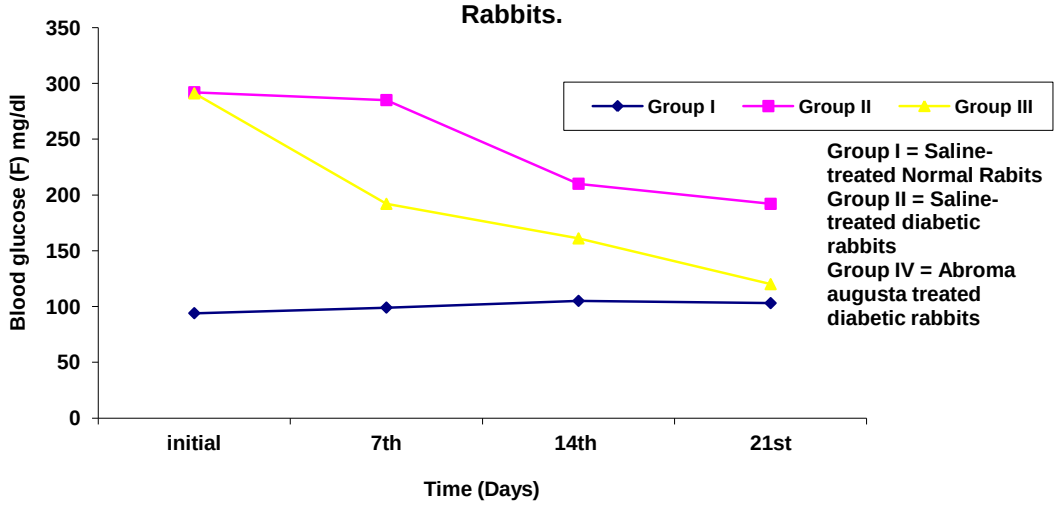
Effect of *Abroma augusta* on behavioural patterns

Alloxan-induced diabetic rabbits (Group III) showed improvement in behaviour comparable to saline treated normal healthy rabbits (Group I). The behavioural improvement was significant in contrast to saline treated alloxanized diabetic rabbits (Group II).

Effect of *Abroma augusta* on Biochemical parameters

The biochemical parameters viz. blood sugar (F), blood urea and serum creatinine levels showed a significant improvement in Group III rabbits by the oral administration of *Abroma augusta*. The blood sugar level of Group III rabbits decreased consistently from an initial value of 291 ± 10.50 mg/dl to 120.25 ± 3.89 mg/dl upto 21st day. The blood sugar level of Group III rabbits was almost comparable to Group I rabbits and significant to Group II rabbits (Fig. 67).

Fig 67: Effect of Abroma augusta on the Blood Glucose of Alloxanized Rabbits.



Blood urea and serum creatinine levels in Group III rabbits decreased from an initial values of 46.75 ± 1.65 mg/dl and 3.10 ± 0.14 mg/dl to 24.75 ± 0.62 mg/dl and 1.93 ± 0.13 mg/dl respectively upto 21st day of the treatment. These values were significant in contrast to Group II rabbits which showed a value of 36.1 ± 1.16 mg/dl and 2.11 ± 0.14 mg/dl respectively and almost comparable to Group I rabbits [Fig. 68 and 69].

Fig 68: Effect of Abroma augustaon the Blood Urea of Alloxanized Rabbits.

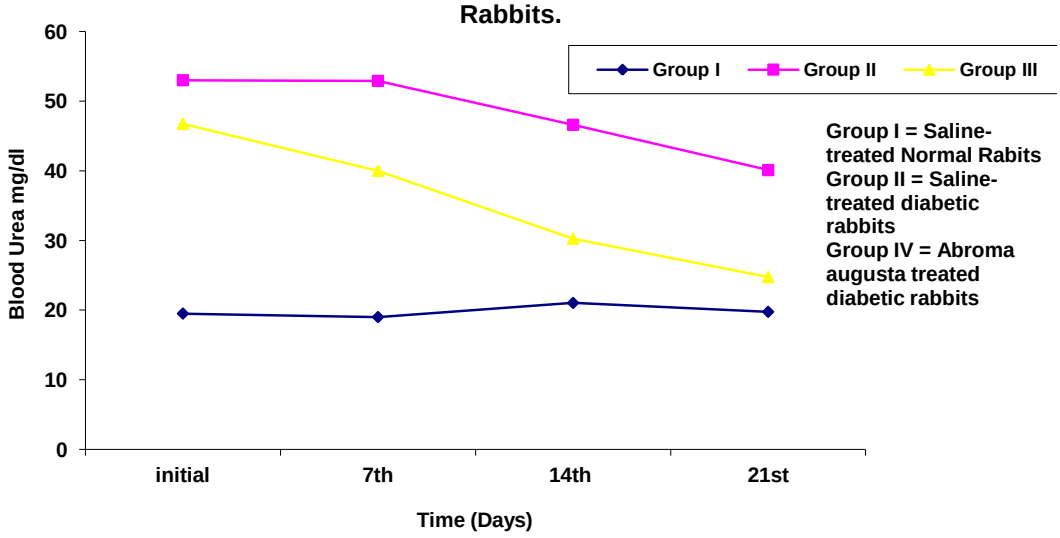
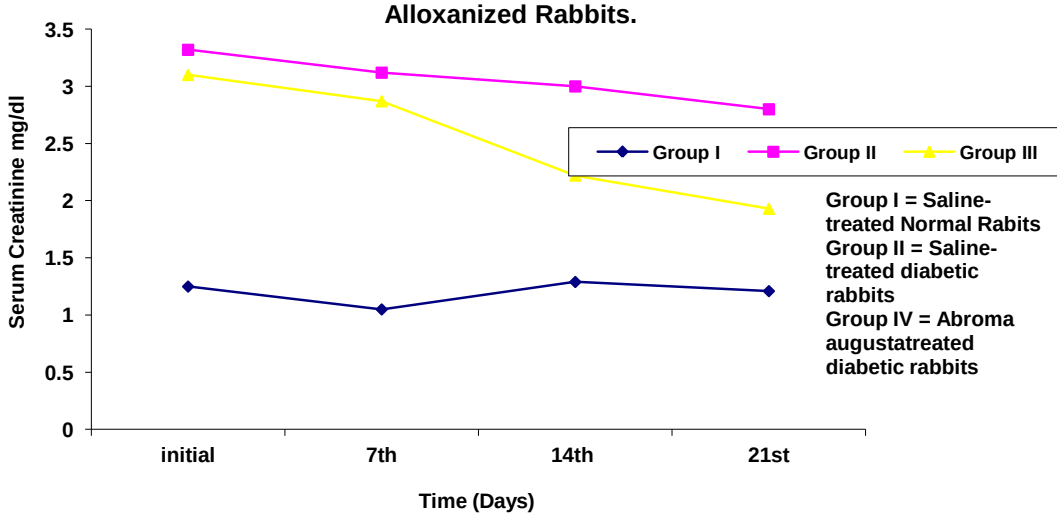


Fig 69: Effect of Abroma augustaon the Serum Creatinine of Alloxanized Rabbits.



An improvement of blood sugar, blood urea and serum creatinine was thus observed in Group III rabbits in contrast to Group II rabbits (Table III). The comparative changes in bio-chemical parameters of Group I, Group II and Group III rabbits showed a level of significance.

Effect of *Abroma augusta* on Histomorphology of Diabetic Rabbits

Haematoxylin and Eosin stained sections of pancreas of Group III rabbits showed comparatively normal histomorphology in contrast to Group II

rabbits. However, using modified Gomori's aldehyde fuchsin stain (Halmi, 1952) the number of beta cells were found to be less in comparison to Group I rabbits but significant in contrast to Group II rabbits (Fig. 70, p.126 and 84, p.95). The other organs stained with Haematoxylin and Eosin in Group III rabbits showed congestion and mild degenerative changes [Fig. 71, p.127] in kidneys, congestion and haemorrhage [Fig. 72, p.127] in lungs, degenerative changes [Fig. 73, p.128] in liver and haemorrhage in subendocardial portion [Fig. 74, p.128] in heart. However, alimentary canal and brain sections of Group III rabbits showed normal histomorphology.

Effect of Glimiperide on Diabetic Rabbits

The efficacy of Glimiperide given orally @ 2 mg/kg body weight to alloxan-induced diabetic rabbits showed improvement in diabetic complications with regard to behavioural, biochemical and histomorphological alterations.

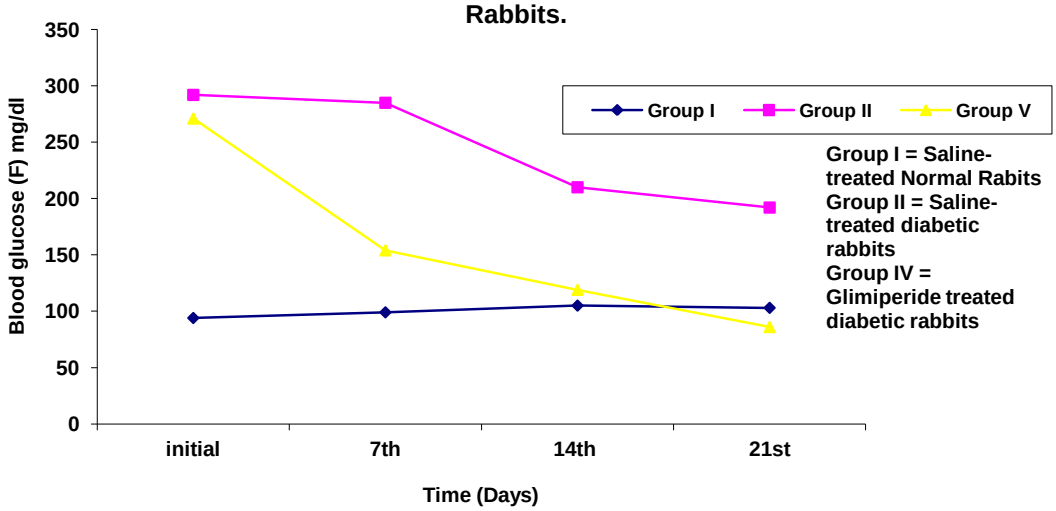
Effect of Glimiperide on Behaviour

The Glimiperide treated diabetic rabbits (Group V) showed a significant improvement in behaviour in contrast to alloxan-induced diabetic (Group II) rabbits which exhibited polyuria, polydipsia, lethargy, dullness and a tendency to lie down. Further, Group V rabbits showed an active behaviour comparable to saline-treated normal (Group I) rabbits.

Effect of Glimiperide on Biochemical Patterns

The biochemical levels showed a significant improvement in Group V rabbits in comparison to Group II rabbits. The blood sugar level of Group V rabbits decreased steadily upto 21^{st} day reaching 86 ± 8.39 mg/dl in contrast to Group II rabbits with a level of 192 ± 9.41 mg/dl. A decrease of blood sugar from 271 ± 9.11 mg/dl to 86 ± 8.39 mg/dl upto 21^{st} day was observed during the experimental period. The blood sugar level of Group V rabbits was almost comparable to Group I rabbits (Fig. 75).

Fig 75: Effect of Glimiperide on the Blood Glucose of Alloxanized Rabbits.



Blood urea and serum creatinine levels decreased consistently in Group V rabbits. A significant decrease of blood urea and serum creatinine from 46.25 ± 1.54 mg/dl and 3.13 ± 0.08 mg/dl to 18.75 ± 0.84 mg/dl and 0.98 ± 0.05 mg/dl respectively was observed upto 21st day in Group V rabbits in contrast to Group II rabbits which showed a fluctuation during the entire period and in the 21st day the values were 40.1 ± 1.16 mg/dl and 2.80 ± 0.14 mg/dl respectively. The improvement in blood urea and serum creatinine levels in Goup V rabbits was almost comparable to Group I rabbits [Fig. 76 and 77].

Fig 76: Effect of Glimiperide on the Blood Urea of Alloxanized Rabbits.

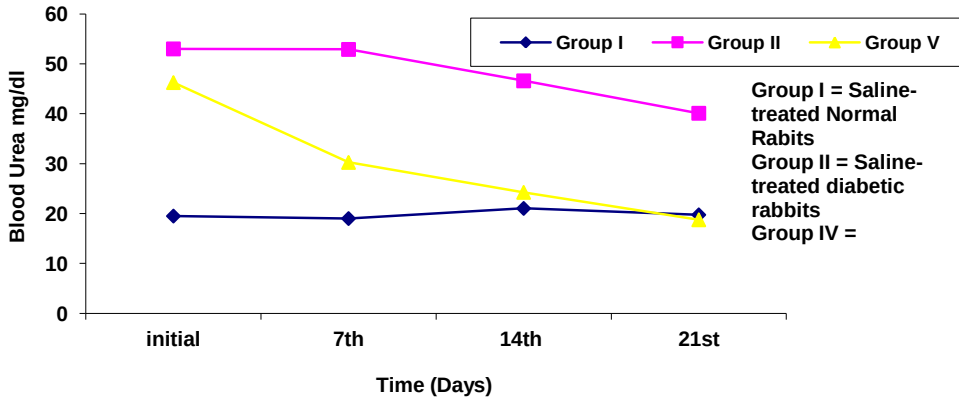
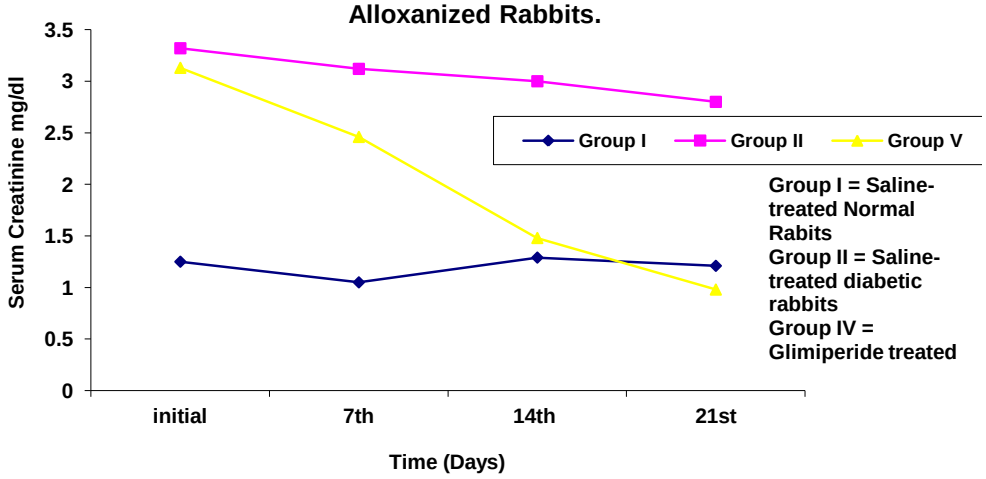


Fig 77: Effect of Glimiperide on the Serum Creatinine of Alloxanized Rabbits.



Effect of Glimiperide on Histomorphological Alterations

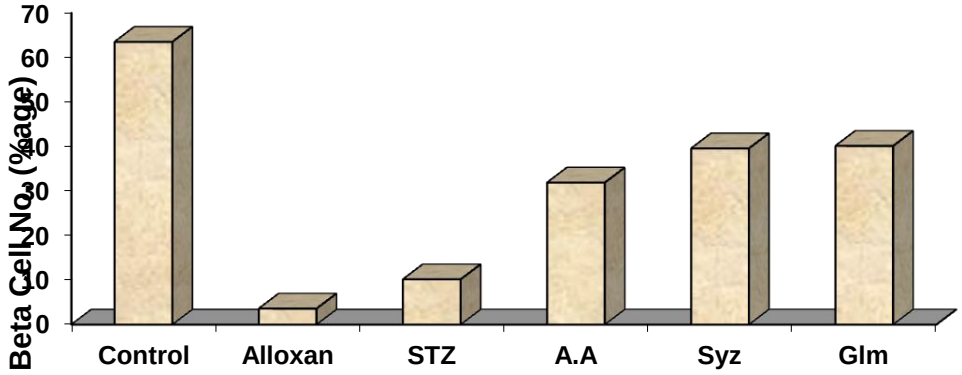
Haematoxylin and Eosin stained sections of different organs of Group V rabbits showed amelioration in histomorphological alterations when compared to Group II rabbits. The pancreatic sections showed normal islets (Fig. 78, p.129) but using modified Gomori's aldehyde fuchsin stain (Halmi, 1952) the number of beta cells was found to be less than Group I rabbits but significant to Group II rabbits (Fig. 79, p.129 and 84, p.95). The other H&E stained sections of Group V rabbits showed mild degenerative changes [Fig. 80, p.130] in kidneys, haemorrhage in heart [Fig. 81, p.130], congestion and haemorrhage [Fig. 82, p.131] in lungs and mild congestion but regenerative hepatocytes [Fig. 83, p.131] in liver. These histomorphological changes were significant in comparison to Group II rabbits. However, brain and alimentary canal of Group V rabbits did not show any histopathology.

Quantitative Study of Beta Cells

Histological examination of the pancreatic islets in all groups of rabbits using modified Gomori's aldehyde fuchsin (Halmi, 1952) showed that majority of the beta cells in alloxan-induced diabetic rabbits were greatly shrunken and

even some cells coalesced into almost homogenous debris in which individual cells could not be recognized. In streptozotocin-induced diabetic rabbits the number of beta cells were comparatively more as compared to alloxan-induced diabetic rabbits. However, in both the groups of rabbits the number of beta cells was reduced when compared with saline-treated (control) rabbits. The other groups of rabbits viz, diabetic-treated showed a higher percentage of beta cells than untreated groups of rabbits. The relative percentage of beta cells in different groups of rabbits is given in figure 84.

Fig 84: Comparative percentage of beta cells in different groups of rabbits



Control	=	Saline-treated healthy rabbits
Alloxan	=	Alloxan-induced diabetic rabbits
STZ	=	Streptozotocin-induced diabetic rabbits
A.A	=	*Abroma augusta* treated alloxanized diabetic rabbits
Syz	=	*Syzygium jambolanum* treated alloxanized diabetic rabbits
Glm	=	Glimiperide treated alloxanized diabetic rabbits

TABLE-I: Changes in Blood Glucose (F), Blood Urea, Serum Creatinine and Body Weight Profile of Alloxan-induced Diabetic Rabbits

Parameters	Initial Value		WEEKS											
			1st		2nd		3rd		4th		5th		6th	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Blood Glucose (F) (mg/dl)	103.25 ± 3.19	103.75 ± 3.63	106.75 ± 4.71	149 ± 6.26	98 ± 5.18	192.5 ± 6.88	101.5 ± 4.97	195.25 ± 7.41	94.5 ± 5.28	234.75 ± 8.68	99.5 ± 4.80	257.5 ± 9.19	105.25 ± 4.65	292.75 ± 8.87*
Blood Urea (mg/dl)	19.75 ± 0.84	21.08 ± 1.27	20 ± 1.07	31.75 ± 1.17	20.5 ± 1.12	37.25 ± 1.69	19.25 ± 1.17	42.5 ± 1.04	19.5 ± 0.64	46.25 ± 1.43	19 ± 0.89	50 ± 1.08	21.03 ± 1.00	53 ± 1.54*
Serum Creatinine (mg/dl)	0.91 ± 0.04	1.52 ± 0.25	0.97 ± 0.05	2.26 ± 0.13	0.98 ± 0.46	2.78 ± 0.18	0.94 ± 0.08	2.97 ± 0.13	0.95 ± 0.09	3.29 ± 0.14	0.99 ± 0.10	3.15 ± 0.11	0.99 ± 0.09	3.32 ± 0.16*
Body Weight (Kg)	1.15 ± 0.09	1.68 ± 0.11	1.21 ± 0.07	1.46 ± 0.02	1.29 ± 0.06	1.56 ± 0.04	1.35 ± 0.07	1.52 ± 0.02	1.45 ± 0.07	1.55 ± 0.03	1.51 ± 0.06	1.63 ± 0.01	1.58 ± 0.05	1.65 ± 0.02**

C = Control (Saline-treated normal rabbits); T = Treated (alloxan-induced diabetic rabbits). Values are mean ± SEM; *p < 0.001, **p < 0.50 compared to control.

Table – II: Changes in Blood Sugar (F), Blood Urea and Serum Creatinine and Body Weight of Streptozotocin-Induced Diabetic Rabbits

Parameters	Initial Value		DAYS							
			2nd		5th		10th		15th	
	C	T	C	T	C	T	C	T	C	T
Blood Glucose (mg/dl)	100 ±5.08	96.25 ±8.16	102 ±3.37	233 ±9.17	99 ±2.68	198 ±6.97	100 ±3.32	182 ±8.21	101 ±2.73	159 ±6.51*
Blood Urea (mg/dl)	18.75 ±0.62	20.5 ±1.32	17.5 ±0.86	39.75 ±0.84	18.5 ±0.64	36.75 ±1.10	17.5 ±1.01	28.25 ±1.51	17.74 ±1.46	25 ±2.07**
Serum Creatinine (mg/dl)	0.93 ±0.07	1.02 ±0.12	0.94 ±0.04	2.25 ±0.32	0.95 ±0.09	1.98 ±0.21	0.92 ±0.08	1.87 ±0.18	0.95 ±0.087	1.54 ±0.19**
Body Weight (Kg)	1.177 ±0.06	1.275 ±0.14	1.242 ±0.07	1.227 ±0.10	1.302 ±0.05	1.251 ±0.07	1.320 ±0.05	1.270 ±0.08	1.377 ±0.06	1.380 ±0.06

C = Control (Saline-treated normal rabbits); T = Treated (Streptozotocin-induced diabetic rabbits). Values are mean ± SEM; *p < 0.01, **p < 0.10 compared to control.

