Chapter 1.
Hyperglycemia and Testicular Functions
1.1 INTRODUCTION

For many centuries doctors have been familiar with a disease that features sweet urine. Historically, knowledgable doctors faced with a patient who was losing weight, was constantly thirsty, and was passing for more than the usual amount of urine, always felt obliged to check the urine for sweetness. In some European languages diabetes mellitus used to be known as the "Sugar Sickness".

The term "diabetes" derives from Greek word meaning 'to go through'. This refer to the fact that a person with untreated diabetes drinks a lot and passes a lot of urine. There are a number of different kinds of diabetes, but the one usually referred to as the full name diabetes mellitus. 'Mellitus' comes from the latine word 'mel' meaning 'honey'.

The hypothetical antidiabetic substance thought to be secreted by the islets was named 'Insulin' in 1909 by DeMeyer, but definitive proof of its existence was not obtained until 1922 when Frederick Banting and Chastles Best successfully extracted insulin from dog pancreas (Banting and Best, 1922 et al., 1962).

"Diabetes is a chronic hereditary disease characterised by abnormally high level of glucose in blood and later excretion in urine. The basic defect is an absolute or relative lack of insulin which leads to abnormalities of metabolism of carbohydrates, protein and fats. There are following types of diabetes
1) The type - I or insulin - dependent diabetes, in which the sufferer produces little or no insulin, affect about 1% of the population. It commonly occurs during youth. This type of
diabetes is commonly called as juvenile diabetes. It requires life long treatment, constant checking of the level of sugar in the blood, and a regular watch for complications. Insulin, being a protein which is digested in the bowel, cannot be given by mouth or it would be broken down before it reaches the blood stream, so injection is necessary at least once a day, often twice.

2) The type - II diabetes, often called maturity-onset diabetes is usually associated with obesity and can be regarded as a condition in which the amount of insulin produced by the pancreas and may be near normal - is not enough for the excessive bulk of tissue in the body.

Many cases of type - II diabetes can be cured simply by dieting and weight loss. This reduces the sugar demands but, possibly more important, is also makes lowered demand on the insulin supply. Other cases require oral anti-diabetic drugs which stimulate the pancreas to produce more insulin.

Non insulin dependent diabetes mellitus type II diabetes is characterised by retention of endogenous pancreatic insulin secretion, which altered secretory dynamics, the absence of ketosis and insulin resistance due to diminished target cell response to insulin and obesity (Pfreifffer et al., 1981; Rizza et al., 1981, Olefsky, 1981).

Type II diabetes is genetically based. The primary defect is not known and may differ among patient. Obesity, a common precursor of type II diabetes, is associated with insulin resistance (decreased binding of insulin to cells membranes accompanied by decreased number of receptors, the highly specific sites of insulin cell interaction responsible for activating transmembrane glucose transport and use) (Olefsky, 1990).
Stresses that affect the cells of the body seem to set the stage for diabetes in people. Stresses can be emotional or physical such as surgery or a serious infection, an accident or emotional shock. Many medications affect the body in stressful way. Pregnancy also place extra stresses on the body and diabetes is often diagnosed in pregnant women who have repeated miscarriages.

Insufficiency of insulin either absolute or relative is considered to be the basic cause for both human and experimental diabetes. The insulin insufficiency not only causes disturbances of carbohydrates, but the occurrences of abnormalities of lipid and protein have been well recognised (Eder 1979). Increase in serum non-esterified free fatty acid, cholesterol and tryglycerides is the characteristic of experimental diabetes (Schein et al., 1971; Topping and Targ 1975, Gupta and Dixit, 1981).

Since the diabetes was known to interfere the male reproduction causing its deleterious effects in human testes. The diabetic hazards on the reproductive system of diabetic man have been reported by Irisawa et al., 1966, Faerman et al., 1972, Kolodny et al., 1974. Impaired fertility accompanied by pathological changes in the testes and male accessory organ were reported in streptozotocin diabetic rats (Oksanne, 1975; Paz et al., 1978, Dixit 1978a; Gupta and Dixit et al., 1981) in alloxan diabetic rats and dogs (Soularias et al., 1948, Dixit et al., 1980). Complication of long term diabetes are chiefly vascular in nature and may lead to marked disability due to involvement of large vessel (Ganda, 1980) in the brain, kidneys (Mauer et al., 1981) and extremities (Scaffidi et al., 1975); Paz et al., (1978b) and Paz, Homonnai,
freely soluble in water and lightly acidic with 
pka. 6.63. On standing of room temperature 
it decomposes to alloxation, oxolate ,urea, 
carbon dioxide and other chemical substances 
but at low PH, the aqueous solution of 
Alloxan is fairly stable. Alloxan acts as a 
diabetogen in rats, mice, rabbits, dogs, sheep 
and monkey, cat, man, turtile, pigeon 
(Lukens, 1948) and mouse. (Lazaraw, 1947). 
But guinea pigs are resistance to alloxan 
(West and Highest, 1948a,b; Johnson, 
1950 a,b).

In normal fasted animals the blood 
glucose level after alloxan injection 
fluctuates in triphasic pattern. There is early 
marked hyperglycemia of short duration 
followed by hyperglycemia of long duration. 
Permanent diabetes produced by alloxan was 
found to be associated with ketoacidosis in 
dogs and cats and require, insulin treatment 
for survival, whereas with rat and mice 
spontaneous remission was reported 
(Gondos and Bevier, 1995).

After a diabetogenic dose of alloxan a 
massive necrosis of β- cells in the islets of 
langerhans is observed in most mammals and 
certain other species (Bailley et al.,1944; 
Duff,1945; Lukens, 1948; Lazarus anal 
Volk, 1962). The effects of alloxan were so 
specific that besides slight and most 
reversible changes in the kidney and adrenal 
medulla (Hard and Carr, 1994) no other 
histological changes were encountered.

The main disadvantage of alloxan is its 
high over all toxicity, especially its 
nephrohepato and capillar toxicity. However, 
the secondary dearrangement in diabetes 
mellitus is man are faithfully reproduced in 
alloxan diabetics and hence alloxan diabetes 
is still used as on of the favorable 
experimental model.
Goals of therapy:

Goals of therapy in diabetes include receiving the symptoms of hyperglycemia and hypoglycemia attaining optimal body composition, and identifying and managing long-term diabetic complications and their consequences. Target glucose levels (Gerich, 1989) should be adjusted to suit individual needs. (Management of Type II diabetes mellitus, 1988)

The importance of tight control (i.e., maintaining the blood glucose level as close to normal as possible to prevent or delay vascular and neurologic complications) is supported by evidence from animal studies and from natural history, cross-sectional and epidemiologic studies (Klein et al., 1988). In addition renal transplantation as well as up to 2 years of continuous subcutaneous insulin infusion in patients with type I diabetes were shown to benefit glomerular structure and function. Symptomatic improvement in nerve function and lessening of neuropathic pain were also observed. However randomized controlled trails of the effect of treatment of the progress of established micro vascular and macro vascular complications in type II diabetes (Knatterud et al., 1982 and Miettinen et al., 1985) and of retinopathy in Type I diabetes (Westphal and Goetz, 1990, Siperstein, 1988). The risks and benefits of sustained near normoglycemia in Type I diabetes are being examined in the diabetes control and complications. Trial of prospective 10-year program sponsored by US National Institutes of Health. Until the results are available it is sensitive to assume that the onset or progress of micro vascular complication may be delayed or reduced by glycemic control (DCCT Research Group, 1990; Blood glucose control in diabetics, 1990).
Table 2: Organ Weights of Male albino rat

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<th>Treatment Group</th>
<th>Testis</th>
<th>Epididymides</th>
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<td>± 0.19</td>
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1.3.2 Histological Observations

i. Pancreas

Control: (Fig. 1)

Pancreas is a diffused gland consisting of branched tubules whose terminal swelling are called alveoli. Alveoli form groups of glandular cells, acini. Each acinus in section appears as a ring of columnar cells having large nuclei and clear non-granular cytoplasm. The cells surrounded a central space or lumen. The lumen of the acini and tubules are connected by small pancreatic ductules which unites to form a large pancreatic duct which finally opens into the common bile duct. The cells of the lobules or acini secrete the pancreatic juice which contains enzymes responsible for digestion of protein, carbohydrates and fats. Interspersed between the acini are present, small isolated groups of cells known as pancreatic islets or islets of Langerhans. Three type of cells can be distinguished in these islets. The α cells are stained by acidic dyes and the β cells by basic dyes. The β cells secretes the hormones insulin which influences the metabolism of glucose. The α cells secrete another hormone called glucagon. It causes glycogenolysis and elevates the blood glucose contents.

Diabetic: (Fig. 2)

A 48 hour of diabetic induction, degranulation of β-cells. Their population was reduced.

Intact Control: (Fig. 1)

Micrograph showing islets of Langerhans surrounded by pancreatic acini. β-cell at centre have dark staining and α-cell which are at periphery have light staining. Haematoxylin and Eosin × 200.

Diabetic: (Fig. 2)

Microphotograph showing vacuolization in α-cell and degranulation of β-cell. Haematoxylin and Eosin × 200.
Intact Control: (Fig. 3)

The testes in control animals present a picture with all successive stages of spermatogenesis i.e. spermatogonia, and secondary spermatocytes, spermatids and spermatozoa. In the centre of the tubule, bunches of spermatozoa can be seen adhered to sertoli cells. In the intertubular spaces healthy Leydig Cell, connective tissue and blood vessels are present. Control testes showed active spermatogenesis.