Table – III: Effect of various drugs on Blood Sugar (F), Blood Urea and Serum Creatinine of Alloxan-Induced Diabetic Rabbits

Factor Group	Initial Value			DAYS								
				7th			14th			21st		
	BS(F)	BU	SC	BS(F)	BU	SC	BS(F)	BU	SC	BS(F)	BU	SC
NC	94 ±6.06	19.5 ±0.64	1.25 ±0.20	99 ±5.95	19 ±1.46	1.05 ±0.20	105 ±4.66	21.03 ±1.58	1.29 ±0.25	103 ±3.19	19.75 ±0.84	1.21 ±0.04
DC	292 ±10.60	53 ±2.11	3.32 ±0.16	285 ±9.46	52.9 ±1.80	3.12 ±0.16	210 ±8.83	46.6 ±1.25	3.00 ±0.15	192 ±9.41	40.1 ±1.16	2.80 ±0.14
Abroma augusta treated	291 ±10.50	46.75 ±1.65	3.10 ±0.14	192 ±4.02	40 ±1.57	2.87 ±0.05	161 ±3.32	30.25 ±1.10	2.22 ±0.09	120 ±3.89**	24.75 ±0.62**	1.93 ±0.13*
Syzygium jambolanum treated	298 ±7.90	49.75 ±1.31	3.10 ±0.10	162 ±5.76	40.25 ±1.10	2.75 ±0.06	141 ±5.90	28.5 ±0.95	2.02 ±0.02	111 ±4.43#	22 ±0.91#	1.75 ±0.08#
Glimiperide treated	271 ±9.11	46.25 ±1.54	3.13 ±0.08	154 ±4.96	30.25 ±1.79	2.46 ±0.05	119 ±4.29	24.25 ±0.84	1.48 ±0.17	86 ±8.39+	18.75 ±0.84++	0.98 ±0.05+

Values represent mean ± SEM; Each experiment was performed on a group of four rabbits; BS(F): Blood Sugar (Fasting); BU: Blood Urea; SC: Serum Creatinine; NC: Non-diabetic control rabbits treated with normal saline; DC: Diabetic rabbits (Alloxanized) treated with normal saline; *$p < 0.02$, **$p < 0.01$, #$p < 0.01$, +$p < 0.01$, ++$p < 0.001$ compared with DC.

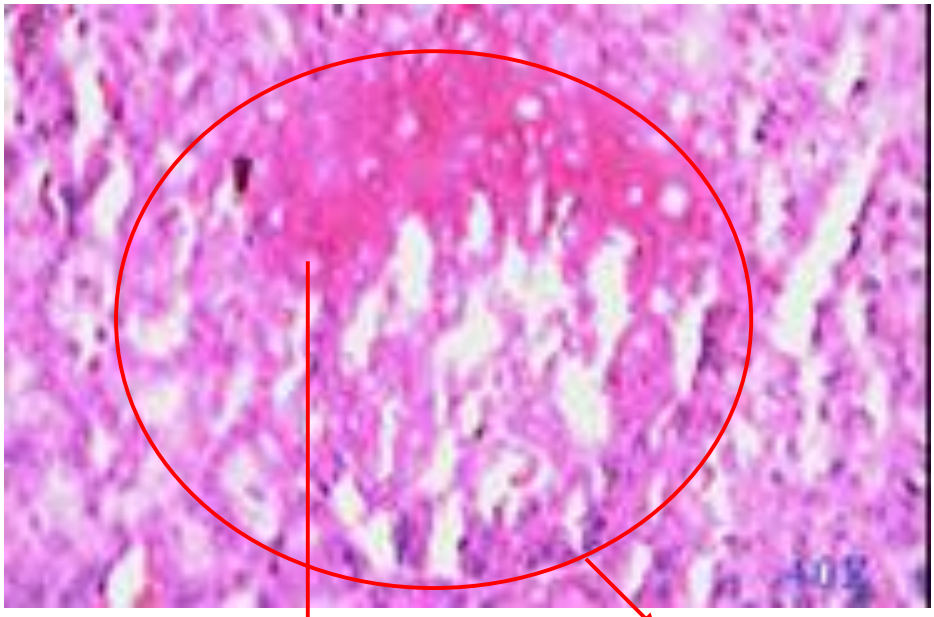


Fig. 5

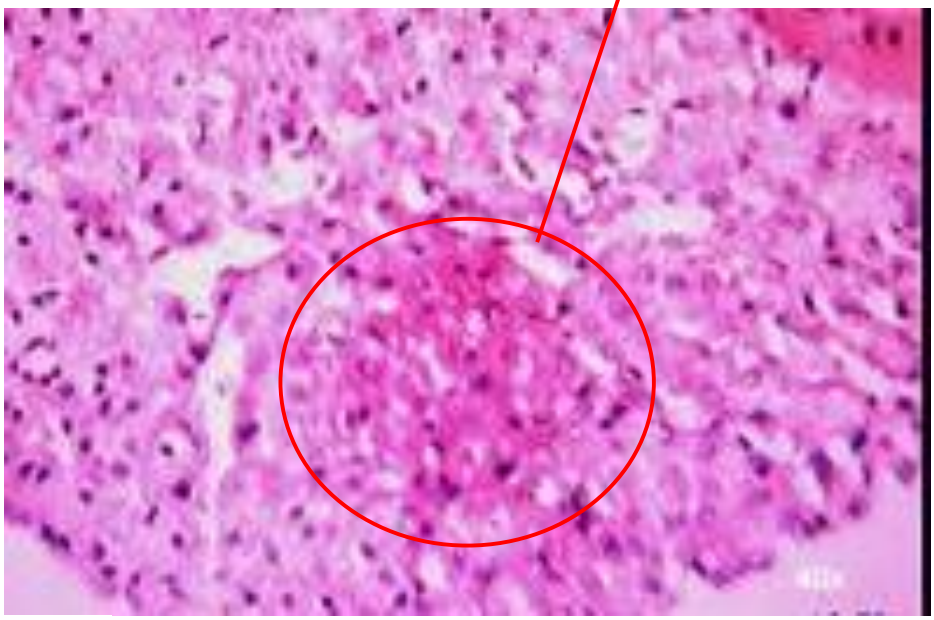


Fig. 6

Degenerated Neuron

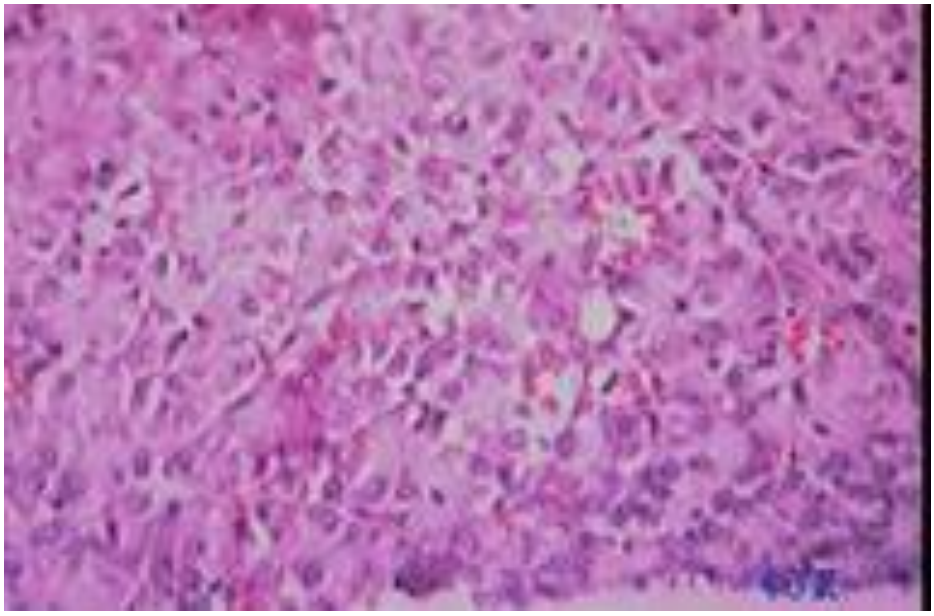


Fig. 7

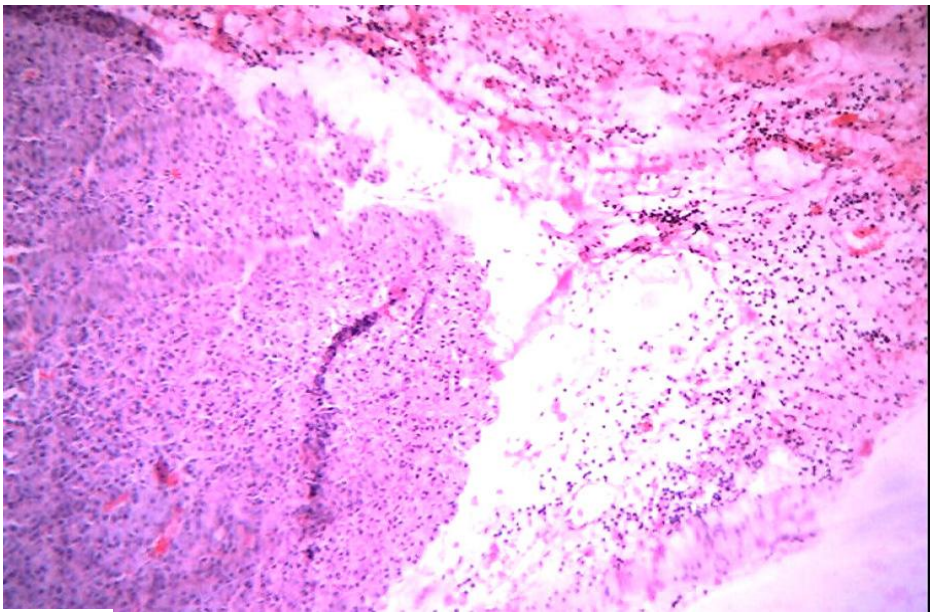


Fig. 8

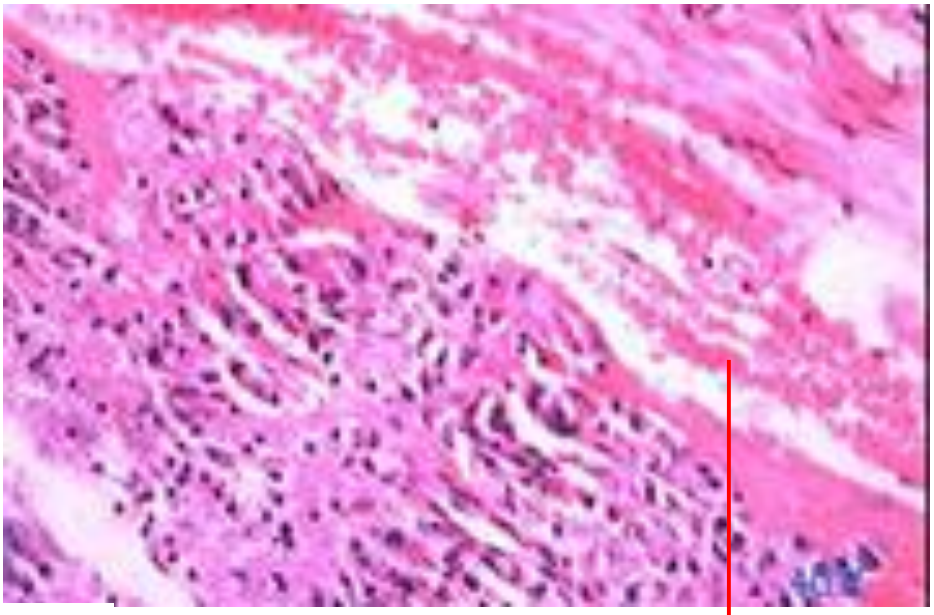


Fig. 9

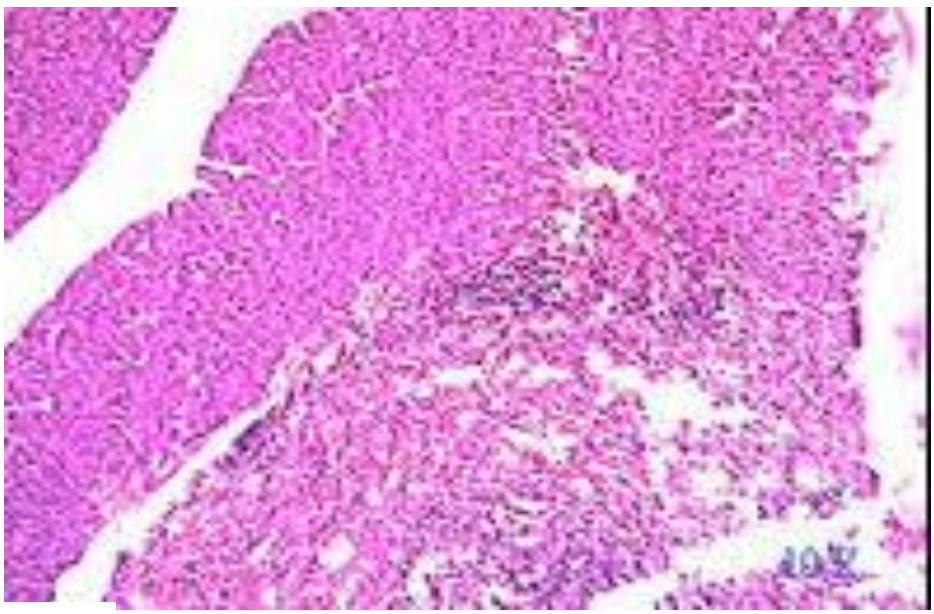


Fig. 10

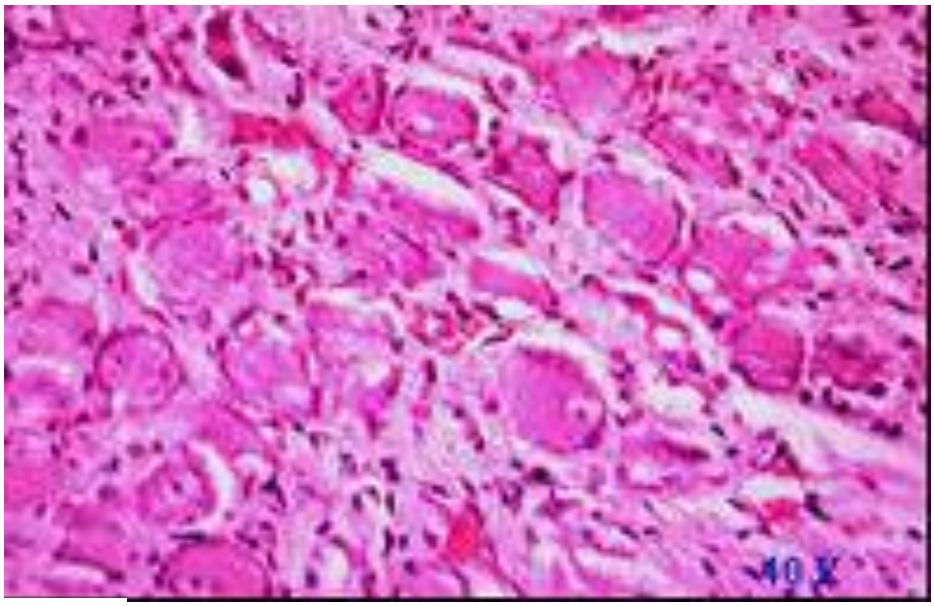


Fig. 11

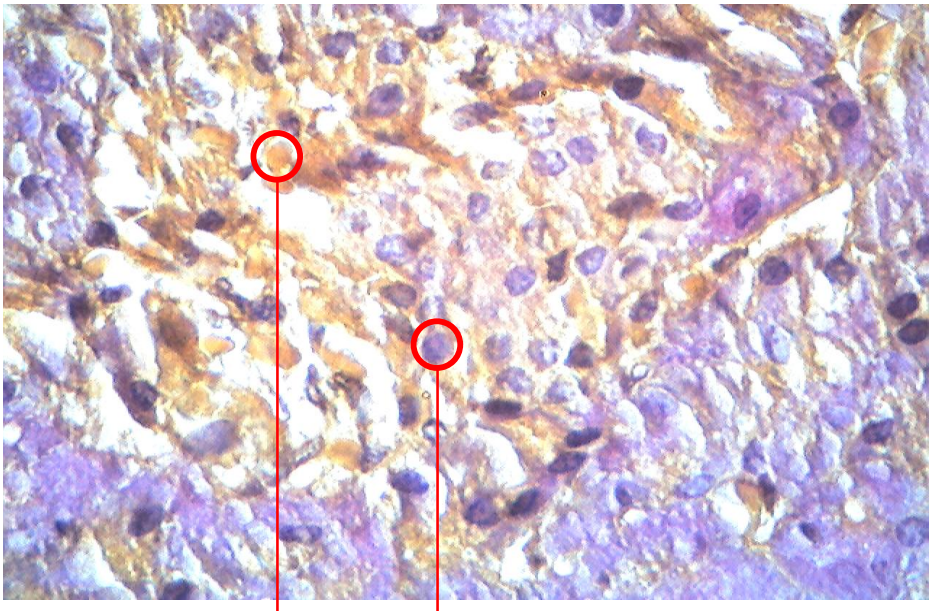


Fig. 12

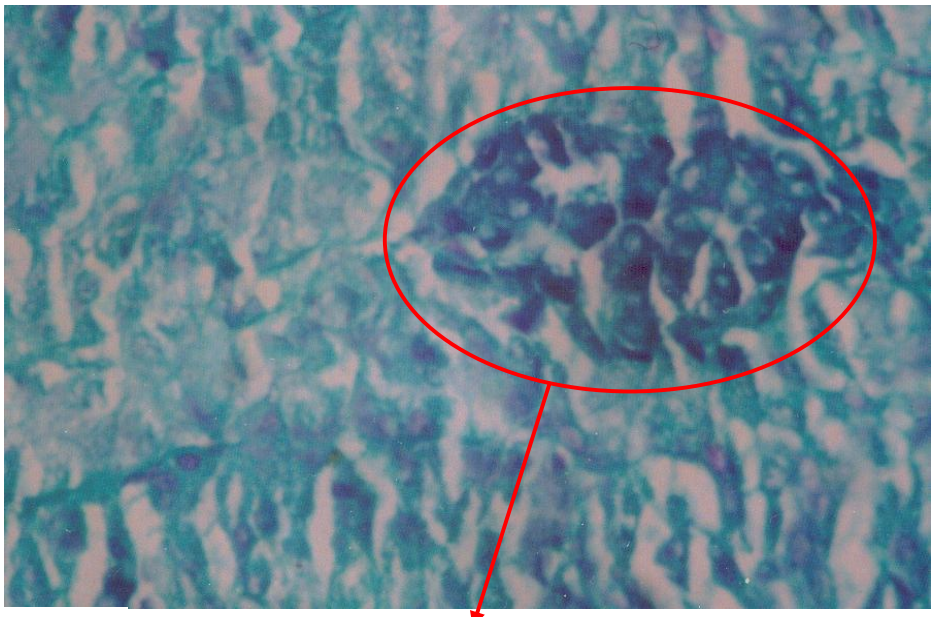


Fig. 13

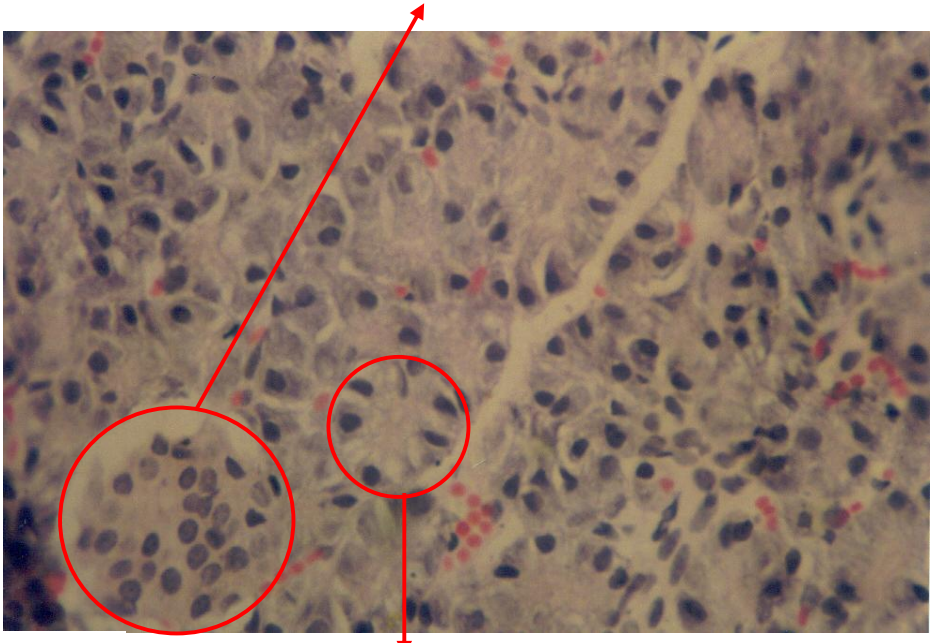


Fig. 14

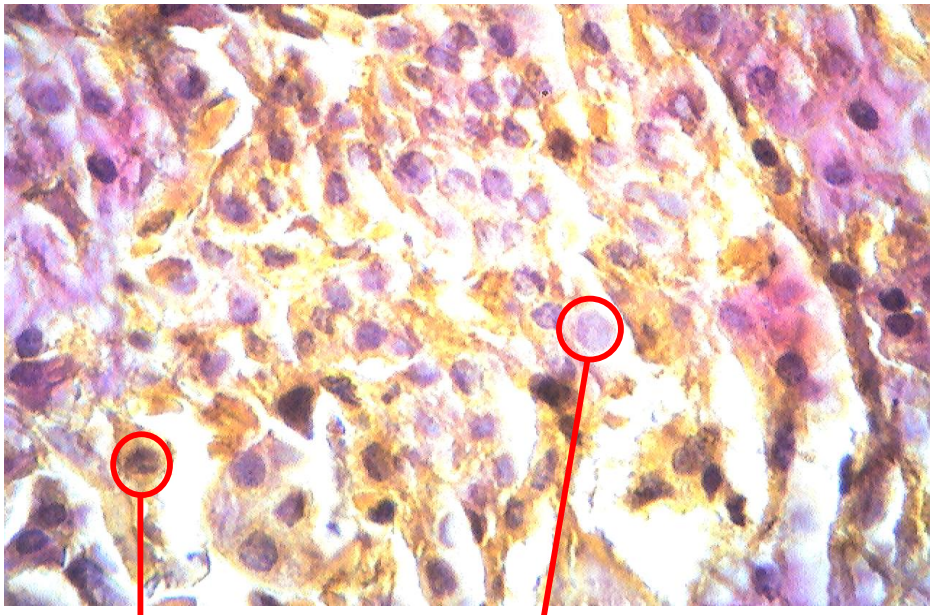


Fig. 15

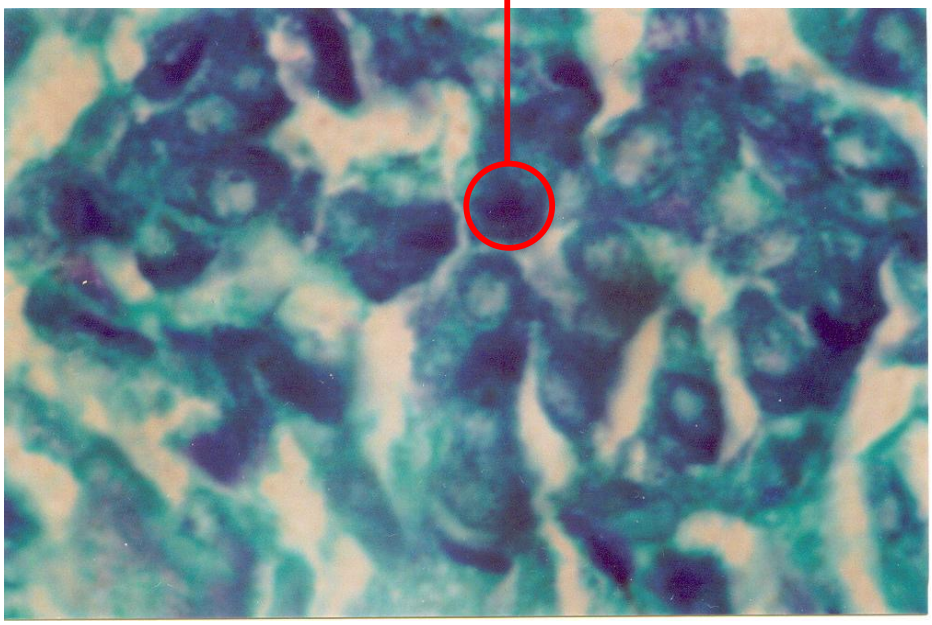


Fig. 16

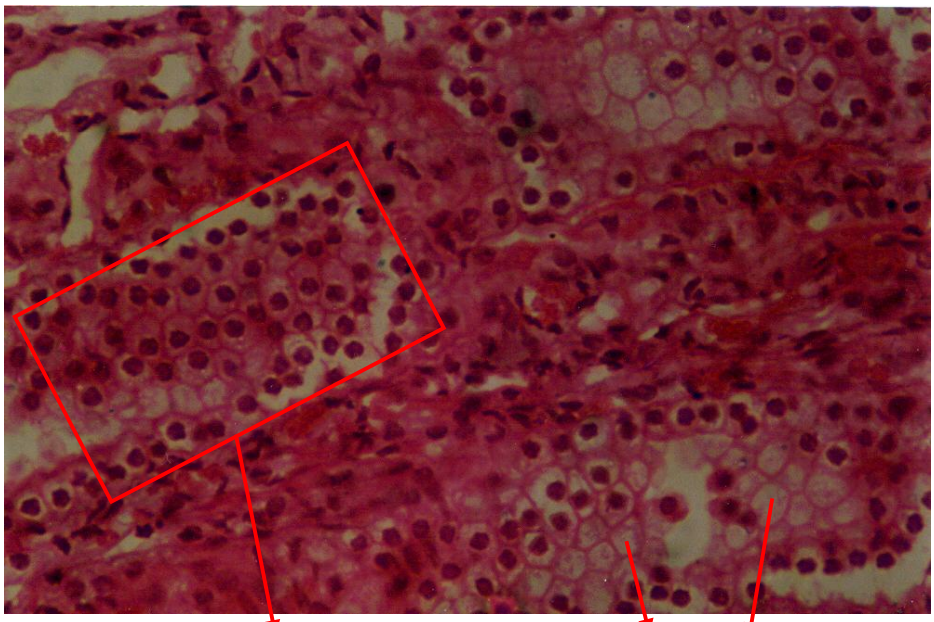


Fig. 17

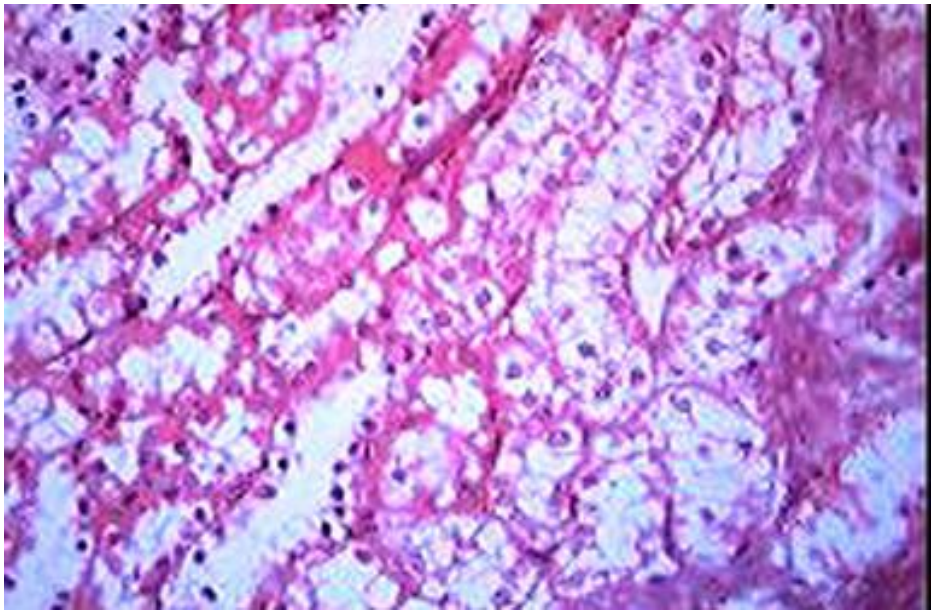


Fig. 18

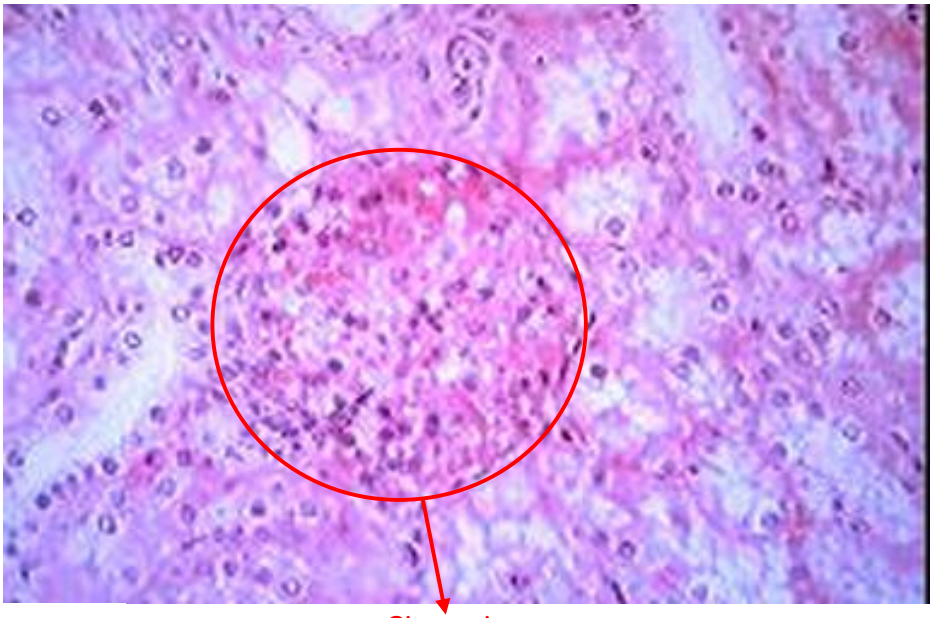


Fig. 19

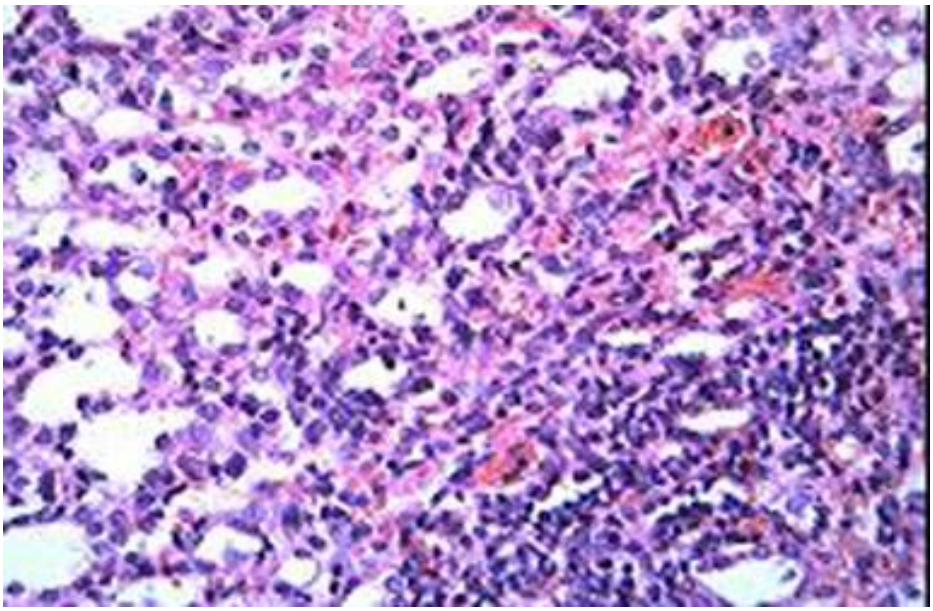