Diabetic: (Fig. 4)

After 48 hours of induction of diabetes in albino rats the relative amount of interstitial tissue was decreased. Spermatocytes were completely absent.

Functional Leydig cells in the interstitial tissue could be seen. The seminiferous tubule diameter was decreased when compared with control.

*Fig. 3*

![Seminiferous tubule and spermatozoa](image)

*Fig. 4*

Microphotograph showing normal spermatogenesis. Eosin and Haematoxylin x 200

Microphotograph showing reduced seminiferous tubule diameter and degenerating spermatogenic cells. Haematoxylin and Eosin x 200.
Intact Control: (Fig. 5)

Cauda Epididymides show enlarged tubules. The structure is lined with pseudostratified epithelium with low columnar cell. The lumen was filled with a large number of mature spermatozoa.

Diabetic: (Fig. 6)

In alloxan induced diabetic rats the tubular diameter was reduced. Epithelial cell heights were low in comparison with control. Stereo cilia were few in number and the sperms were absent. Intertubular stroma was increased.

Intact Control: (Fig. 5)

Micrograph showing normal epithelial lining. The lumen is full of sperms.

Haematoxylin and Eosin × 200

Diabetic: (Fig. 6)

Microphotograph showing reduction in size of tubule and epithelial cell height. The lumen is devoid of sperm.

Haematoxylin and Eosin × 200.
iv. CAPUT EPIDIDYMIDES

Intact (Control): (Fig. 7)

The caput epididymides of control rats are lined with columnar epithelium. The columnar cell were provided with stereocilia which helps in forming the pathway for the secretion of cells into the lumen. The lumen was filled with spermatozoa. The intertubular stroma was filled with connective tissues.

Diabetic:

Histological observations of caput epididymides of diabetic rats showed shrinkage tubules and reduced epithelial cell height. Stereocilia were few in number and the lumen was devoid of spermatozoa.

Fig. 7

Intact Control: (Fig. 7)

Micrograph showing tubules lined by columnar epithelium with stereocilia. The lumen is full of sperms. Haematoxylin and Eosin × 200

Diabetic: (Fig. 8)

Microphotograph showing reduction in epithelial cell height and tubular size. Also the lumen is devoid of sperms. Haematoxylin and Eosin × 200.
v. VAS DEFERENS:

Intact Control: (Fig. 9)

Vas deferens conducts spermatozoa from epididymis to urethra. A thick walled muscular tubule consisting of inner and outer longitudinal layers and an intermediate circular layer lined by pseudo epithelium are its peculiar characters. The epithelial and its supporting lumina propria forms the longitudinal folds. The lumen was full of mature spermatozoa.

Diabetic: (Fig. 10)

In diabetic rats some changes were noticed after 48 hours of duration. The epithelium was normal. Stereocillia were present. Lumen was shrunken and devoid of sperm.

Fig. 9

Fig. 10

Intact Control: (Fig. 9)

Micrograph showing normal histoarchitecture and lumen is filled spermatozoa. Haematoxylin and Eosin × 200

Diabetic: (Fig. 10)

Microphotograph showing normal histoarchitecture but there is reduction of spermatozoa in lumen. Haematoxylin and Eosin × 200.
vi. VENTRAL PROSTATE

Intact + control: (Fig. 11)

The ventral prostate is surrounded by a vacuole of fibroblastic tissue containing numerous smooth muscle fibres in its inner layer. From the capsule broad septa penetrate into the interior to form the unusually abundant stroma which separates the scattered tubules. The stroma is rich in elastic fibres and contains numerous smooth muscle fibres which course in various direction.

Diabetic: (Fig. 12)

In alloxan induced diabetic rats the ventral prostate showed reduced glandular lumen. The epithelial wall was reduced with vacuolated cytoplasm with no papillary formation. The lumen was devoid of secretory material.

Intact Control: (Fig. 11)

Micrograph showing less amount of fibromuscular stroma and normal epithelium. The lumen is full of secretion. Haematoxylin and Eosin × 200

Diabetic: (Fig. 12)

Microphotograph showing reduced tubular size and secretion in lumen is absent. Haematoxylin and Eosin × 200.
Intact Control: (Fig. 13)

The mucosa was folded in a complicated manner, forming numerous irregular chamber for crypts many of which were continued as glandular tubules. The epithelium was pseudo-stratified, being composed of cuboidal or columnar cells and irregularly shaped basal cells. The cells contained yellow pigment. The lamina propria was rich in elastic fibres and forms a continuous layer around the vesicle outside the lamina propria were smooth muscles, which could be divided into circular and longitudinal layers.

Diabetic: (Fig. 14)

The epithelium of seminal vesicle of diabetic rats was atrophied. Secretions were less in amount. The muscle and connective tissues were thick.

Intact Control: (Fig. 13)
Micrograph showing normal secretory activity is conspicuous as luminal space is full of secretion. Haematoxylin and Eosin x 200

Diabetic: (Fig. 14)
Micrograph showing reduction in epithelial cell height and observe of secretory material in the lumen. Haematoxylin and Eosin x 200.
1.3.3 SPERM DYNAMICS: Table 3

a) Sperm Motility:

The motility of sperm in cauda-epididymides of diabetic rats was significantly reduced when compared with control group.

b) SPERM DENSITY

i) Cauda epididymides:

In diabetic rats sperm density was reduced significantly in comparison with intact control group.

ii) Testes:

A slight significant reduction in sperm density in the testes was noted of diabetic rats, when compared with control group.

iii) Seminiferous tubule diameter

The seminiferous tubule diameter was slightly significantly reduced in diabetic rats when compared with intact control group.

iv) Leydig cell nuclear diameter:

In diabetic rats the Leydig cell nuclear diameter was significantly reduced when compared with intact control group.

1.3.4. EPITHELIAL CELL HEIGHT:

a) Caput epididymides:

Diabetic rats showed a significant reduction in the epithelial cell height of caput, when compared with intact control group.

b) Cauda epididymides:

The epithelial cell height of cauda showed a significant reduction in diabetic rats, when compared with intact control group.

c) Seminal vesicle:

Alloxan induced diabetic rats resulted in the reduction of epithelial cell height significantly of seminal vesicle when compared with intact control group.
Table No. 3 Histomtrical Parameters Fertility Test and Sperm Dynamics of male albino rats

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Sperm Density in (million/ml) Testis</th>
<th>Sperm Density in (million/ml) Cauda</th>
<th>Sperm Motility (%) Cauda</th>
<th>Seminiferous tubules Diameter</th>
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<tr>
<td>Intact ©</td>
<td>5.19</td>
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<td>67.17</td>
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<td></td>
<td>0.27</td>
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<td>Diabetic</td>
<td>3.48</td>
<td>22.47</td>
<td>32.28</td>
<td>245.80</td>
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<td></td>
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<td>1.92</td>
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<td>4.89</td>
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<table>
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<th>Treatment Group</th>
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<td>32.05</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>1.86</td>
<td>1.86</td>
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1.3.5 TISSUE BIOCHEMISTRY: Table 4

a) Fructose:

An elevation in the fructose level of seminal vesicle was noticed significantly in alloxan induced diabetic rats at 48 hours, when compared with diabetic control group.

b) Ascorbic Acid:

A significant decrease in the adrenal gland, ascorbic acid was noticed at 48 hours in alloxan induced diabetic rats, when compared with intact control group.

c) CHOLESTEROL:

i) Testes:

The testicular cholesterol showed a significant increase at 48 hours in alloxan induced diabetic rats, when compared with intact control group.

ii) Adrenal glands:

A significant elevations were recorded in adrenal gland cholesterol level at 48 hours in hyperglycemic rats in comparison to the control group.

d) GLYCOGEN:

i) Testes:

A significant decrease in testicular glycogen at 48 hours was noticed in alloxan induced diabetic rats in comparison with the control group.

e) PROTEIN:

i) Testes:

Testicular proteins were depleted significantly in diabetic rats at 48 hours in comparing with the control group.

ii) Caput Epididymides:

Protein concentration of the caput epididymies depleted significantly in diabetic rats at 48 hours duration, when compared with intact control group.

iii) Cauda Epididymides:

A significant decrease in cauda epididymides protein of diabetic rats at 48 hours were recorded on comparing with diabetic rats control group.
iv) Seminal vesicle:

At 48 hours a highly significant reduction in seminal vesicular proteins was seen in diabetic rats, when compared with intact control group.

v) Ventral prostate:

A significant decrease in protein of ventral prostate contents was noticed in alloxan induced diabetic rats at 48 hours in comparing with diabetic control group.

vi) Vas deference:

The vas deference protein content showed significant decrease at 48 hour comparing with intact control group.

f) SIALIC ACID:

i) Testes:

A highly significant decrease in testicular acid content was observed in alloxan induced rats at 48 hours when compared with intact control group.

ii) Caput epididymides:

A significant decrease was observed in sialic acid content of caput epididymides at 48 hours in alloxan induced diabetic rats comparison to the control group.

ii) Cauda epididymides:

In alloxan induced diabetic rats a significant decrease in sialic acid contents of cauda epididymides was seen when compared with intact control group.

iv) Seminal vesicle:

The seminal vesicle sialic acid content showed a significant decrease at 48 hours after alloxan induced diabetic, when compared with intact control group.

v) Ventral prostate:

The level of sialic acid in the ventral prostate of diabetic rats was significantly low at 48 hours, on comparison with that control group.
vi) Vag deference:

A significant lowering of vag deference was observed in hyperglycemic rats, when compared with intact control group.