Fig. 20

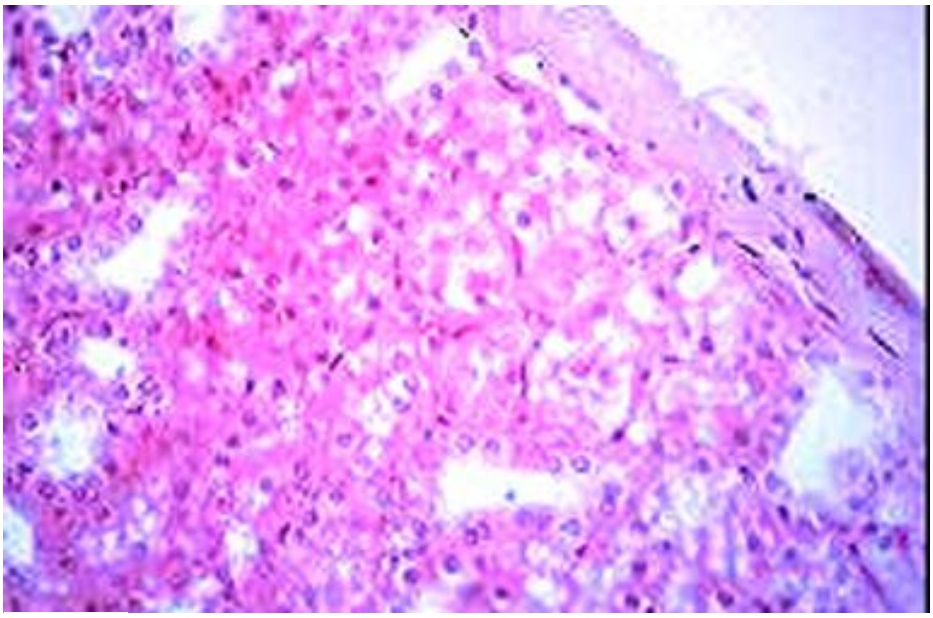


Fig. 21

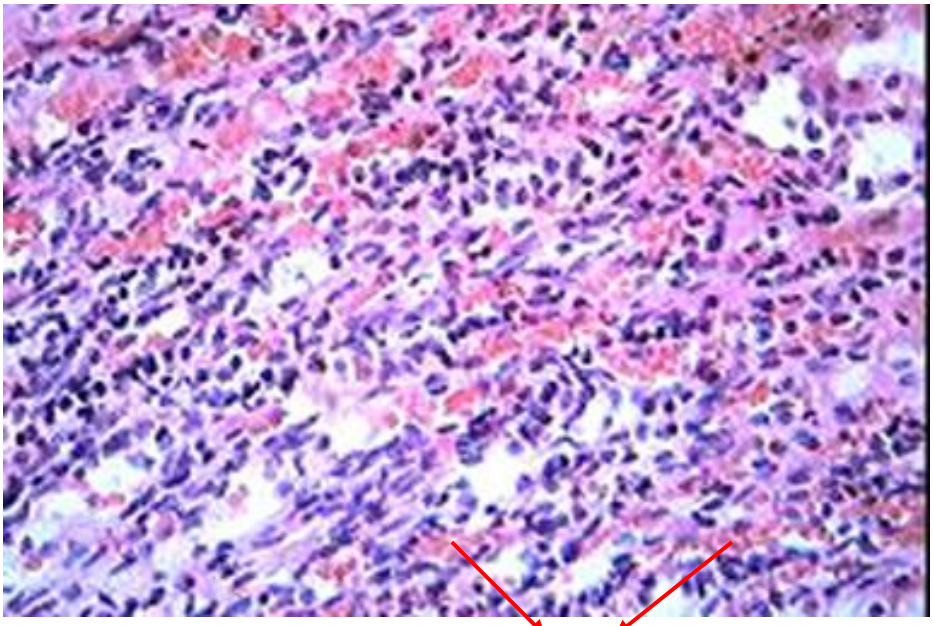


Fig. 22

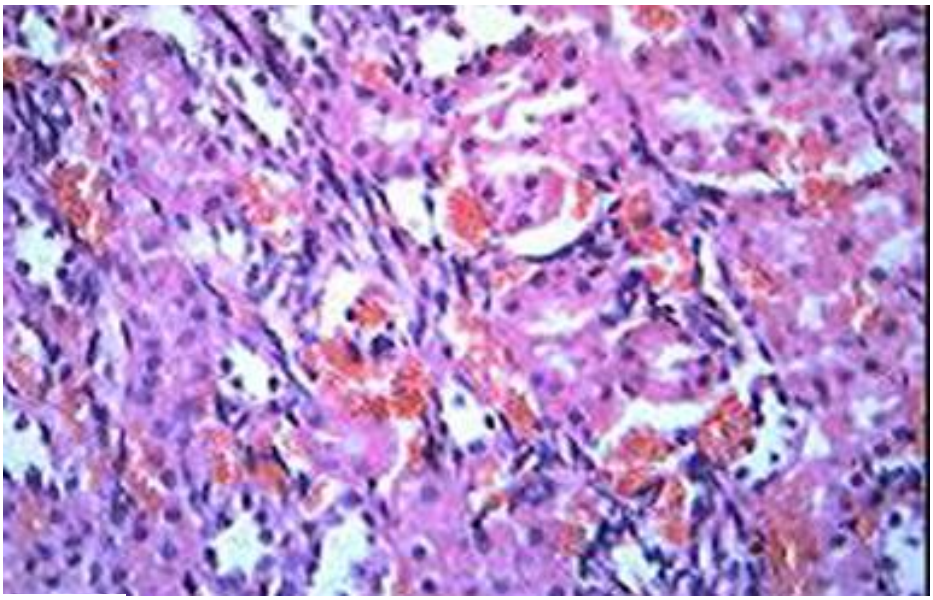


Fig. 23

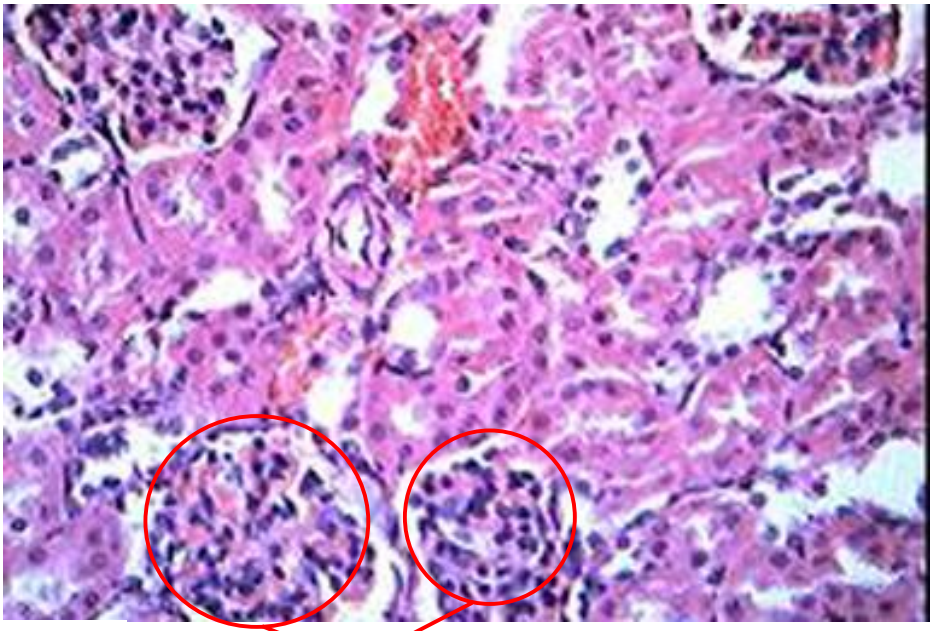


Fig. 24

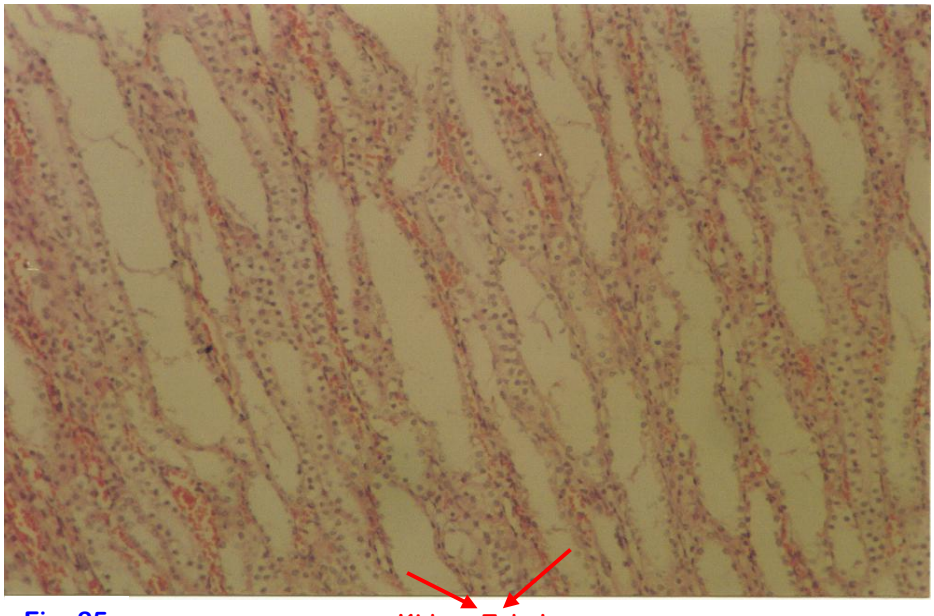


Fig. 25

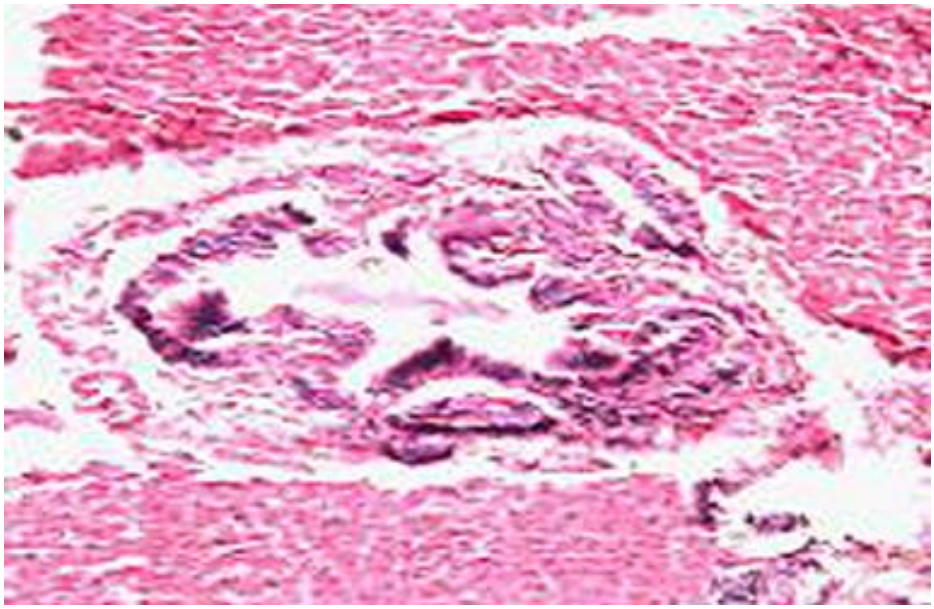


Fig. 26

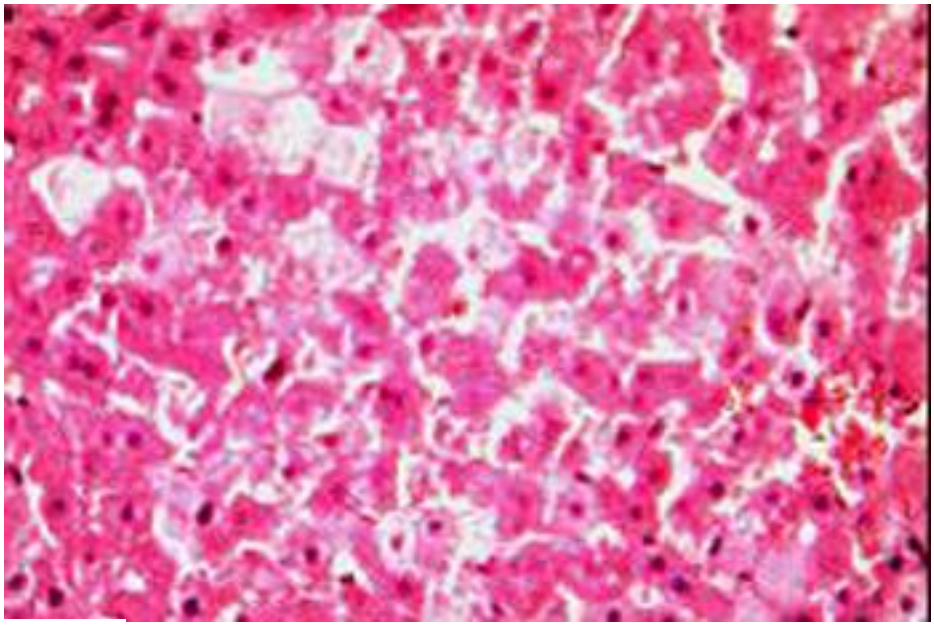


Fig. 27

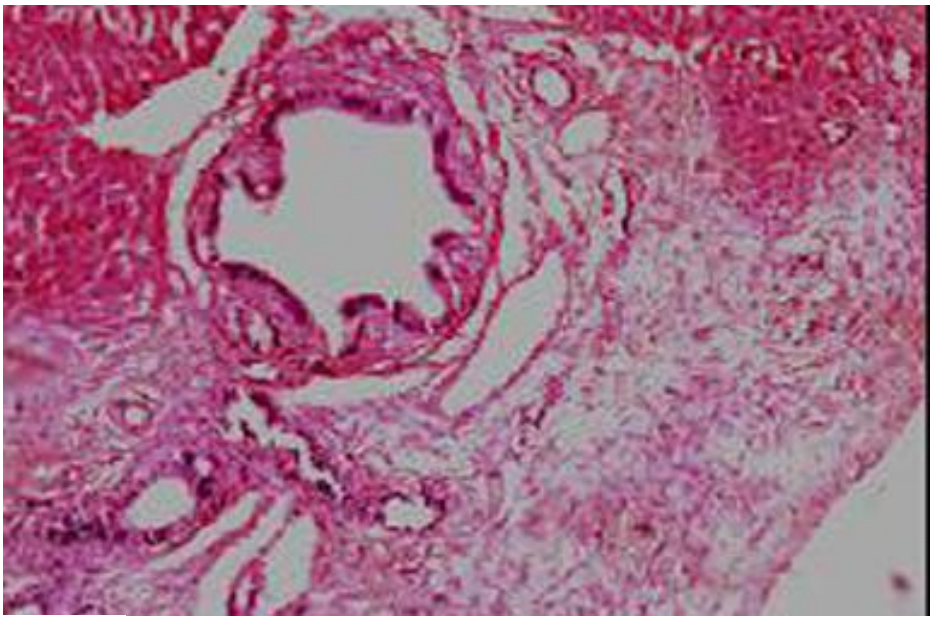


Fig. 28

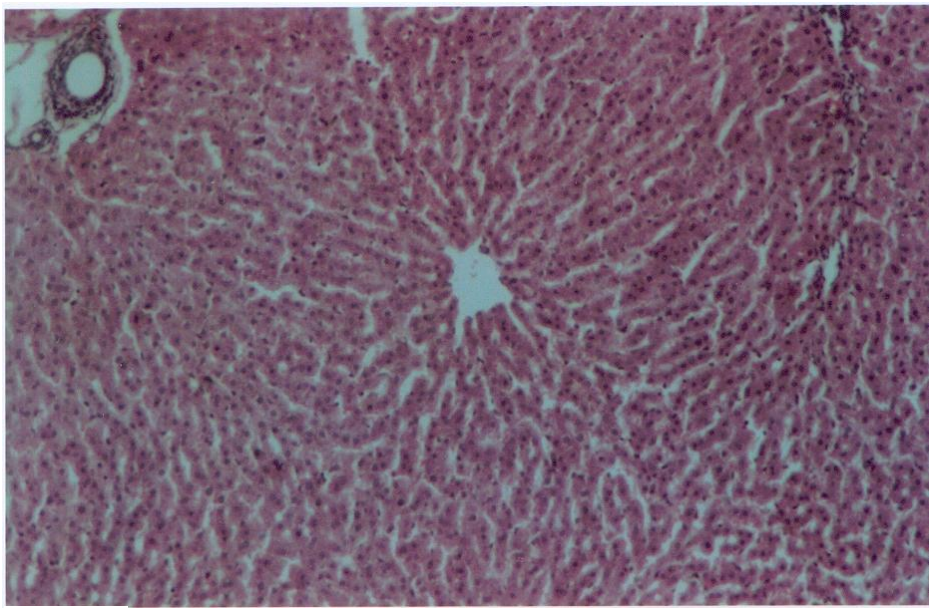


Fig. 29

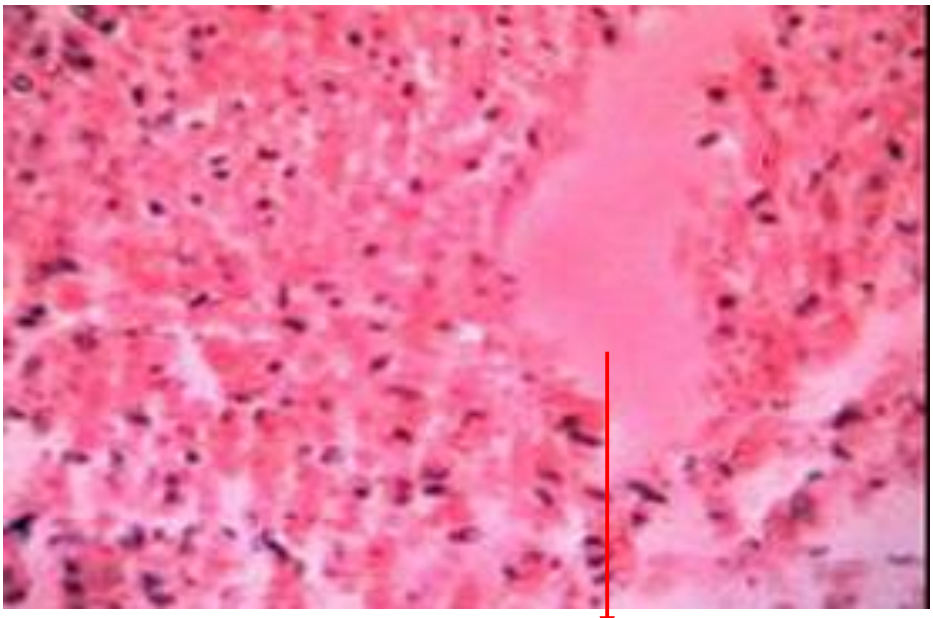


Fig. 30

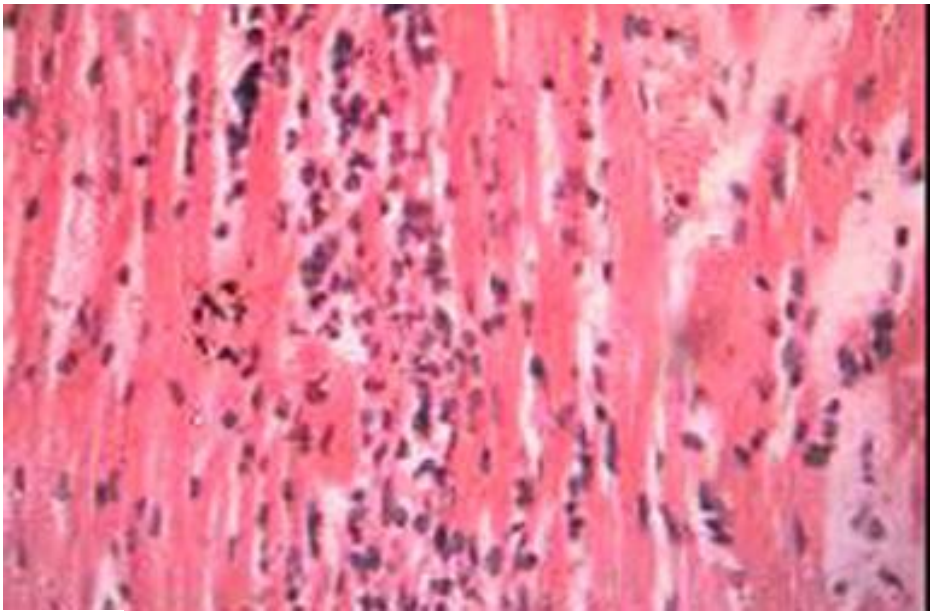


Fig. 31

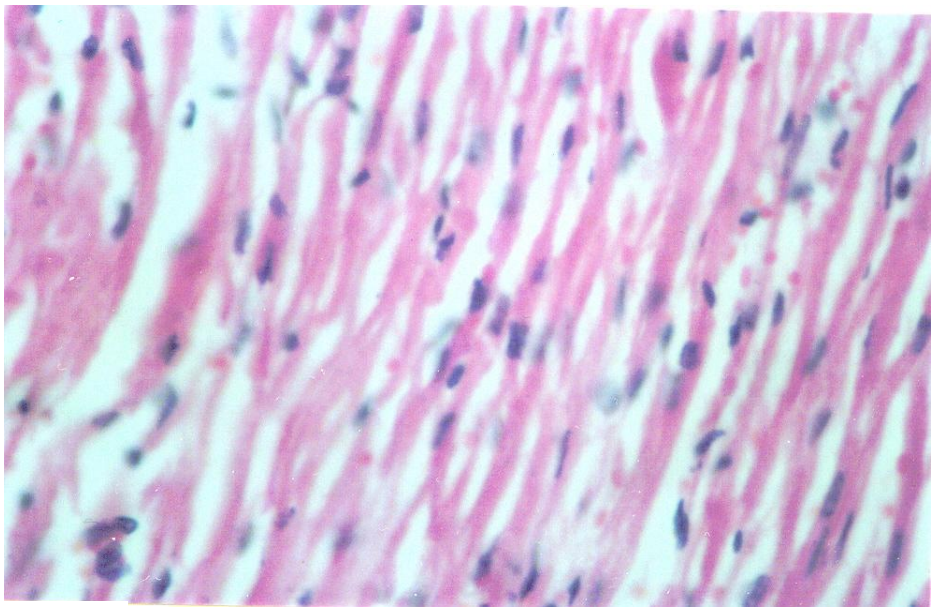


Fig. 32

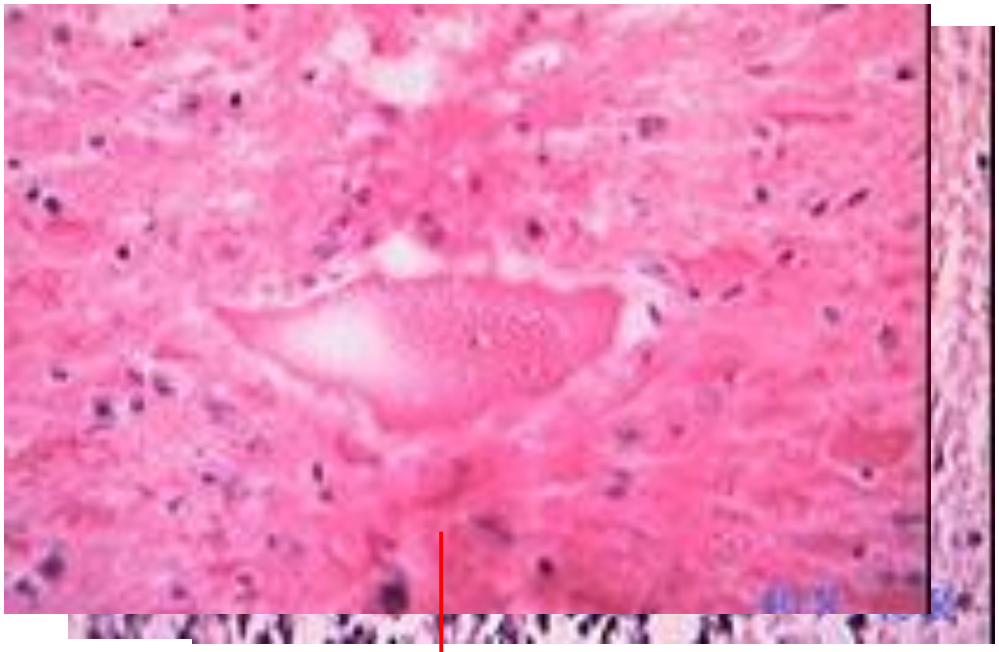


Fig. 35

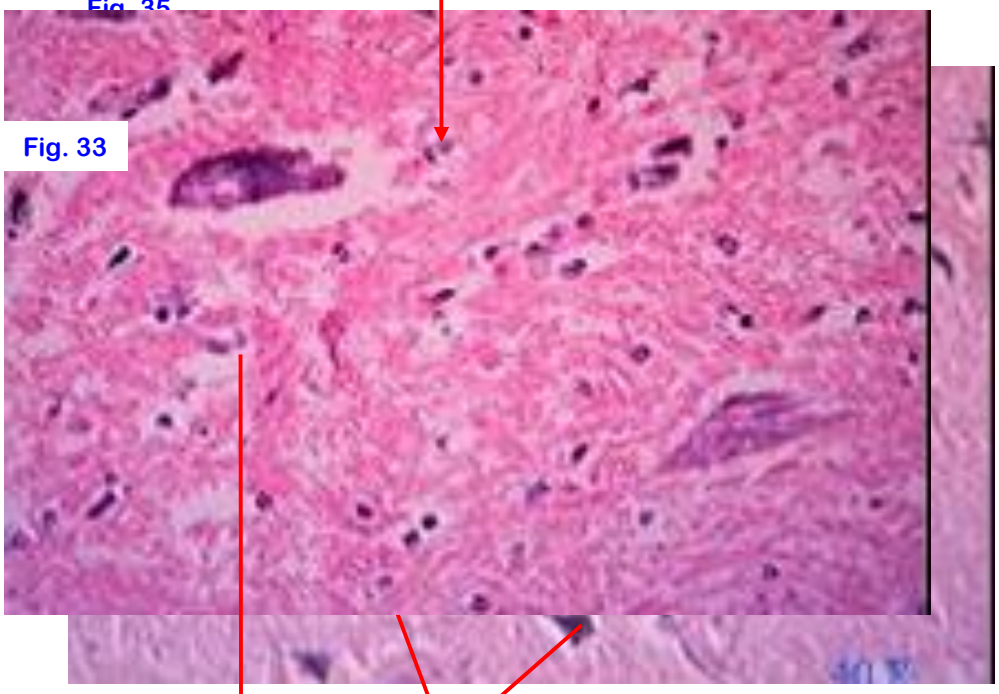


Fig. 33

Fig. 36

Neurons

Fig. 34

Degenerated Neuron

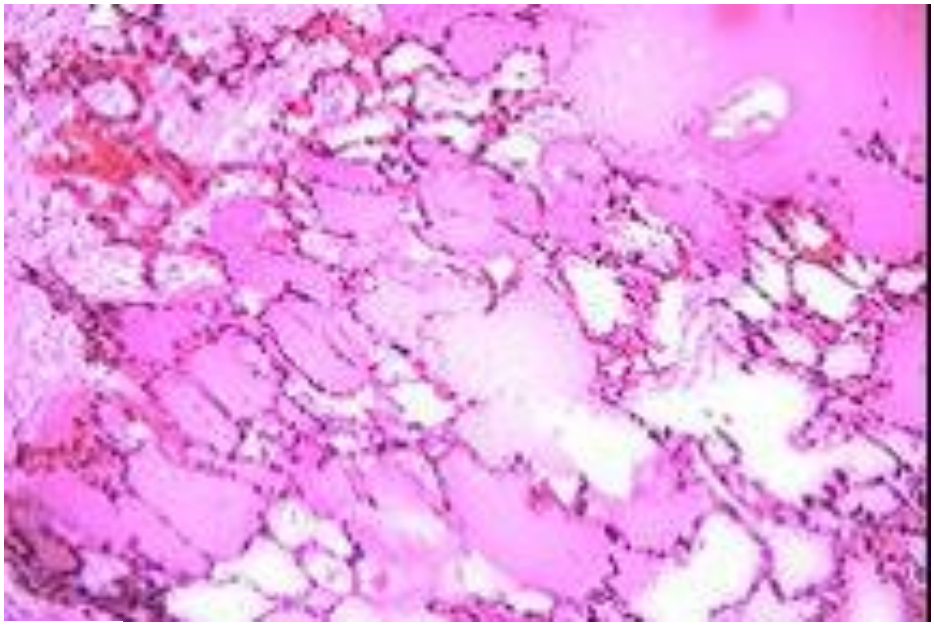


Fig. 37

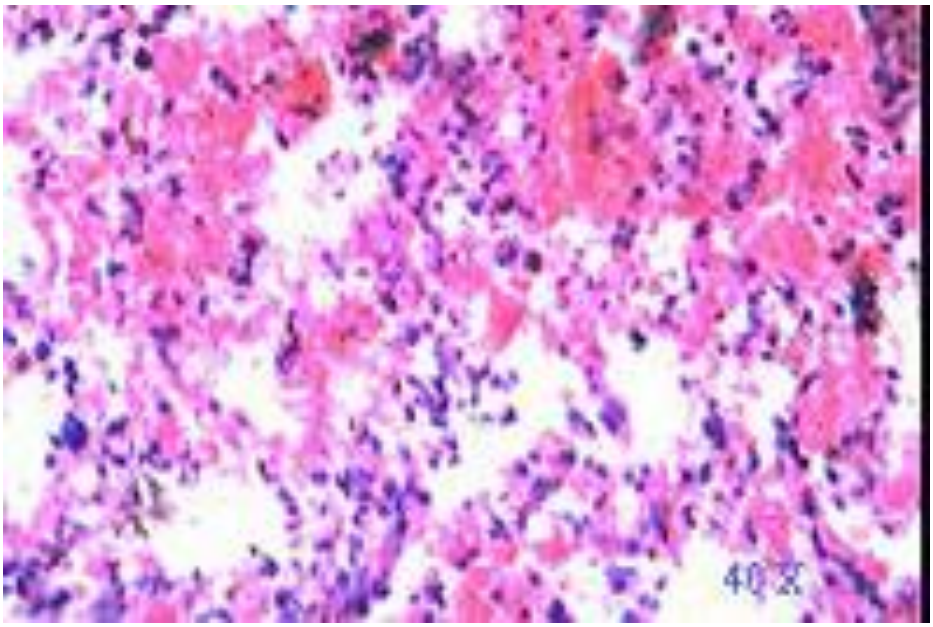


Fig. 38

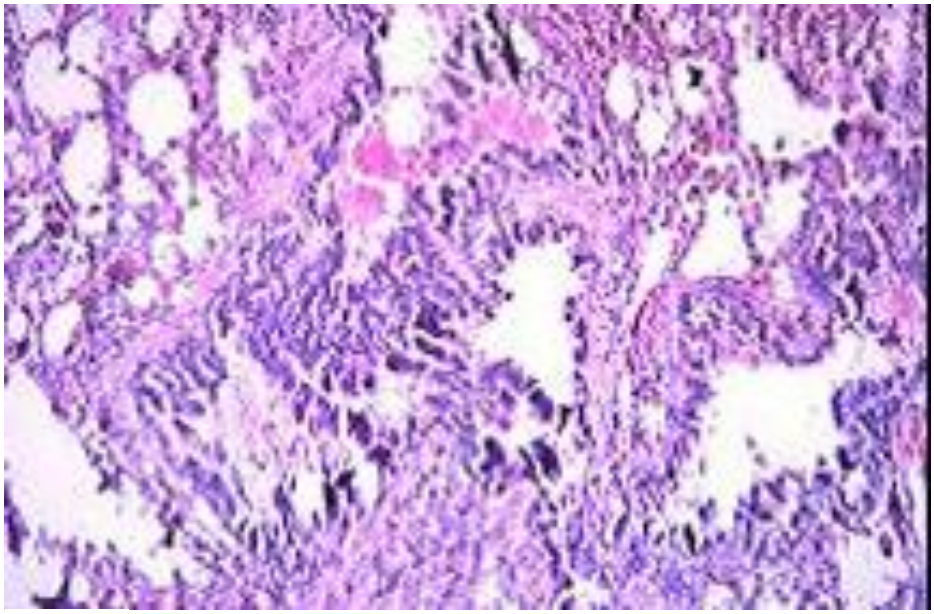


Fig. 39

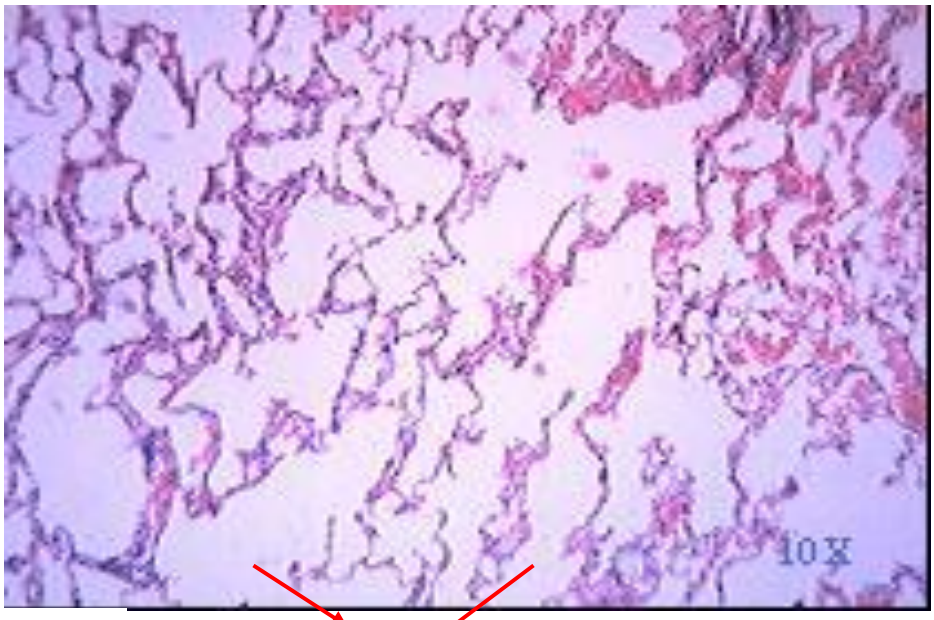


Fig. 40

Emphysema

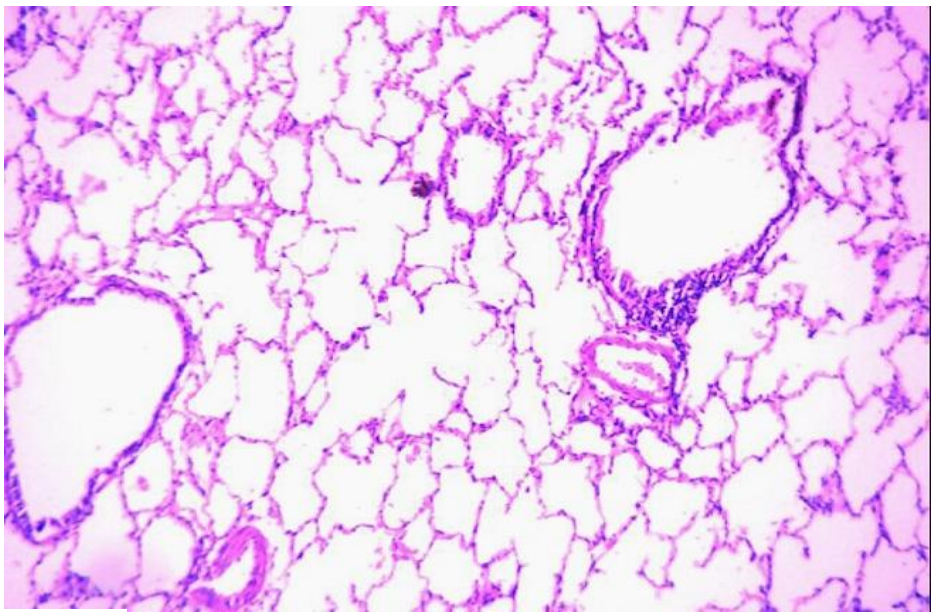


Fig. 41

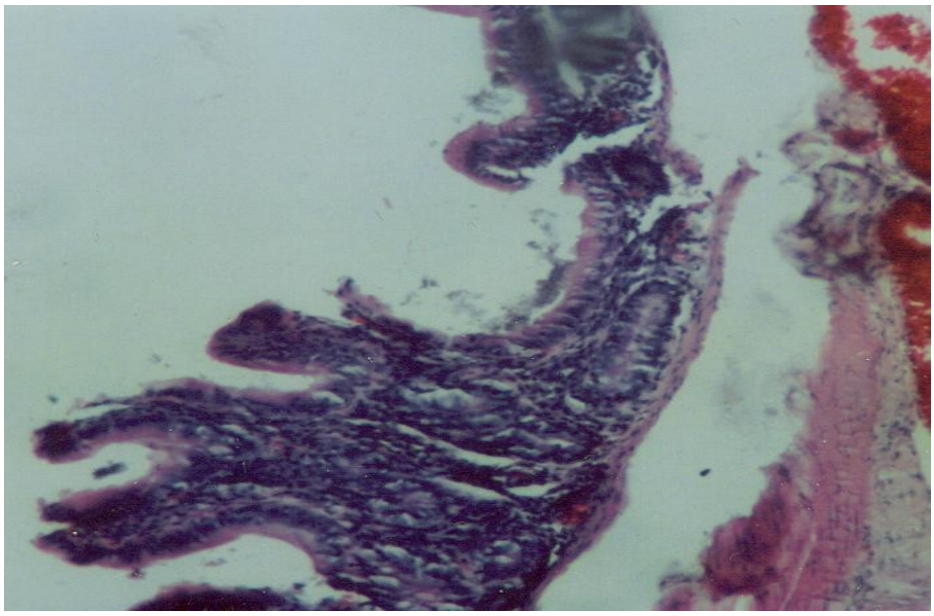


Fig. 42

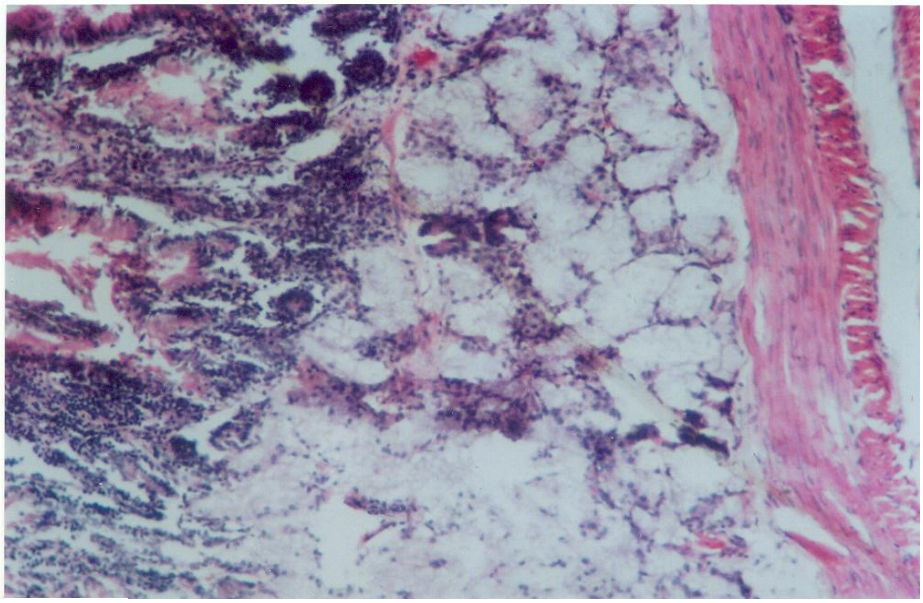


Fig. 43

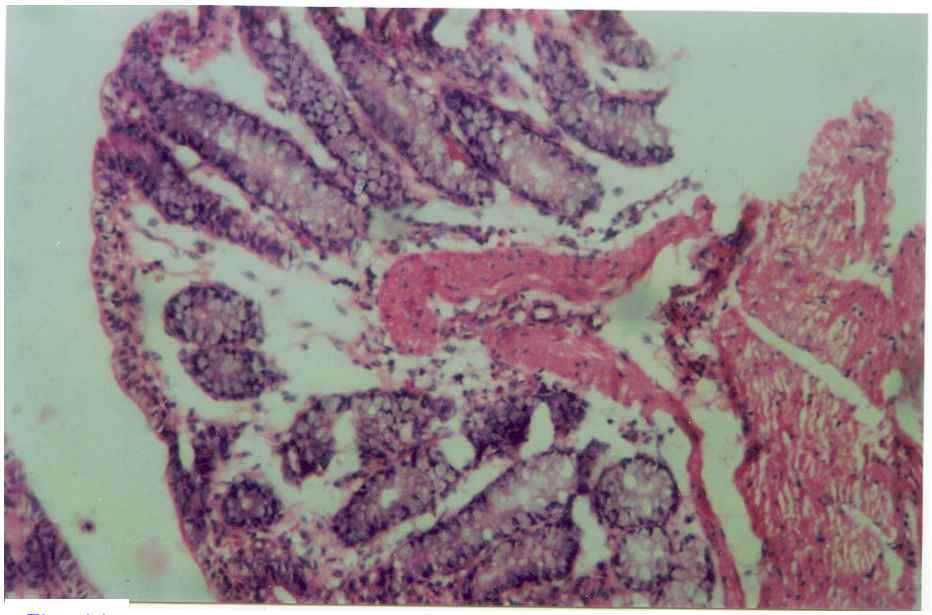


Fig. 44

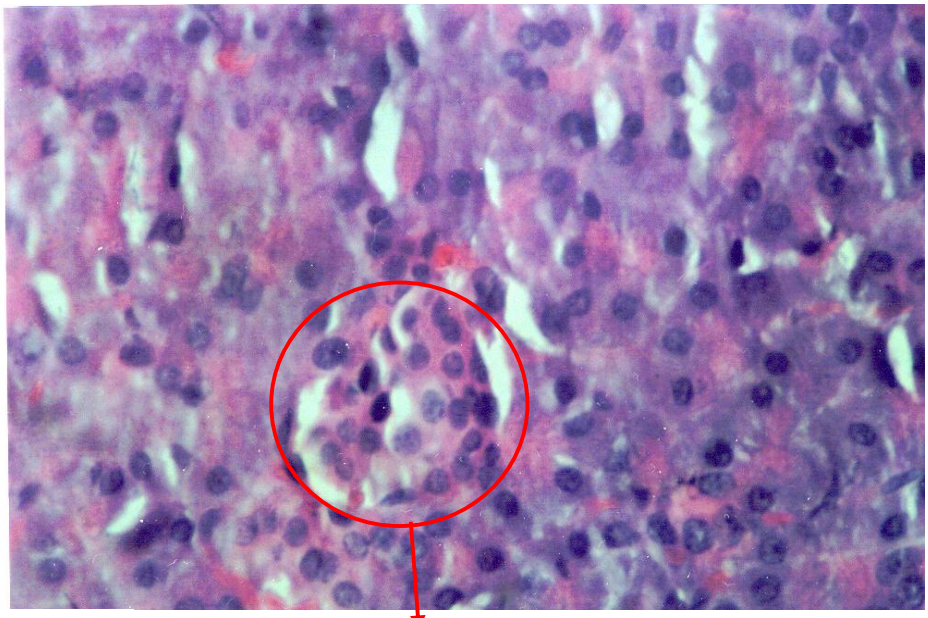


Fig. 49

Islet of Langerhan

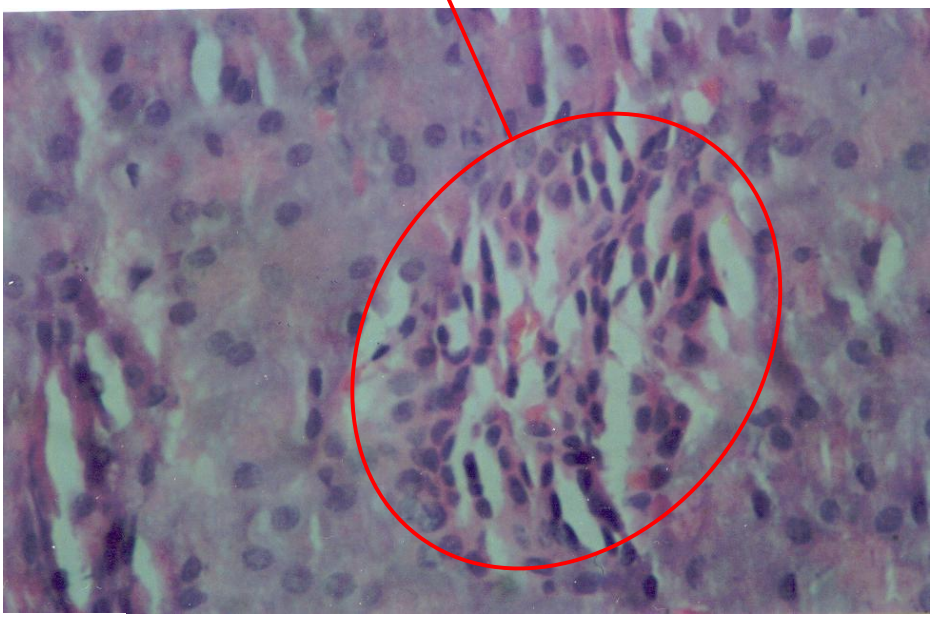


Fig. 50

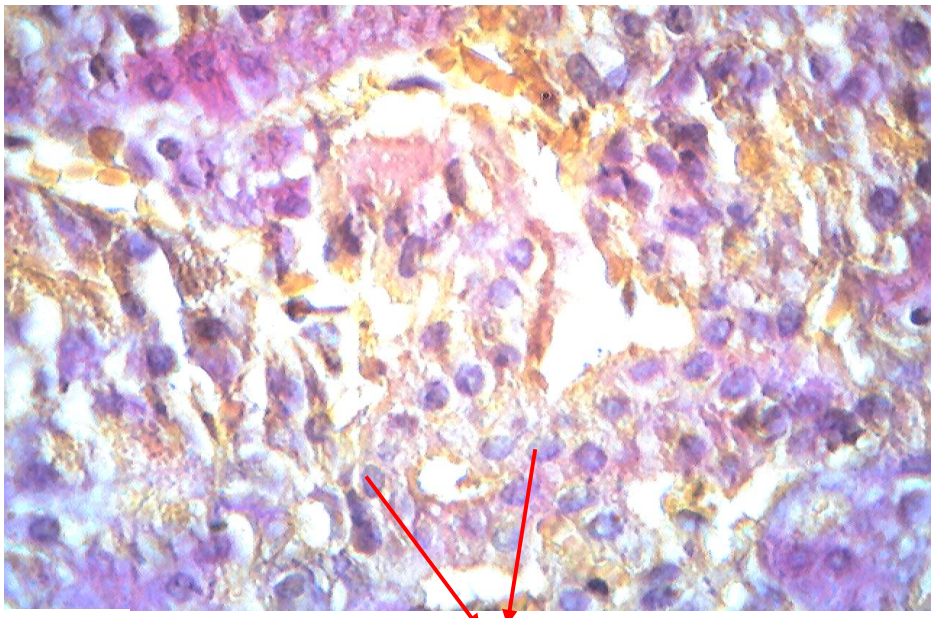


Fig. 51

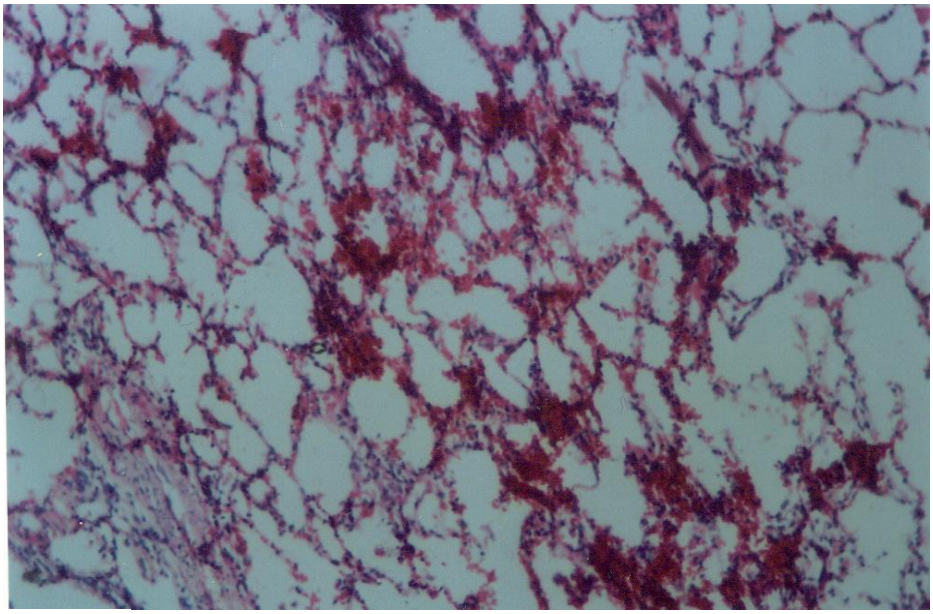


Fig. 52

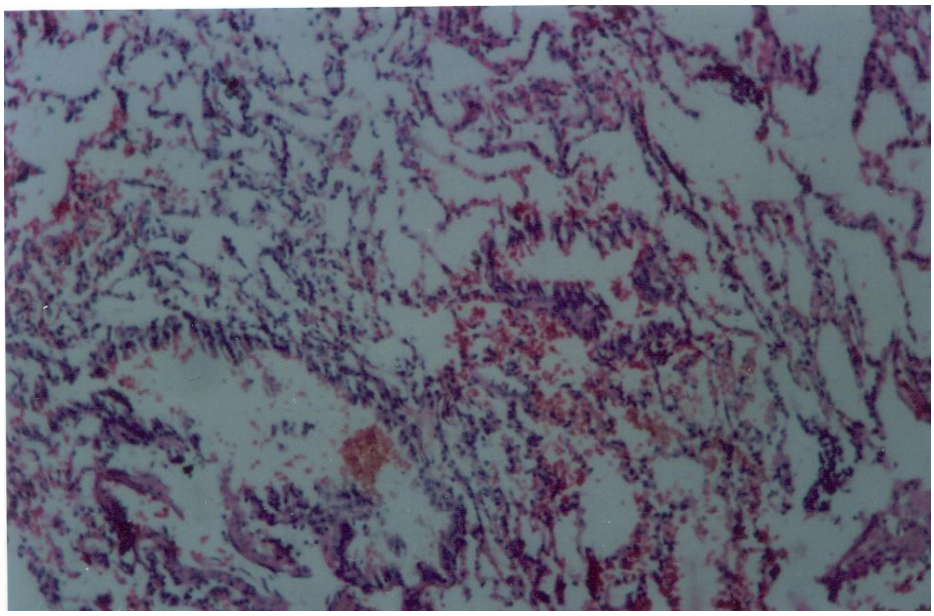


Fig. 53

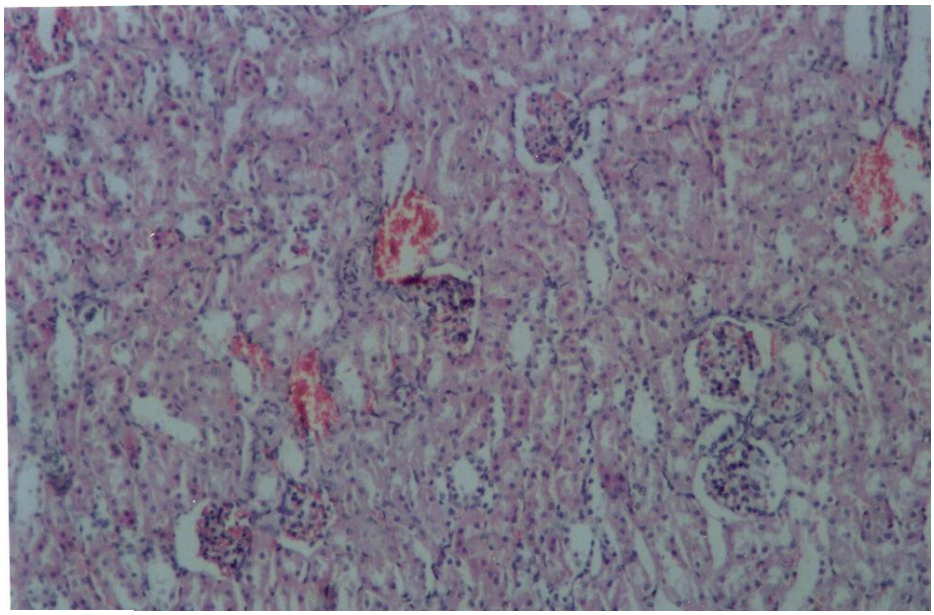


Fig. 54

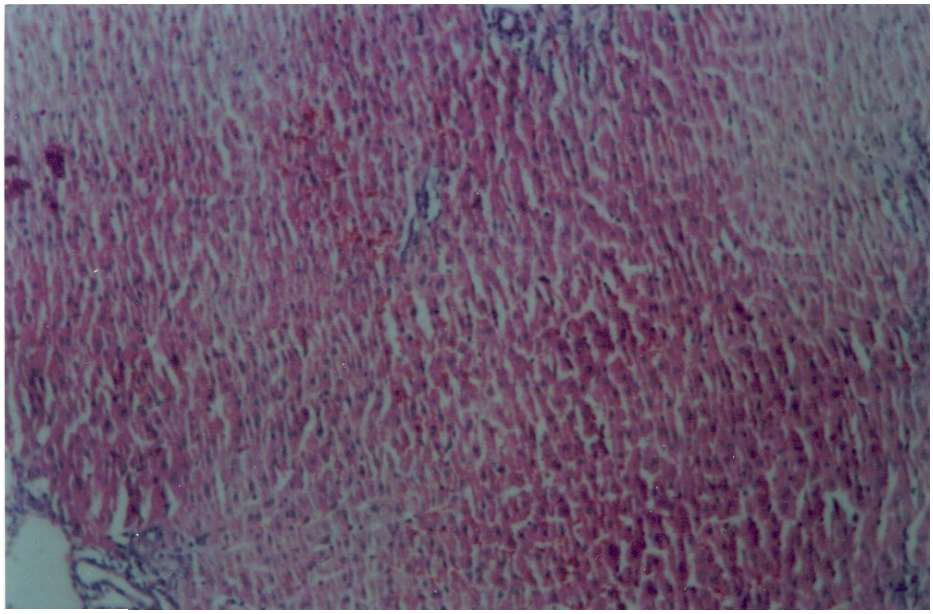


Fig. 55

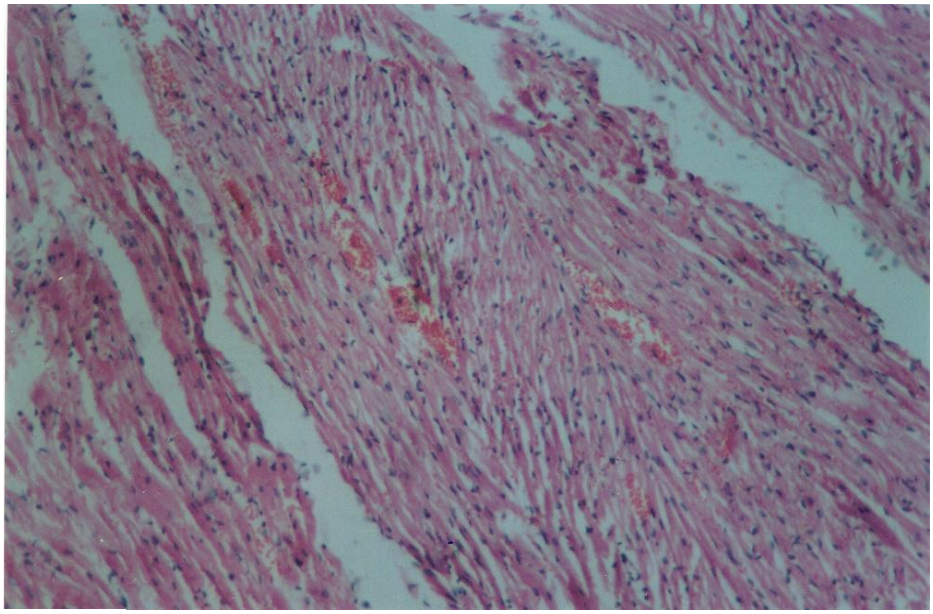