<table>
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<tr>
<th>Treatment Group</th>
<th>Fructose (mg/gm) Sem. Ves.</th>
<th>Ascorbic Acid Adrenal</th>
<th>Cholesterol (mg/gm) Testis</th>
<th>Cholesterol (mg/gm) Adrenal</th>
<th>Glycogen (mg/gm) Testis</th>
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**Table No. 4 Tissue Biochemistry**

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<th>Cauda</th>
<th>Seminal Vesicle</th>
<th>Ventral Prostate</th>
<th>Vag Deference</th>
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<tbody>
<tr>
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**Protein (mg/gm)**

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<th>Cauda</th>
<th>Seminal Vesicle</th>
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**Sialic Acid (mg/gm)**

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</table>
1.3.6. Serum biochemistry : Table 5

i) Protein :

Significant decrease was noted in the serum proteins after 48 hours of diabetic inductions, when compared with control group.

ii) Cholesterol :

The serum cholesterol level significantly increased in alloxan induced diabetic rats in comparison to control group.

iii) HDL cholesterol :

Alloxan induced diabetic rats resulted in the reduction of serum HDL cholesterol levels which were found to be a highly significant, when compared with control group.

iv) Triglycerides :

Diabetes induced by alloxan injection increased the triglycerides level significantly after 48 hours when compared with intact control group.

v) Phospholipids :

Serum phospholipids were elevated significantly in alloxan induced diabetic rats at 48 hours in comparison with that of the control group.

vi) Serum glutamic oxaloacetic transaminase (SGOT) :

The SGOT level in diabetic animals were increased significantly in diabetic rats, when compared with intact control group.

vii) Serum glutamic pyruvic transaminase (SGPT) :

The SGPT level remained significantly high in alloxan diabetic rats after 48 hours when compared with intact control group.
### Table No. 5 Serum Biochemistry of Male Albino Rats

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<tr>
<th>Treatment Group</th>
<th>Protein (mg/100 ml)</th>
<th>Cholesterol (mg/100 ml)</th>
<th>HDL Cholesterol (mg/100 ml)</th>
<th>Triglyceride (mg/100 ml)</th>
<th>Phospholipids (mg/100 ml)</th>
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<table>
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<td>37.55</td>
</tr>
<tr>
<td></td>
<td>3.58</td>
<td>2.97</td>
<td>0.98</td>
<td>2.90</td>
</tr>
</tbody>
</table>

### 1.3.7. HAEMATOLOGICAL PARAMETERS:

**Table 6**

e) **Haematology:**

The RBC, WBC, haematocrite and haemoglobin of diabetics rats were within the normal range in comparison to intact control group.

e) **Blood glucose:**

Single dose of intraperitoneal administration of alloxan to rats caused a highly significant increase in blood glucose, when compared with intact control group.
Table No. 6 Haematological Parameters of Male albino Rats

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>RBC (million/mm³)</th>
<th>WBC (million/mm³)</th>
<th>Haemoglobin (gm%)</th>
<th>Haematocrit</th>
<th>Blood Sugar (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>4.43</td>
<td>8182.83</td>
<td>13.92</td>
<td>39.90</td>
<td>93.32</td>
</tr>
<tr>
<td></td>
<td>0.58</td>
<td>350.16</td>
<td>0.44</td>
<td>2.31</td>
<td>4.07</td>
</tr>
<tr>
<td>Diabetic</td>
<td>4.72</td>
<td>7923.17</td>
<td>12.93</td>
<td>32.77</td>
<td>420.35</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>217.04</td>
<td>0.73</td>
<td>2.13</td>
<td>13.29</td>
</tr>
</tbody>
</table>

1.3.8 Testicular Cell Population Dynamics:

i) Spermatogonia:

The spermatogonia count was within normal when alloxan (15mg/100 gm) administered to intact rats comparison with control group.

ii) Spermatocyte (Primary):

A slight significant decrease in spermatocyte (primary) count were observed in diabetic treatment group (15mg/100 gm b.w. of alloxan) after 48 hours when compared with control group.

iii) Spermatocyte (secondary):

Alloxan treated to intact rats (15mg/100 gm b.w.) resulted in significant reduction in secondary spermatocyte count, when compared with control group.

iv) Spermatids:

The spermatids counts was a significantly decreased, after alloxan administered to intact rats (15mg/100gm b.w.) in comparison to control group.

v) Fibroblast:

The fibroblast count was within normal range in alloxan (15mg/100gm b.w.) administered to intact rats in comparison with
control group.