Fig. 56

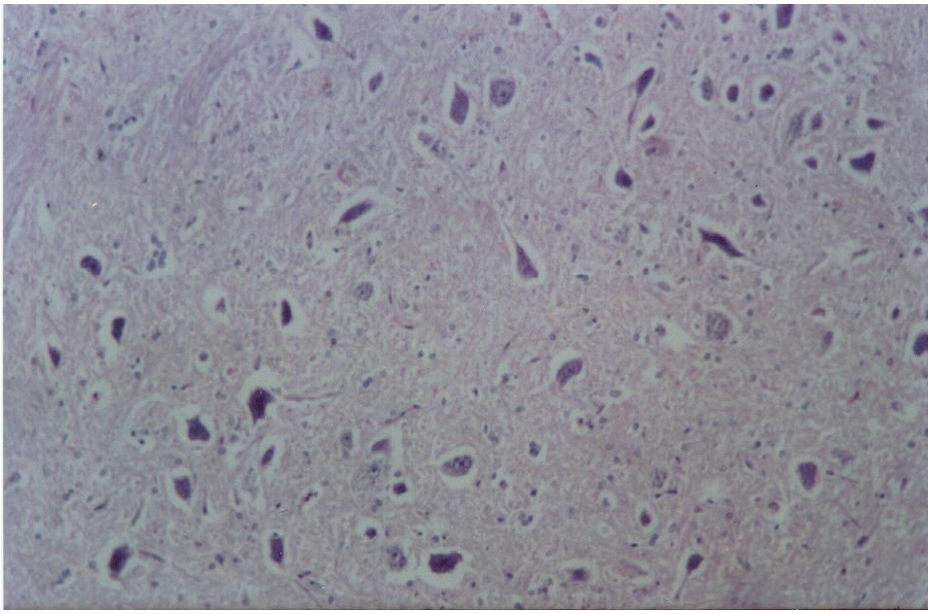


Fig. 57

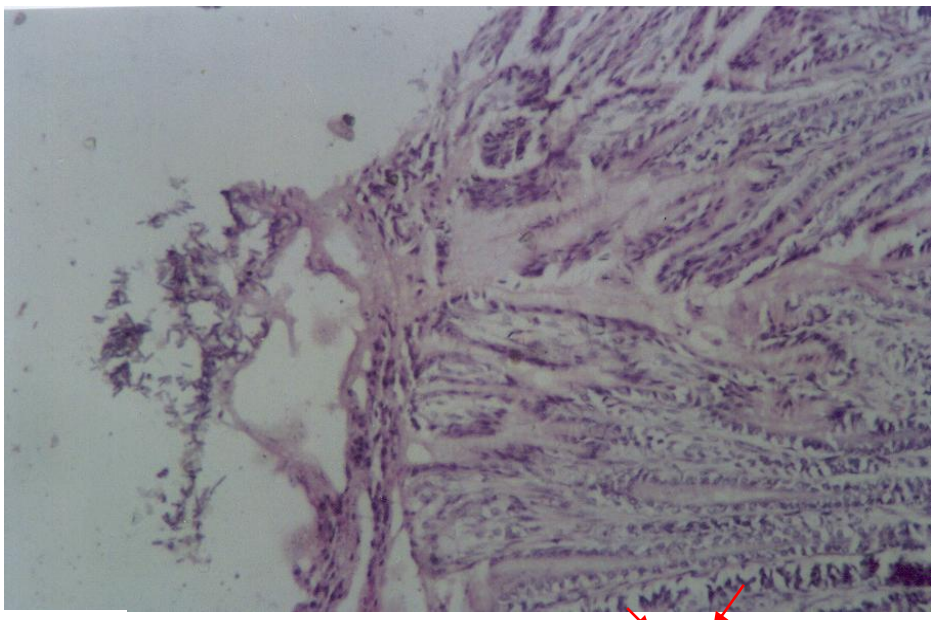


Fig. 58

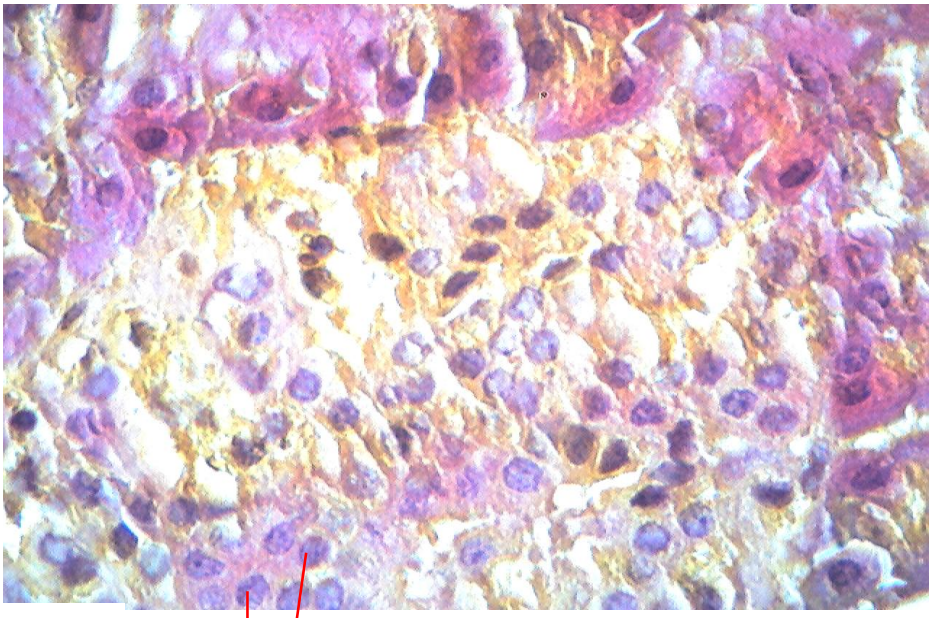


Fig. 62

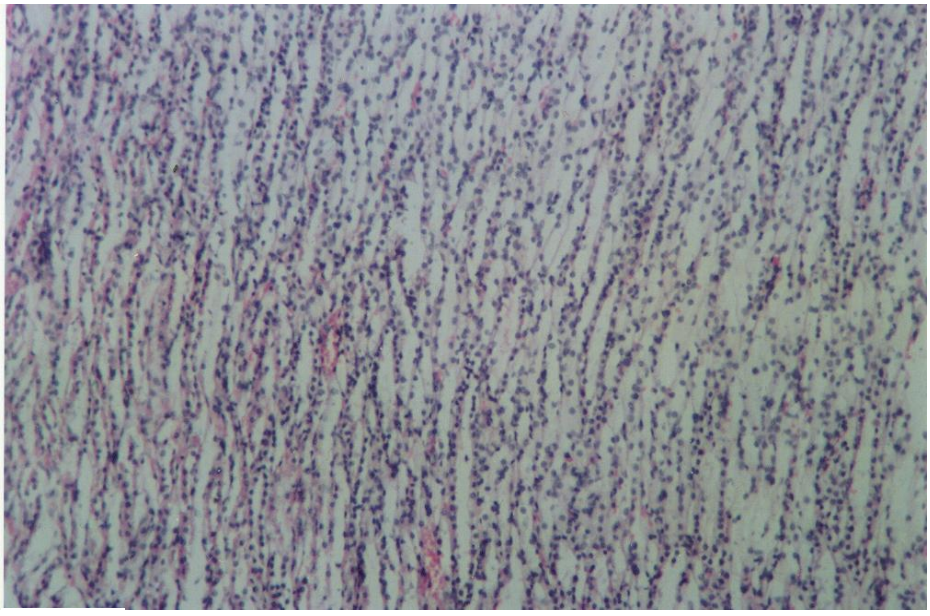


Fig. 63

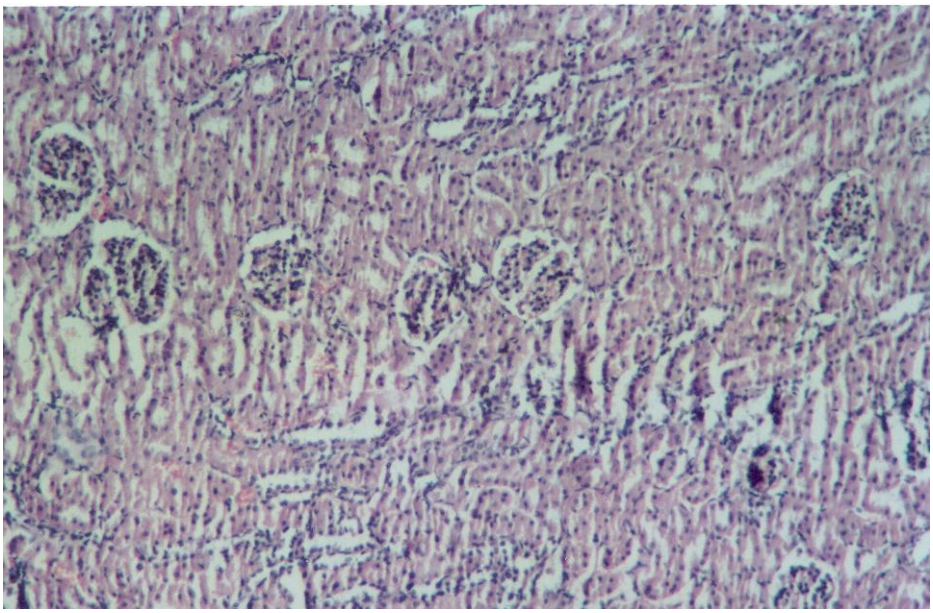
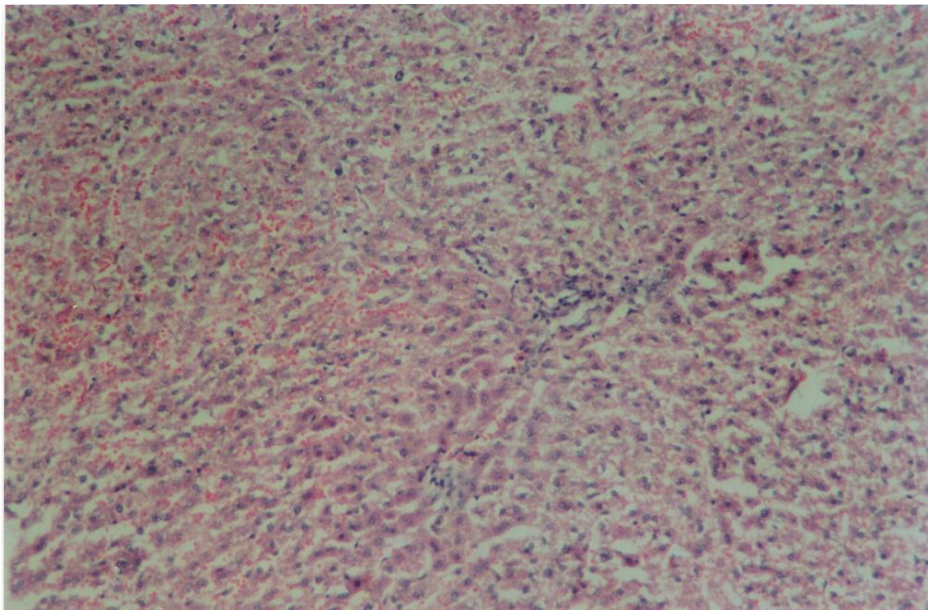


Fig. 64



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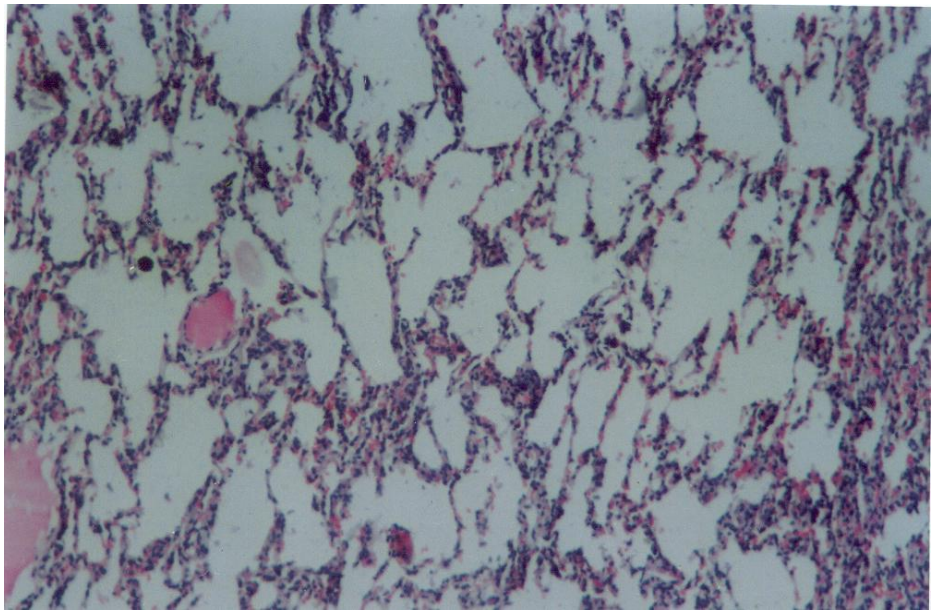


Fig. 66

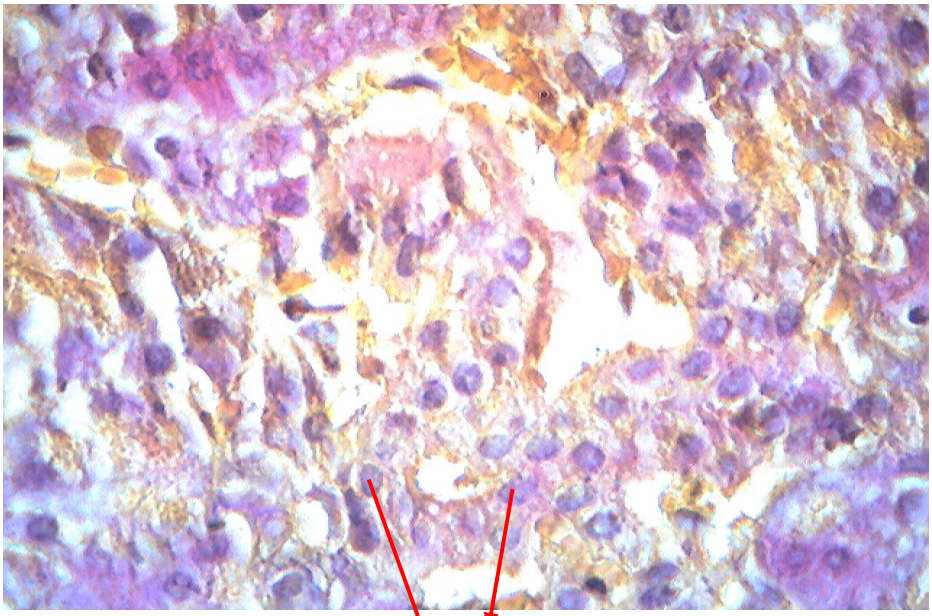
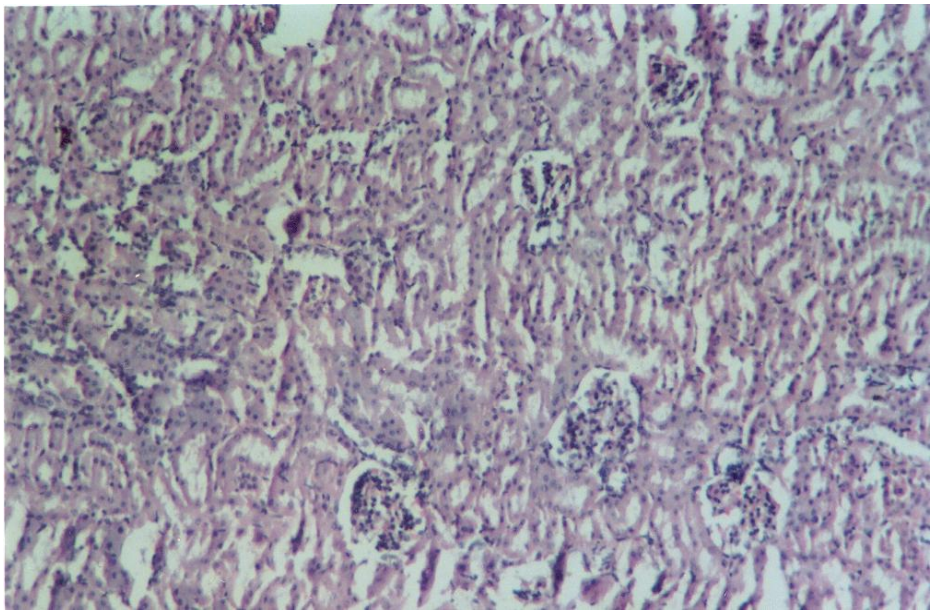


Fig. 70



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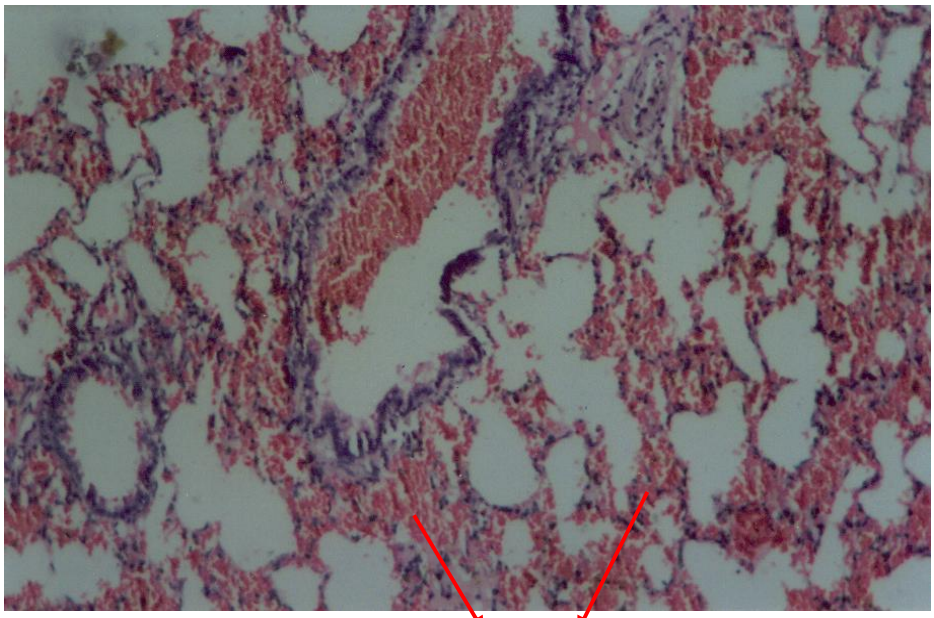

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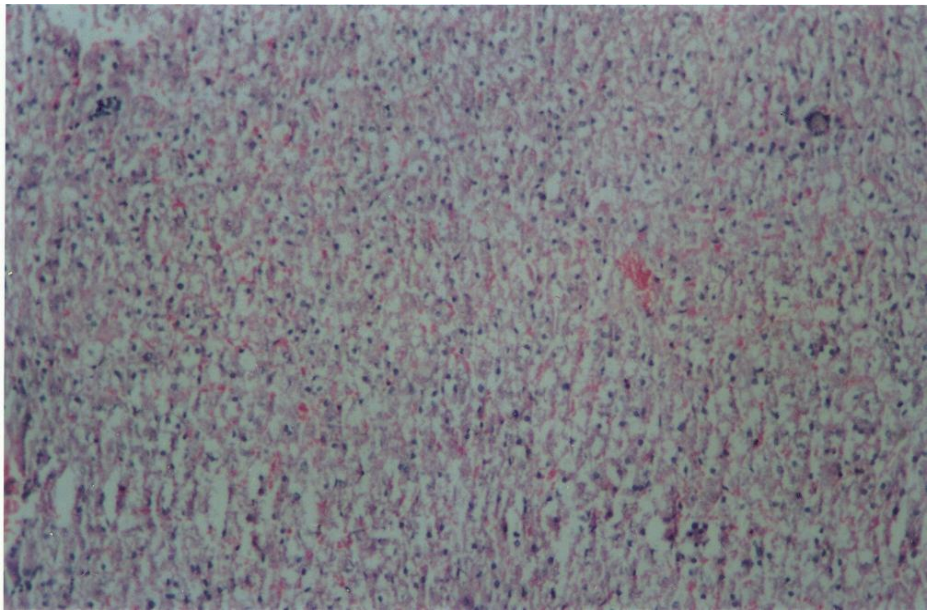

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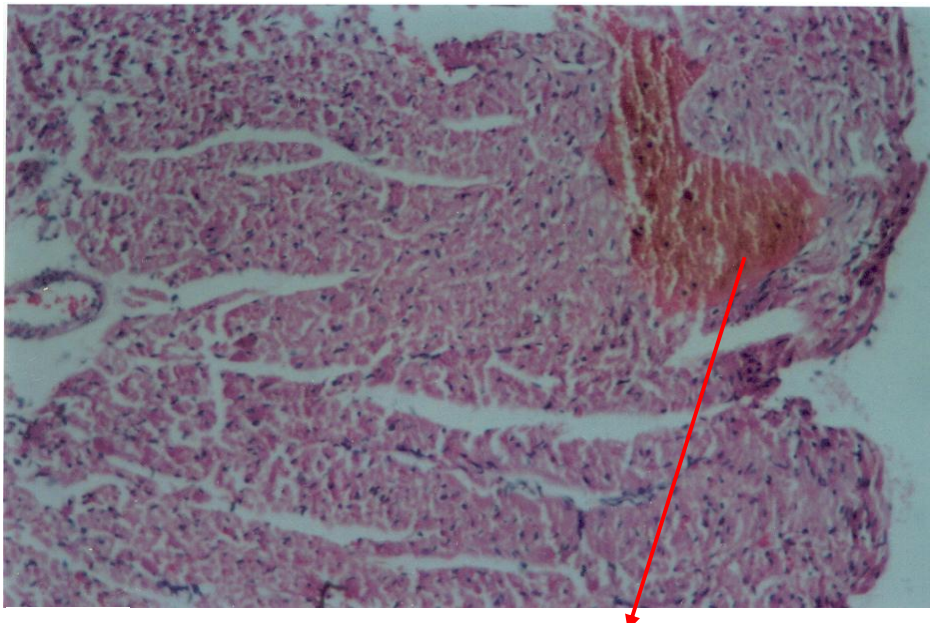