vi) Immature Cells:

Diabetic rats showed a significant change in immature Leydig cell counts when compared with control group.

vii) Mature Leydig cells:

The counts of mature Leydig cell of diabetic treated rats showed a highly significant decrease when compared with control group.

viii) Degenerating Cells:

Alloxan (15mg/100 gm b.w.) when administered to intact resulted in a highly significant increased in degenerating cell count in comparison with control group.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Spermatogonia</th>
<th>Spermatocytes (Primary)</th>
<th>Spermatocytes (Secondary)</th>
<th>Spermatids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact ©</td>
<td>21.80 0.19</td>
<td>17.05 0.24</td>
<td>57.10 0.32</td>
<td>138.13 1.11</td>
</tr>
<tr>
<td>Diabetic</td>
<td>19.92 0.15</td>
<td>15.18 0.22</td>
<td>35.32 0.28</td>
<td>52.40 2.99</td>
</tr>
</tbody>
</table>

**Table: Testicular Cell Population dynamics of Male albino Rat**

**Germinal Cell Type**

**Interstitial Cell Type**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Fibroblast</th>
<th>Immature Leydig Cells</th>
<th>Mature Leydig Cells</th>
<th>Degenerating Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact ©</td>
<td>54.30 1.57</td>
<td>51.18 0.94</td>
<td>63.15 1.22</td>
<td>17.16 0.20</td>
</tr>
<tr>
<td>Diabetic</td>
<td>50.25 0.69</td>
<td>38.68 3.77</td>
<td>29.22 0.13</td>
<td>69.11 0.38</td>
</tr>
</tbody>
</table>
1.4 DISCUSSION

Alloxan induced diabetic rats showed a reduction in the weight of testes and sex accessories as has been reported by Dixit et.al., (1980). Loss of body weight was recorded in diabetic rats (Oksanen, 1975; Dixit et al., 1980; Dixit and Gupta, 1981)

Reduction in the weight of testes and sex accessories viz, epididymides, spermatids and spermatozoa in the testes and the reduced secretory activity of the seminal vesicle and ventral prostate. This could be due to disturbed glucose metabolism (Chinoy et al., 1977) Defective spermatogenesis in the testes is reported to be associated with diabetes of longer duration (Foglia et al., 1969; Rosenmann et al., 1974; Oksanen, 1975).

Severe hyperglycaemia resulted in testicular dysfunctioning in the adult albino rat. The degenerative changes in the seminiferous tubules have been reported in alloxan diabetic rats. These results are in agreement with the findings of Irisawa et al., 1966, Foglia et al., 1969.

Scaffidi et al., (1975) reported testicular lesions in diabetic rats range from premature sloughing of germinal epithelium to complete cessation of spermatogenesis. Decreased seminiferous tubule diameter in the testes of diabetic rats reflects the reduced functional state of the laydig cells. Reduced Laydig cell populations in the diabetic man and experimental animals was shown by Faerman et al., 1972. The breakdown of Leydig cell components in the diabetic rats was reported by Orth et al., 1979.

A reduction of serum LH and testosterone levels caused the depression of
testicular function in diabetic rats has been ascribed to a partial blockage of GnRH and LH secretion (Paz and Homonnai 1979b) on the other hand Murray et al., (1978) reported no significant reduction in plasma LH and lowered FSH levels in streptozotocin diabetic rats. FSH acts synergistically with LH in stimulating androgen production, the depression levels of FSH could play a role in the lowered output of testosterone by diabetic animals. Spontaneous release of the testosterone from the Leydig cells is known to be under LH control (Hauger et al., 1977). A deficit glucose utilization by the anterior pituitary has been demonstrated in vitro in insulin deficit rats (Goodnez and Freinkel, 1961) and their pituitary glycogen content is increased (Debit anal Lazarow, 1962). Thus the suggestion that the diabetic condition results in a reduction of pituitary gonadotropins. Thus this disturbed hormones level is assumed to cause the testicular dysfunctions plug a combination of metabolic dearrangement, microangiopathy and peripheral neuropathy.

Paz and Homonnai (1979) studies the environment of insulin in the reproduction of streptozotocin diabetic rats. They reported that insulin was enough for restoration of accessory gland activity in contrast to its failure to preserve spermatogenesis and restore fertility. Paz et al., (1978) reported the improved fertility of the streptozotocin diabetic male rats following treatment with insulin and human chorionic gonadotropins (HCG). Scafidi et al., (1975) reported the protective role of insulin and hCG on testicular damage in diabetic rats whereas testosterone did not have this effect.

In the present study the sperm motility
of diabetic rats was reduced. These results are in harmony with the findings of Klebanow and Macleod, 1960; Bartak et.al., 1975; Leibel and Garner, 1980.