Fig. 74

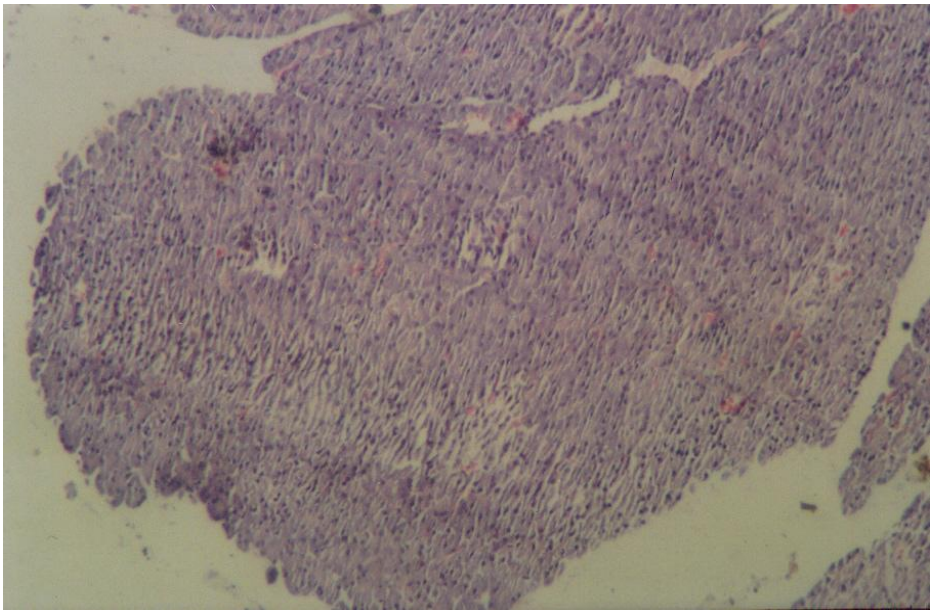

Fig. 78

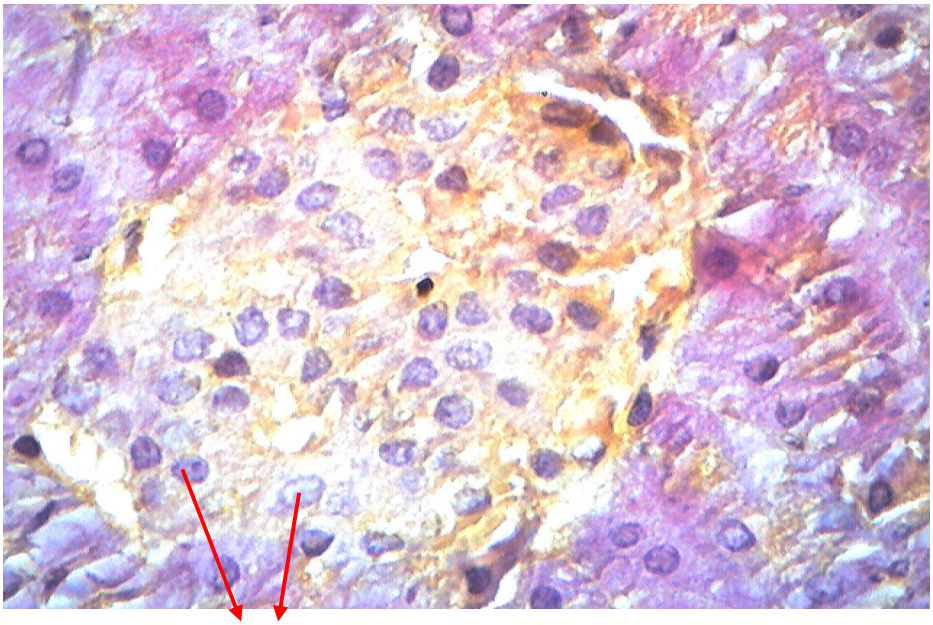

Fig. 79

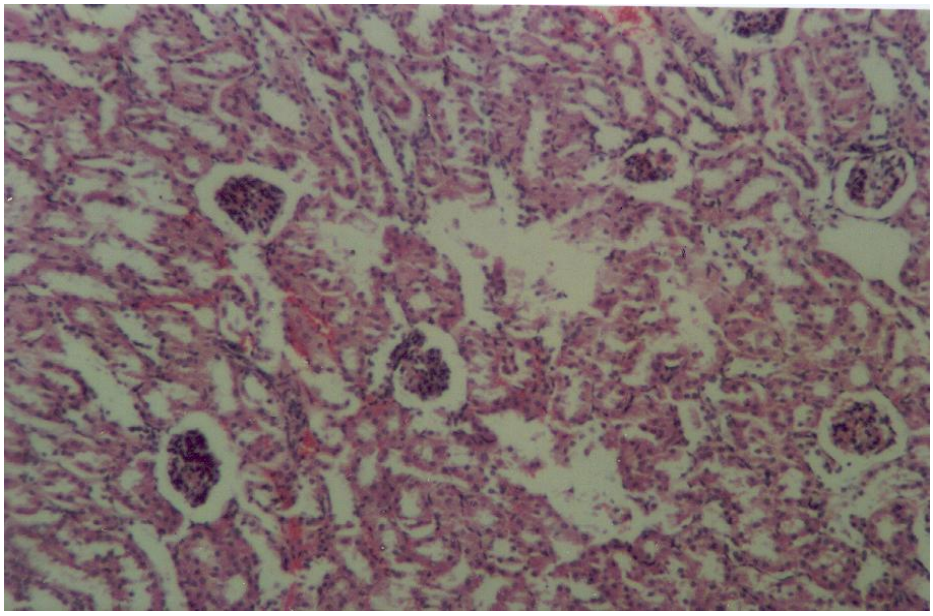

Fig. 80

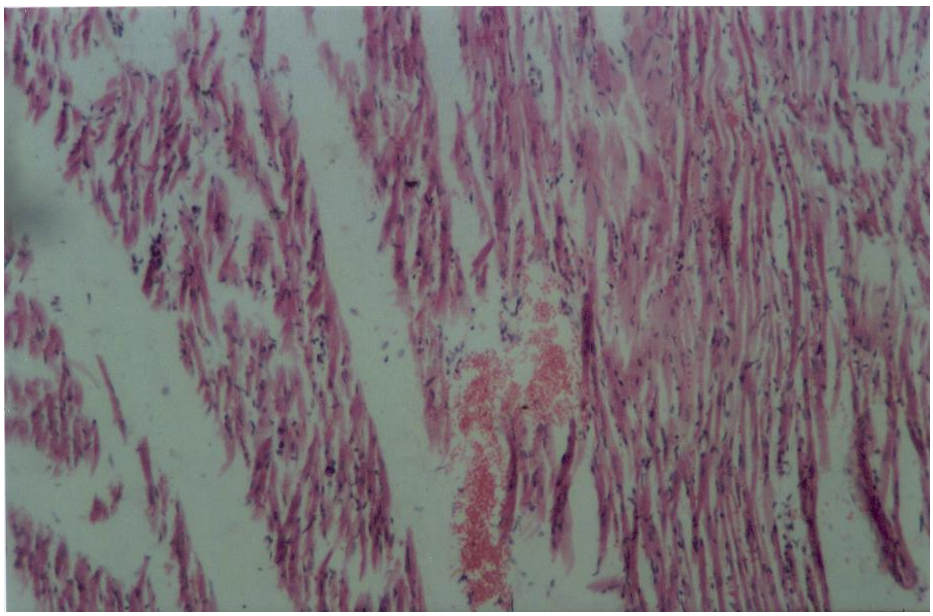

Fig. 81

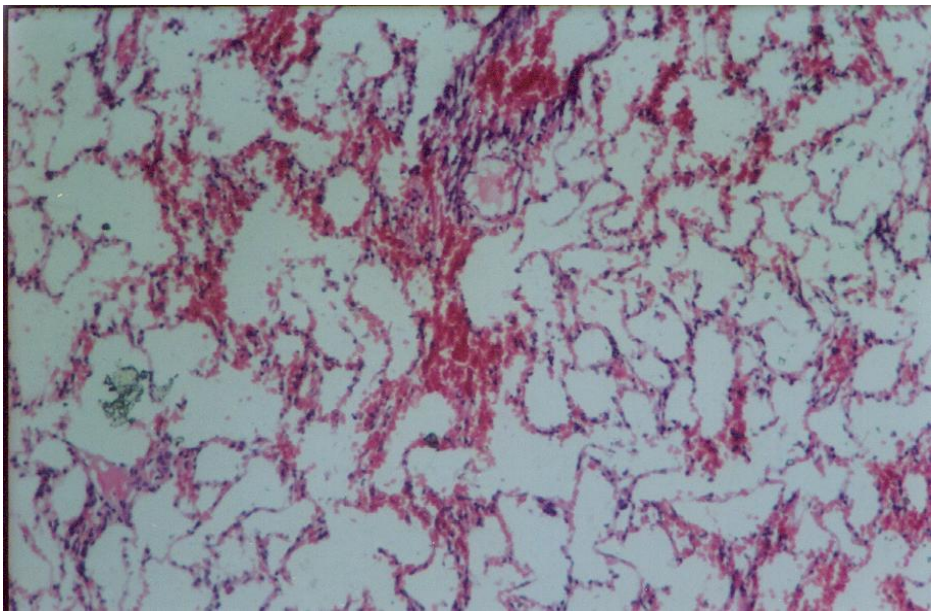

Fig. 82

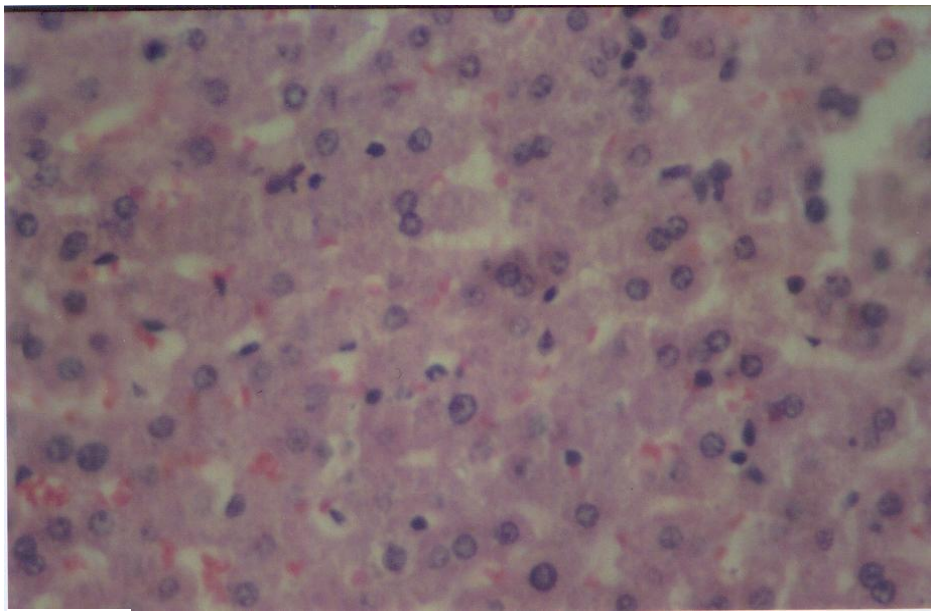

Fig. 83

Alloxan-induced diabetes mellitus in rabbits was confirmed by elevated blood sugar (F) level on first week after intraperitoneal administration of alloxan, followed by persistent hyperglycemia during the entire period of the experiment. Keen and NgTang (1982) reported that the minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration. Establishment of diabetes mellitus in rabbits in the present study, induced by alloxan administration, might be attributable to specific irreversible toxic effects of alloxan on β cells of pancreas (Dunn *et al.,* 1943; Lukenes, 1948). Fisher and Herman (1982) reported that alloxan is rapidly reduced in the body forming dialuric acid, that undergoes auto-oxidation yielding detectable amounts of hydrogen peroxide, superoxide anion ($^{-}\cdot O_2$) and hydroxyl free radicals ($^{-}\cdot H_2$); the latter being produced by metal catalyzed Haber-Weiss reaction. These reduced species of oxygen particularly the extremely reactive OH radical, are believed to initiate alloxan based attack on β cells. The deleterious effects of alloxan causing hyperglycemia, might be due to rapid inhibition of insulin secretary mechanism (Grodsky *et al.,* 1982). Malaisse (1982) suggested that alloxan and its metabolites have a tendency to concentrate in pancreatic islet tissue relative to some other tissue and that the selective cytotoxicity of alloxan was due to the function of three factors: efficient uptake, oxidant production by redox coupling of the drug with intracellular reductant (ascorbate and thiols) coupled with low levels of glutathione peroxidase in the islets of Langerhan's. In

vivo and in vitro experiments have demonstrated that alloxan elevates cytosolic free Ca^{2+} concentration in pancreatic beta cells (Kim *et al.*, 1994; Park *et al.*, 1995). This effect arises due to alloxan-induced calcium influx from extra cellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx might result from the ability of alloxan to depolarize pancreatic beta cells (Dean and Mathews, 1972). Depolarization of the cell membrane opens voltage-dependent calcium channels and enhances calcium entry into cells. A stimulatory effect on mitochondrial Ca^{2+} efflux with simultaneous inhibitory action on Ca^{2+} uptake by mitochondria was also found to be exerted by alloxan (Nelson and Boquist, 1982; Lenzen *et al.*, 1992). The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by H_2O_2 since it exerts a similar effect on calcium concentration in beta cells (Park *et al.*, 1995). The exaggerated concentration of Ca^{2+} contributes to supraphysiological insulin release and together with reactive oxygen species, causes damage of pancreatic beta cells (Kim *et al.*, 1994).

Fisher (1985) reported that a number of agents such as radical scavengers (e.g., Dimethyl urea); chelators of metal catalysts, a variety of relevant enzymes (e.g., Superoxide dimutase), nicotineamide and its analogues given to animals prior to alloxan administration prevented the diabetogenic effect of alloxan. Further, adrenergic agents (e.g., epinephrine, and clonidine) were shown to protect alloxan-induced diabetes (Nakadate *et al.*, 1983). Schauberger and his associates (1977) have observed that methanol, ethanol, n-propanol and n-butanol pretreatment of mice protects the animals from alloxan-induced diabetes. Pretreatment of mice with n-butanol causes hyperglycemia at the time of alloxan administration (Heikkila *et al.,* 1976) and glucose administration is known to protect animals from alloxan-induced β cell necrosis (Bhattacharya, 1953; Rossini *et al.,* 1975).

The four doses of intraperitoneal administration of alloxan given to fasted rabbits caused elevation of blood glucose upto sixth weeks. In the seventh week the blood glucose was almost constant. The multiple low doses of alloxan given at periodical intervals might be responsible for destruction of beta cells and establishment of diabetes mellitus. Similar methods for establishment of diabetes in fasted rabbits were followed by earlier workers (Rastogi *et al.*, 1998*;* Baqui *et al.,* 2005). Katsumata *et al.* (1992) reported that the intraperitoneal dose below 150 mg/kg b.w. is insufficient for inducing diabetes in the rat. Further, fasted animals have been reported to be more susceptible to alloxan (Katsumata *et al.,* 1992; Szkudelski *et al.,* 1998). The multiple low doses of alloxan were given because too high doses of alloxan administration cause loss of animals due to kidney tubular cell necrotic toxicity (Lenzen *et al.,* 1996). The blood sugar level in all the rabbits was on peak in the sixth week, plateaued on the seventh week and later on started to show fluctuations with a decreasing tendency. Alloxan, in low doses, has been reported to produce non-insulin dependent diabetes mellitus (NIDDM) like state which can progress to a gradual recovery or to an insulin dependent diabetes mellitus (IDDM) stage (Cooperstein and Watkins, 1981; Bailey and Flatt, 1991).

The changes in other biochemical parameters viz. blood urea and serum creatinine in alloxanized diabetic rabbits also increased consistently with blood sugar level. Blood urea and serum creatinine levels were on peak upto sixth week in consonance with blood sugar level and later on showed a fluctuations. The changes in blood urea and serum creatinine have been reported in rabbits following alloxan administration by other workers (Dubey *et al.,* 1994; Baqui *et al.,* 2005). These biochemical changes, which are indicative of renal damage, might be due to increased renal threshold for hyperglycemia. Deekert and Grenfel (1991) reported that the severity of renal disease positively correlates with the levels of blood urea and serum creatinine. Salah *et al.,* (2004) have characterized

the development of diabetic nephropathy by a progressive increase in albuminuria and a late decline in glomerular filtration rate, leading eventually to end stage renal failure.

The clinical signs of hyperglycemia in rabbits, as observed in the present study were polyuria, polydipsia, general weakness, lethargy and decreased physical activity. These findings are in agreement with earlier observations recorded by other workers in sheep (McCandlers *et al.,* 1984), goats (Prasad *et al.,* 1985), dogs (Nelson *et al.,* 1990; Rao *et al.,* 1998) and rats (Balasubramanian, 1991). The signs of polyuria and polydipsia might have been caused by the excessive fluid intake required to carry the increased glucose levels in the blood and exceeding the renal threshold (Doxy *et al.*, 1985). Similarly, the absorption of water by kidneys is inhibited by the osmotic diuresis, thus, resulting in polyuria. Nelson (1985) had observed the symptoms of a diabetic dog as polyuria, polydipsia, polyphagia and weight loss. Sandhu *et al.,* (2000) characterized alloxan induced diabetes mellitus in dogs by vomiting, polydipsia, polyuria, inappetence, dehydration, hypothermia, dullness, depression, hind leg weakness and recumbency followed by death.

The reduction in body weight, as observed in alloxan induced diabetic rabbits, is in consonance with earlier reports (McCandlers *et al.,* 1984; Prasad *et al.,* 1985; Mir *et al.,* 1995). The decrease in body weight might be due to insulin insufficiency leading to decreased accumulation of body reserve and an increased mobilization of endogenous energy store particularly fat (Edward, 1977). The alterations in body weight of diabetic rabbits were along with the changes of blood sugar, blood urea and serum creatinine throughout the experimental study. The disturbances in carbohydrate, lipid and protein metabolism are characteristic of diabetes mellitus (Milne, 1987).

Histopathological examination that revealed necrosis, degeneration and vacuolation of beta cells of islets of Langerhan's was due to the cytotoxicity of alloxan. These pathological features of beta cells induced by alloxan have been experimentally observed in animals (Sandhu *et al.*, 2000; Szkudelski, 2001; Mir *et al.*, 2005). The action of alloxan in the pancreas is preceded by its rapid uptake by the beta cells (Boquist *et al.*, 1983). Rapid uptake by insulin- secreting cells has been proposed to be one of the important features determining alloxan diabetogenecity. The other histomorphological changes in five-month-old diabetic rabbits showing chronic pancereatitis, haemorrhage, proliferation of fibroblasts and disorganization of pancreatic acini have been reported earlier (Thomson, 1989). Further, in long standing diabetes mellitus interstitial fibrosis of the exocrine tissue has been reported (Doniach *et al.*, 1973; Rahier *et al.*, 1983 a).

The kidneys, which excrete the waste products of metabolism and regulate the body concentration of water and salt, indicated impaired structural and functional activity in alloxan induced diabetic rabbits. Nephrosis, occlusion of tubules and degenerative changes in kidney observed in the present study are in agreement with other workers (Nakayama *et al.*, 1986; Bansal *et al.*, 1994; Mir and Baqui, 2005; Mir *et al.*, 2005). Thickening of the glomerular basement membrane and capillaries of diabetics have been reported earlier (Heidland *et al.*, 1996; Rabkin *et al.*, 1996) and might contribute to end stage renal damage. In the present study the long-term effects of diabetes on kidneys indicated chronic nephritis, interstitial nephritis and chronic changes in medullary sites. Previous studies on the long-term effects of diabetes in experimental animals show glomerular nephropathy along with tubular and interstitial abnormalities (Rasch, 1979; Hirose *et al.*, 1982). Further, histologic studies on kidneys showed proliferation of polygonal cells and resulted in occluding the lumen at some sites. Bulut and his associates (2001) have reported that glomerular capillaries entirely

fill the renal corpuscle along with mesangial cell proliferation and hypertrophy in alloxan-induced diabetic rabbits. In diabetic dogs, degeneration of glomeruli and tubular epithelium along with the presence of hyaline casts, mildly sclerotic glomerulus and coagulative necrosis of tubular epithelium has been reported (Sandhu *et al.,* 2000). The structural changes in kidneys could be attributed to altered metabolism in diabetes (Rasch, 1980) and the subsequent effects on the increased renal threshold for hyperglycemia (Mir *et al.,* 2005). Further, studies have shown that good metabolic control is beneficial in slowing the progression of nephropathy in diabetes, and if the duration of diabetes is prolonged before reinstitution of normoglycemia, nephropathy is not easily reversed (Floretto *et al.,* 1998; Renu *et al.,* 2004). Contrary to it diabetic nephropathy accounts for considerable morbidity and mortality even in patients with well-controlled blood sugar values (Grenfal, 1991).

In the present study histomorphological changes of liver in alloxan-induced diabetic rabbits showed degenerative changes such as hepatosis, biliary hyperplasia and chronic hepatitis. The pathoanatomical changes in liver of alloxan and streptozotocin induced diabetic animals has been previously reported (Herman *et al.,* 1999; Sandhu *et al.,* 2000). Liver, an insulin dependent tissue, playing a pivotal role in glucose and lipid homoeostasis, is severely affected during diabetes (Seifter and England, 1982). There is a profound alteration in the concentration and composition of lipid (Sochor *et al.,* 1985). Changes in glucose metabolism such as decreased glycolysis, impeded glycogenesis and increased gluconeogenesis in diabetic liver have been reported (Baquer, 1998). Further studies suggest that untreated diabetic liver result in hyperglycemia which in turn is known to activate isoforms of protein kinase C (PKC) in several tissues (Porte and Schwartz, 1996) and in hepatocytes, PKC is an intermediate step in the insulin transduction pathway that activates mitogen activated protein kinase (Adachi *et*

al., 1996). Mitogen activated protein kinase leads to decreased apoptosis and hyperplasia and finally results in diabetic hepatomegaly (Herman *et al.,* 1999).

The histopathological changes in heart such as histocyte proliferation, haemorrhage and myocarditis (inflammation of cells) observed in the present study could be attributed to the subsequent effects of hyperglycemia which induces degenerative changes in the tissues along with cardiomyopathy and nephropathy by oxygen free radicals (Oberley, 1988). Mechanisms that contribute to the formation of free radicals in diabetes mellitus include not only increased non-enzymic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defense systems (Griesmacher *et al.,* 1995). The evidence indicates that oxidative stress is increased in diabetes due to over production of reactive oxygen species and decreased efficiency of antioxidant defenses. Oxidative stress as well as non-enzymic glycosylation, is considered as a major factor contributing to the extent of chronic diabetic complications (Yaki, 1984; Griesmacher *et al.,* 1995; Gul *et al.,* 2000). Previous studies in diabetic patients had shown the higher prevalence and severity of atherosclerosis compared to non-diabetic population (Keen *et al.,* 1999) contributing to mortality and morbidity among diabetic subjects (Pyorala *et al.,* 1987). Furthermore, in diabetic subjects hyperglycemia, insulin resistance, abnormal lipid profile, oxidative modification of lipoproteins, increased blood pressure and altered rate fibrinolysis have been found to accelerate pathophysiology (Arvind *et al.,* 2002).

The present experiment demonstrated degenerative changes in neurons and edema in brain sections of alloxan-induced diabetic rabbits. A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals (Tomlinson *et al.,* 1992; Ozturk *et al.,* 1996). The variety of alterations, called diabetic neuropathy, affects the brain, spinal cord

and peripheral nerves (Gallego *et al.,* 2003). Diabetes aggravates brain damage in experimental and clinical subjects, accelerates maturation of neuronal damage, increases infarct volume and induces post-ischemic seizures (Muranyi *et al.,* 2003). Diabetic neuropathy has been related to excessive generation of sorbitol by aldose reductase due to maintained hyperglycemia, altered metabolism of phosphoinositides and reduced Na/K-ATPase activity (Greene *et al.*, 1987; Tomlinson *et al.*, 1992).

The histomorphological study of lung in the present study demonstrated significant alterations. These included edema, haemorrhage, bronchial hyperplasia and emphysema in the five month old diabetic rabbits. These findings were correlated with the severity of diabetes mellitus. There is the possibility that these morphological alterations observed in the lung after the alloxan treatment might be due to the direct action of alloxan per se, since it has been reported that a large dose of alloxan produced pulmonary oedema (Houssay, 1947; Aufdermaur, 1948) and changes in both the capillary endothelium and the alveolar epithelium of the lungs (Cottrell *et al.,* 1967). Sandhu *et al.* (2000) observed edema, collapse of alveoli, congestion and haemorrhage in lung sections of alloxan-induced diabetic dogs. The pulmonary emphysema observed in the present study is an additional observation. Therefore, the alteration in the lung of the rabbits treated with multiple doses of alloxan are most likely due to severity of hyperglycemia for a prolonged period. Previous studies have demonstrated that diabetic lungs in rats show depressed glucose oxidation (Morishige *et al.*, 1977) and a reduced rate of glucose incorporation into neutral lipids and phospholipids (Moxley and Longmore, 1975). These observations suggest that the disorder of glucose metabolism in diabetes mellitus may lead to a disturbance of the synthesis of the pulmonary surfactant in the lungs (Sugahara *et al.*, 1981). The present findings in the lungs of diabetic rabbits indicate pulmonary dysfunction in diabetic animals.

The present study further, revealed histomorphological changes in the gut of diabetic rabbits such as intestinal congestion and gastritis which might be attributed to the development of both macrovascular and microvascular complications reported in diabetes (Brownlee, 2001). Hypertrophy and hyperplasia of intestinal epithelium in diabetic animals are linked to absorptive abnormalities (Younoszai *et al.,* 1993). Previous studies in alloxan and streptozotocin induced diabetes in experimental animals have reported degeneration and necrosis at the tips of intestinal villi (Sandhu *et al.,* 2000) and greater intestinal weight with higher tissue water content (Schedl and Wilson, 2005).

Alloxan-induced diabetes mellitus serves as pathological model for detecting various diseases that are associated with diabetes. Experimental models of diabetes developed from chemical induction with alloxan have been most widely used (Rerup, 1970). Administration of alloxan to different animals produces, via necrosis of islets, several features common to those observed in human diabetes (Lukens, 1948; Gaulton *et al.,* 1985; Quan *et al.,* 2001). In the present study elevation of glucose in the blood established at the beginning of experiment and monitored in the course of the experiment was a conclusive proof of the experimentally produced pathological condition with the help of alloxan. Similarly, the increase in blood urea and serum creatinine of alloxan induced diabetic rabbits confirms the subsequent effects of hyperglycemia on the biochemical alterations of kidneys and indicates that effects could extend to other organs/tissues of animals. The decrease in body weight observed in the present experiment by alloxan is indicator of insulin insufficiency leading to decreased accumulations of fat and increased lipolysis. When comparing the biochemical parameters for glucose, blood urea and serum creatinine, and body weight of alloxanized diabetic rabbits with those of the saline-treated normal rabbits, statistical evaluation of the variations of all the parameters showed that the

changes had been significant both at the beginning and at the end of the experiment. Further, excess of the renal threshold for glucose leading to polyuria and polydipsia and other behaviour changes such as dullness, lethargy etc observed throughout the experimental study, also gives evidence of the diabetes production. Furthermore, the subsequent effects of hyperglycemia due to complete destruction of beta cell of pancreatic islets caused deleterious effects on other tissues of the animals. The pathomorphological features observed in pancreas, kidneys, liver, heart, lungs, brain and gut caused by alloxan were significant in contrast to normal rabbits.

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al.,* 2000). Intravenous administration of streptozotocin (65 mg/kg b.w.) in the present study effectively induced diabetes mellitus in rabbits and is in consonance with earlier methods of induction (Kedar and Chakrabarti, 1983; Tawfeeg and Sherif, 2001). The elevation of blood sugar level on day 2^{nd} confirmed the establishment of diabetes mellitus in rabbits which is attributed to its selective cytoxicity on beta cells and subsequently impairs glucose oxidation (Bedoya *et al.,* 1996). Two hours after injection of streptozotocin, the hyperglycemia is observed with a concomitant drop in blood insulin followed by hypoglycemia about six hours due to decrease in blood insulin levels (West *et al.,* 1996). The blood sugar level of the rabbits was on peak on day 2^{nd} after streptozotocin administration followed by changes with a decreasing tendency. The changes in blood glucose and insulin concentrations reflect abnormalities in beta cell function (Bedoya *et al.,* 1996). The fluctuations in the blood sugar might also be attributed to the sensitivity to streptozotocin that varies with species, strain, sex and nutritional state and there are batch differences in activity (Okamato, 1981). When administered intravenously, plasma levels of

streptozotocin rapidly decrease within 15 minutes and concentrate in the liver and kidneys (Sicor Pharmaceticals, 2003). Twenty percent of the drug is metabolized and/or excreted by the kidneys (Sicor Pharmaceuticals, 2003). The changes in blood urea and serum creatinine observed in the present study could be attributed to the functional and/or morphological changes in the kidneys (Alderson *et al.*, 2004). Kedar and Chakrabarti (1983) had reported elevated levels of blood sugar to 340 mg percent associated with glycolysis, ureamia, hypercholesterolemia, hypertriglyceridemia and loss of body weight in rabbits by a single intravenous injection of streptozotocin (65 mg/kg). Further, a significant increase of total protein excreted, albuminuria, glycosuria, and urinary urea levels indicated impaired renal function (Alderson *et al.,* 2004).

Streptozotocin effectively induced diabetes in rabbits characterized by polydipsia, polyuria, weight loss, decreased physical activities and hyperglycemia, which is in agreement with earlier findings (Calabresi and Chabner, 1985; Shenoy and Goyal, 2002). In streptozotocin induced diabetes there is excess of fatty acids in the serum, which promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Bopanna *et al.,* 1997).

The decrease in celularity within islets of Langerhan's observed in the present study reflects the cytotoxity of streptozotocin (Papaccio *et al.,* 2000; Szkudelski, 2001). The reduction in the number of beta cells was also confirmed in rabbits using special stains. Streptozotocin destroys beta cells selectively and a single adequate dose produces lasting hyperglycemia and insulin deficiency

(Szaleczky *et al.,* 1999). Previous studies have reported that streptozotocin enters the beta cells via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenecity of steptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after strptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, beta cells undergo destruction by necrosis (Szkudelski, 2001). Other studies indicated that cytotoxic effects of streptozotocin are dependent upon DNA alkylation by site-specific action with DNA bases (Benneth and Pegg, 1981) and by free-radical generation during streptozotocin metabolism (Bolzan and Bianchi, 2002). In the present study beta cells in some islets were found to be fusiform. The change in the shape of cells can be attributed to the partial damage of streptozotocin due to inadequate dose. Aybar *et al.,* (2001) have reported that use of lower dose of streptozotocin produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic.

The histomorphological study of the lungs observed in the present study indicated alterations such as congestion and haemorrhage in alveoli and bronchioles. Lung damage in streptozotocin induced diabetic hamsters has been reported (Popov and Simionescu, 1997). It is postulated that hyperglycemia affects the lungs by damaging capillaries and by the non-enzymatic glycosylation of collagen (Bell *et al.,* 1988). Hyperglycemia appears to cause cellular stress by a number of mechanisms, which could be detrimental to the lung (Brownlee, 2001). Firstly, hyperglycemia increase movement of glucose through polyol pathway and

sorbitols are produced which in turn causes osmotic stress to cells and dihydronicotine amide adenine dinuleotide phosphate (NADPH) is consumed, depleting intracellular glutathione. Secondly, hyperglycemia increases concentrations of advanced glycation end products. These golycosylated proteins are formed by non-enzymatic reactions, and changes in protein structure may alter their cellular functions. Thirdly, glucose activates various isomers of protein kinase C which in turn affects the expression of nitric oxide, endothelin, nuclear factor kappa B and plasminogen activator inhibitor. Finally, hyperglycemia increases the flux of glucose through the hexasomine pathway effecting inflammatory mediators and insulin resistance. The combined effect of the four mechanisms results in over-production of mitochondrial superoxides, causing cellular stress and damage (Brownlee, 2001).

The morphological study in kidneys of streptozotocin induced diabetic rabbits did not show any significant alteration. It has been reported that streptozotocin does not possess any significant nephrotoxic potential (Floretto *et al.,* 1998). However, the kidney sections showed congestion in the present study, which can be attributed to altered metabolism in diabetes (Rasch, 1980). The changes in the liver in diabetic rabbits induced by streptozotocin have been reported earlier (Mitra *et al.,* 1996). The diabetic liver showed degeneration and congestion. In diabetes, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Glucose 6-phosphatare increases in the liver, facilitating glucose release into the blood. The opposing enzymes which phosphorylate glucose is hexokinase, which is unaffected by insulin and glucokinase, which decrease in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under these circumstances the normal liver would shut off and deposit glycogen (Sheila and James, 1993).

The histopathological changes in the heart of streptozotocin-induced diabetic rabbits showed haemorrhage and cardiomyopathy which could be attributed to the hyperglycemia, which by the formation of oxygen free radicals induces degenerative changes in the tissues along with cardiomyopthy and nephropathy (Oberley, 1988).

In the present study the nervous system of streptozotocin-induced diabetic rabbits showed mild neuronal damage. Diabetes accelerates maturation of neuronal damage, increases infarct volume, and induces postischemic seizures (Muranyi *et al.,* 2003).

Furthermore, histomorphological study of alimentary canal did not show any significant alteration. However, stomach sections showed proliferation of yeasts. Although, the association between diabetes mellitus and increased susceptibility to infection is not supported by strong evidence (Wheat, 1980; Thornton, 1971) but many specific infections are more common in diabetic patients, and some occur almost exclusively in them (Joshi *et al.*, 1999). Further, there is evidence that improving glycemic control in patients improves immune function, which is exemplified by the fact that the efficiency of intracellular billing of microorganisms improves with better glycemic control (Gallacher *et al.,* 1995).

Streptozotocin induced diabetes mellitus in many animal species has been reported to resemble human hyperglycemic nonketotic diabetes mellitus (Weir *et al.,* 1981). This effect has been extensively studied and appears to be mediated through a lowering of beta cell nicotinamide adenine dinucleotide (NAD^+) and results in histopathological alteration of pancreatic islet beta cells (Karunanayake *et al.,* 1974). The present experiment, thus, confirms that a single intravenous injection of streptozotocin is capable of inducting diabetes mellitus in rabbits leading to biochemical, behavioural and structural alterations.

A significant improvement of biochemical indicators viz. blood sugar, blood urea and serum creatinine along with the amelioration of histomorphological changes in alloxan-induced diabetic rabbits by the oral administration of antidiabetic herbal/allopathic drugs was observed in the present study in comparison to saline treated diabetic rabbits. The normoglycemia in the rabbits observed in the present study by the administration of either *Abroma augusta* or *Syzygium jambolanum* might be due to the increased uptake of glucose peripherally and increased sensitivity of insulin (Habib *et al.*, 2005). In a number of studies the antihyperglycemic activities of the *Abroma augusta* and *Szygiumm jambolanum* either alone or in combination with other drugs have been reported (Das and Basu, 1970; Mukherjee and Shah, 1977; Halim, 2003; Bairy *et al.*, 2005). Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances (Collier *et al.*, 1987), some may inhibit insulinas activity (Bhide and Aiman, 1963) and others may increase beta cells in the pancreas by activating regeneration of these cells (Shanmugasundaram *et al.*, 1990; Abdel *et al.*, 1997). The fiber of plants may also interfere with carbohydrate absorption, thereby affecting blood glucose (Nelson *et al.*, 1991). Other studies have reported that administration of herbal products block the absorption of sugar molecules in the intestine and improve the body's ability to use sugar which would help to reduce blood sugar levels (Meir and Yaniv, 1985).

Previous laboratory studies have shown that Abromine, the active constituent of *Abroma augusta* identified as betaine is responsible for antihyperglycemic activity (Das and Basu, 1970; Mukherjee and Shah, 1977). The leaves of the plant contain octacosanol, terasxerol, β-sitosterol acetate and mixture of long chain fatty diols (Mukherjee and Shah, 1977 and 1978).

An antihyperglycemic effect has been reported in experimental and uncontrolled clinical studies on the seeds (Bansal *et al.*, 1981; Nair and

Santhakumari, 1986), fruit (Shrotri *et al*., 1963; Achrekar *et al*., 1991) and leaves (Sepaha and Bose, 1956; Soares, 2000) of Syzygium. Its chemical composition consists of tannins, resins (gambol), terpans (α-pigeon, β-pigeon, limenene), acids (gallic, palmitic, stearic, oleic), steroids (phytosterol), saponinic glycosides (antimelin) and flavanols (Albuquerque, 1989; Correa *et al.,* 1998).

A decline of biochemical indicators such as blood sugar, blood urea and serum creatinine of alloxan-induced diabetic rabbits following glimepiride (Sulphonylurea) treatment observed in the present study is in total agreement with earlier workers (Takada *et al.,* 1996; Krauss *et al.,* 2004). Sulphonylurea bind to specific receptors on beta cells resulting in closure of potassium ATP channels and subsequently open calcium channels leading to an increase in cytoplastic calcium that stimulates insulin release (Pilipson and Steiner, 1995). Glimepiride (a newer sulphonylurea) appears to have a more rapid onset than previous sulphonylureas (both glyburide and glipizide) and consequently less risk of hypoglycemia (Geisen, 1988). Other studies suggest that glimepiride has a potent extra pancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway (Takada *et al.,* 1996).

In the present study the improvement in blood urea and serum creatinine of diabetic rabbits following treatment therapies can be attributed to the recovery of renal function (Tedong *et al.,* 2006), which is explained by the regenerative capability of the renal tubules (Kissane, 1985). Studies have shown that good metabolic control is beneficial in slowing the progression of nephropathy in diabetes, and if the duration of diabetes is prolonged before reinstitution of normoglycemia, nephropathy is not easily reversed (Florretto *et al.,* 1998; Renu *et al.,* 2004). Tedong *et al.,* (2006) have reported that the normoglycemia in diabetic rats with treatment therapies could ameliorate the glomerular and tubular lesions

that characterize diabetic nephropathy and subsequently recover renal morphology and function.

The significant increase in the number of beta cells in the islets of Langerhan's by the application of antidiabetic drugs in comparison to saline treated diabetic rabbits can be attributed to the regenerative effect of plants on pancreatic tissue (Chakravarthy *et al.,* 1980; Shanmugasundaram *et al.,* 1990; Abdel *et al.,* 1997). Increase in pancreatic beta cells mass may result from mitotic proliferation of pre-existing islet cells, or islets may bud off from the ductal system of the pancreas (Slack, 1995), or arise from transformation of the acini into new islets, or may even be derived from the centro-acinar cells (Jindal *et al.,* 1995). There is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative/regenerative phase leading to nesideoblastosis (neogenesis of islets) (Hellerstrom, 1984; Bonner-Weir *et al.,* 1993). According to Waguri *et al.* (1997) the beta cells can regenerate either through differentiation of the precursor cells from the pancreatic duct, or proliferation from existing or surviving mature beta cells. Lipsett and Finegood (2002) reported beta cell neoformation from precursor cells in the pancreatic duct of diabetic animals. Schossler *et al.,* (2004) reported the regeneration of insulin producing cells in the pancreatic duct wall of *Syzgium cumini* treated alloxan-induced diabetic rats. Chakravarthy (1980) reported that *Pterocarpus marsupium* Roxb. acts as hypoglycemic agent by a selective regeneration of beta cells of alloxan damaged pancreas and that its presence can protect the beta cells against the necrotic effect of subsequently administered alloxan. Such evidences corroborate the suggestion that the drugs used in the present study possess the chemical substances that stimulate precursor cell differentiation causing regeneration of beta cells. Rastogi *et al.* (1988) reported β-

cell regeneration with homoeopathic drug *Cephalendra indica* Q in diabetized rats.

The pathological changes of diabetic organs are caused due to the production of oxygen free radicals (Oberley, 1988). Mechanisms that contribute to the formation of free radicals in diabetes mellitus include not only increased non-enzymic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defense systems (Griesmacher *et al.,* 1995). Free radicals meet many of the criteria required for a role in the pathogenesis of diabetic syndrome (Giron *et al.,* 1999). The reversal of oxidative damage shown as a measure of antioxidant enzymes with the antidiabetic compounds indicates that they have possibly antioxidant properties that play a crucial role in the defense against oxygen free radicals (Kaleem et al., 2005). The slight changes observed in the present study in different organs viz, pancreas, kidneys, liver, lungs and heart of group III and Group IV rabbits were significant in comparison to Group II rabbits. The amelioration of histomorphological changes can be attributed to the normoglycemia caused by the chemical substances therapeutic properties that mediate the stimulation of regeneration process and revitalization of remaining beta cells (Diatewa *et al*., 2004).

Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter and England, 1982). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer, 1998). The amelioration of histomorphological changes in the diabetic rabbits following treatment therapies as observed in the present study can be attributed to the increase in glycogen level in liver by an increase in glycogenesis and/or a decrease in glycogenolysis (Tedong *et al.,* 2006). Kamalakkanan *et al.,* (2003) reported that in liver the prevention of depletion of

glycogen is possibly due to stimulation of insulin release from beta cells that activate the glycogen synthase system. Herbomineral preparations have been reported to reverse histopathological changes in pancreas and liver partially by scavenging the free radicals and increasing the islet cell super oxide dimutase activity (Mitra *et al.*, 1996).

Diabetes mellitus is a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normal and continue for a retracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus. In the present experimental study, one group of rabbits was made diabetic by intraperitoneal administration of alloxan (@ 80 mg/kg b.w.) and the other group of rabbits was kept as control (normal healthy) which received normal saline. The establishment of diabetes mellitus in alloxanized rabbits was confirmed by periodical elevated levels of fasting blood glucose, blood urea and serum creatinine. The alloxan-induced diabetic rabbits showed a decline in body weight throughout the experimental period. Further, the diabetic rabbits showed a change in behaviour such as dullness, lethargy, decreased physical activity, a tendency to lie down, polyuria and polydipsia. The subsequent effect of hyperglycemia on tissue morphology of diabetic rabbits revealed degenerative changes in most of the organs. The pancreatic sections showed necrosis, vacuolations, increased eosinophilia, islet congestion, nuclear changes, karyolysis, disappearing of nucleus and rarefaction of nuclear contents. However, in five month old alloxan-induced diabetic rabbits chronic pancreatitis, haemorrhage, proliferation of fibroblasts and disorganization of

pancreatic acini were observed. Using special stains (Halmi, 1952 and Scott, 1952), the number of beta cells in pancreatic islets were found to be highly reduced in contrast to control rabbits. The kidney sections of alloxan induced rabbits showed nephrosis, occlusion of tubules, proliferation of polygonal cells, lower nephron nephrosis and degenerative changes in cortex, subcapsular region, collecting tubules and tubular epithelium. Further, chronic nephritis, interstitial nephritis, tubular nephrosis and chronic changes in medullary sties were observed in five month old diabetic rabbits. The liver sections showed hepatosis (degeneration of hepatocytes), biliary hyperplasia, chronic hepatitis in prolonged diabetic rabbits. Heart sections revealed degenerative changes such as edema, haemorrhage and histocyte proliferation. Myocarditis as also observed in prolonged diabetic rabbits. The brain sections showed degeneration of neurons and edema in cerebellum. The lung sections showed haemorrhage, bronchial hyperplasia and emphysema in prolonged diabetic rabbits. Furthermore, alimentary canal showed intestinal congestion and gastritis. All these histomorphological changes were observed in alloxan-induced diabetic rabbits in comparison to saline-treated (control) rabbits. The pathological changes in tissues were found to be aggravated with the progression of disease.

In another set of experiment, one group of rabbits were made diabetic by intravenous administration of streptozotocin (@ 65 mg/kg b.w.) and the second group of rabbits were kept as control that received normal saline. The onset of diabetes in streptozotocin-induced group of rabbits was checked by raised levels of blood glucose (F), blood urea and serum creatinine. Further, behavioural changes were also observed in streptozotocin-induced rabbits like alloxan diabetic rabbits. However, a decline in body weight was observed initially which latter on, showed an increased trend throughout the experimental period. The subsequent effects of streptozotocin-induced diabetes in different organs of rabbits showed histomorphological changes. Pancreatic sections

showed slight congestion and mild degenerative changes in the acini. The acinar epithelium showed swelling and the cells within islets were fusiform and diminished in number. Using special stains (Halmi, 1952 and Scott, 1952) for quantitative study of islet cells, the number of beta cells were found to be reduced in comparison to saline-treated normal rabbits. The lung sections showed congestion and haemorrhage in alveoli and bronchioles. Further, congestion in kidneys, degeneration and congestion in liver, haemorrhage and myopathy in heart, and mild neuronal damage were observed in the streptozotocin-induced diabetic rabbits. However, no histopathological findings were observed in the alimentary canal but stomach sections showed proliferation of yeasts.

The experimental study was further extended to elucidate the therapeutic efficacy of antidiabetic drugs on biochemical, behavioural and histomorphological parameters of rabbits. For this purpose, rabbits were made diabetic by intraperitoneal administration of alloxan (@ 80 mg/kg b.w.). When diabetes mellitus was well established which was confirmed by periodical estimation of blood glucose, blood urea and serum creatinine, the rabbits with a blood sugar (F) level above 250 mg/dl were selected for therapeutic study. The diabetic rabbits were divided into four groups viz., group II, group III, group IV and group VI excluding control (group I) rabbits. Group I served as normal control and received normal saline orally. Group II were untreated alloxanized diabetic rabbits and received normal saline orally. Group III alloxanized diabetic rabbits received water extract of *Abroma augusta* @ 2 ml daily. Group IV alloxanized diabetic rabbits received aqueous extract of *Syzygium jambolanum* @ 2 ml daily. Group V alloxanized diabetic rabbits received glimepiride @ 2mg/kg b.w. The assessment of treatment was checked on biochemical, behavioural and histomorphological ameliorations. The drug treated diabetic rabbits showed a significant improvement in behaviour in

contrast to saline-treated diabetic rabbits. The biochemical changes with regard to fasting blood sugar, blood urea and serum creatinine of all the drug treated diabetic rabbits showed a significant improvement comparable to the normal levels. However, glimepiride treated diabetic rabbits showed highly significant improvement in biochemical values followed by *Syzygium jambolanum* treated and *Abroma augusta* treated diabetic rabbits. Furthermore, a significant amelioration of diabetic organs were observed. The quantitative study of beta cells using special stains showed a high regeneration of beta cells in all the treated groups of diabetic rabbits. The highest number of regenerated beta cells were observed in glimepiride treated diabetic rabbits followed by *Syzygium jambolanum* treated and *Abroma augusta* treated diabetic rabbits respectively.

The therapeutic study for the management of diabetes mellitus in rabbits indicates that the drugs contain a variety of herbal/non-herbal ingredients that seem to act on a variety of targets by various modes and mechanisms. The entire experiment presents an overview of diabetic pathogenesis, particularly impaired carbohydrate metabolism leading to hyperglycemia. It analyses how herbal/allopathic medicines and their ingredients correct/manipulate the vitiated homeostasis of carbohydrate metabolism and other related complications particularly biochemical and histomorphological changes.

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