Present investigations provide an evidence for the atrophy of sex accessory glands and epididymides in diabetic rats. (Gupta and Dixit, 1981, Murray et.al., 1981) Epithelial cell height of caput, cauda seminal vesicle and ventral prostate were significantly reduced, this might be due to decreased secretory activity of these glands (Atassi and Lows, 1981) since they are under androgenic control.

The histological changes in the islets of Langerhans after poisoning with alloxan have been investigated in most laboratory animals. Slight but definite changes consisting of diminished granules contents have been reported to occur as early as 5 minutes after alloxan injection (Bailey et.al., 1944). One hour after alloxan injection nuclear pyknosis is evident and shrinkage of the cytoplasm is more marked. The degenerative signs become more intense with time, the nucleus undergoes karyolysis, the cytoplasm disintegrates, the cell boundaries disappear and finally a mass of ibris containing fragments of nuclei witness the last phase of the nacrotizing presents, which is completed at about 24 hours after injection of alloxan in the rats and rabbits (Rerup, 1979). The diabetogenic action of streptozotocin was reported by Evans et.al., (1965) Arison et.al., (1967) and Junod et.al.,(1979) demonstrating the degranulation of pancreatic, β - cells reported the diabetogenic action of streptozotocin on the pancreatic cell function. Malaisse (1984) has observed that deficit or excessive secretion of insulin
results in severe metabolic disturbances such as those encountered in diabetes mellitus or certain hypoglycaemic syndromes. RBC, WBC haematocrit and haemoglobin levels of diabetic rats were within normal range.

A significant fall in serum protein concentration was noticed in alloxan induced diabetic rats. Insulin promotes amino acid uptakes enhances protein synthesis and inhibits protein degradation (Rosen et al. 1981).

Increased plasma cholesterol is reported in diabetic populations (Nestel et al., 1982). Diabetes increases plasma cholesterol in non-human primates (Sadakiro et. al, 1970, Altura et al., 1981). High levels of serum cholesterol have been reported in tolbutamide treated patients (Meenakshi et. al, 1976, Mehta, 1983).

Increased cholesterol in diabetics may be due to the increased synthesis or due to an impairment in the metabolism of cholesterol and storage of the bile salts (Wong and Van Bruggen, 1960).

Diabetic hyperglycemia may be an independent and additional cause of increased cholesterol production. It is also suggested that hypercholesterolaemia in the alloxan diabetic rats is due to the increase in hepatic cholesterol (Wong and Van Bruggen 1960). Insulin administration in diabetic subjects resulted in decreased plasma cholesterol (Tamporlane et. al., 1980).

Similarly a decrease in HDL cholesterol in alloxan diabetic rats was seen. Yamashita and Yamashita (1980) reported that fed sucrose-cholesterol guar gum diet had higher plasma HDL-cholesterol values. These results indicate that a high fibre diet may have beneficial effects on the metabolism of
cholesterol as well as glucose in diabetes. HDL-cholesterol increased to modulate the uptake and removal of cholesterol in the peripheral tissues including arteries and to facilitate the transport of cholesterol to the liver for metabolism and excretion (Miller and Miller, 1975; Carew et al., 1976). In diabetes mellitus there is higher concentration of serum triglycerides. The increased serum triglycerides possibility due to increased availability of fatty acids to the liver (Fredrickson and Gordon, 1958).

Most frequent lipid abnormalities of the diabetes is hyperglyceridaemia (Ganda 1980). Increased triglyceride level have also been reported, in streptozotocin diabetes rats (Nakai et al., 1978). Hypertriglyceridaemia occurs in rats with chemically induced diabetes (Bierman et al., 1975). Increase in plasmatic triglyceride might be due to insulin deficiency (Reaven, 1974). Lipoprotein lipase which appears to be largely responsible for the removal of triglyceride from the plasma was shown to be decreased during insulin deprivation by alloxan and streptozotocin induced diabetes (Sandek and Eder, 1979). Insulin increases the triglyceride storage in adipocytes and also promotes the synthesis of hepatic triglyceride (Sandek and Eder, 1979). Insulin administration in diabetic subjects resulted in decreased plasma triglycerides (Bennion and Grundy, 1977).

Nikkila and Kekki (1973) reported that in absolute insulin deficiency there is an increased concentration and turnover of plasma free fatty acid and decreased activity of adipose tissues, this provides a possible explanation for hypertriglyceridaemia. Excessive triglyceride secretion by the liver may be the most important causative factor
for the hypertriglyceridaemia in insulin deficiency (Murthy and Shipp, 1979). Serum phospholipid content was increased in the present experiments, this might be due to dearranged metabolic control. The increase in phospholipids concentrations in diabetic is due to increased concentration of cephalin and lecithin fractions in the serum (Wales et al., 1971).

Results obtained in the present investigation indicated that diabetic rats showed a significant reduction in the glycogen contents of testes (Ewing et al., 1980) reported that glucose is an essential requirement for proper functioning of the testes. Decrease in testicular glycogen might be due to an effect to accelerate glyconeogenesis. It was supposed that decreases in glycogen contents was brought by inhibition of phosphorylase in activation process. Marked decrease in glycogen content may affect protein synthesis and thus subsequently inhibits spermatogenesis (Dixit and Joshi, 1982).

Cholesterol is an important precursor for testicular steroids (Hall, 1970; Ewing and Brown, 1977). It is required for the normal activity of testis (Ewing et al., 1980). Increase or decrease of cholesterol level has been considered physiologically significant, since cholesterol is involved in inhibition or stimulation of spermatogenesis (Johnson 1970). It is primary substrate for androgen synthesis (Eik-Nes, 1971) Orth. et al., (1979) reported accumulation of lipids in Leydig cells of streptozotocin diabetic rats. He suggested that excess lipid droplets in Leydig cells of streptozotocin diabetic rats with reduced testosterone output could represent an accumulation of stored
cholesterol esters and its less utilization in biosynthesis of androgen. This reduced biosynthesis of androgen may be due to low level of circulating gonadotropins in streptozotocin diabetic rat Murray et al., (1981). The increased level of cholesterol in testes in the present experiments was similar to the findings of Dixit et al., 1980 and Gupta and Dixit 1981 in alloxan diabetic dogs and streptozotocin diabetic rats. An increase in testicular cholesterol may be due to tissue damage (Lacy, 1967).

In orchidectomized animals cholesterol triglyceride and phospholipids levels were increased in liver but when testosterone propionate is given than these parameters are returned to normal level. Lipids especially cholesterol in Leydig cell serves as precursor for androgen synthesis (Sundquisit et al., 1984)

Meenakshi et al., (1976) have reported increased level of cholesterol due to altered androgen synthesis, Rudolf et al., (1967) have reported the hypercholesterolaemia in rats due to suppression of ACTH secretion from adrenal gland. Alloxan increase in adrenal gland cholesterol.

In the present experiment, significant reduction was seen in the concentration of protein in testes, epididymides (Caput and cauda), seminal vesicle, ventral prostate and vas deference. Hunt and Bailey (1961) showed that in alloxan diabetic rat protein synthesis is abnormal and it might be postulated that the effects on the reproductive tract are produced by the pitutary gonadotropin deficiency, resulting from protein malnutrition. Insulin treatment has been shown to restore protein anabolism to
normal values. Decrease in the protein concentration in epididymides may be due to lack of spermatozoa and luminal fluid because fluid contains a number of proteins for maturation (Cameo anal Blaquiers 1976, Brooks and Higgins, 1980).

Decreased concentration of protein in the seminal vesicle and ventral prostate of diabetic rats was seen which was indicative of reduced secretory activity (Gupta and Dixit, 1981). Androgen have a pronounced effect on the synthesis and secretion of proteins of seminal vesicle and ventral prostate (Burn et al., 1979; Soring-mills and Hafez 1980; Spring-Mills, 1980).

Protein metabolism is severely in diabetic rats (Rosen et al. 1981) reported that insulin stimulates the phosphorylation of ribosomal protein. In the absence of insulin protein is converted to glucose at an abnormally high rate (Larner and Hayes, 1975).

According to Cantrecases and Dlliano (1970), sialic acid is involved in transport of glucose across cell membrane and action of insulin to add in restoration of surface membrane sialic acid residue which have been implicated in the transport of glucose.

Sialic acid may have some role in maturation and fertilizing ability of spermatozoa (Prasad et al., 1970; Gupta et al., 1974). The concentration of sialic acid is regulated by androgens (Rajalakshmi 1969, Prasad et al., 1973). The sialic acid concentrations were significantly low in the testes and sex accessories which be due to the inhibition of spermatogenesis in the testes and the absence of spermatozoa in the epididymides and vas deference (Gupta and
Dixit, 1981). Similar results were observed by Braz et al., (1976). Who assumed that decrease in sialic acid was as a result of degenerative changes in testes.

Depletion in adrenal ascorbic acid in diabetic rats is due to hyperactivity of this gland in prompt response to stress (De Nicola et al., 1977, Som et al., 1981) reported that diabetic patients had low ascorbic acid levels. This is due to high turnover of ascorbic acid in the body which is possibly due to increased oxidation of ascorbic acid to dehydro ascorbic acid.

Fructose is the secretory product of seminal vesicle (Rajalakshmi et al., 1973). The results of the present investigation revealed that the seminal vesicle had higher concentration of fructose in diabetic rats.
Epidemiologic evidence suggests that dyslipidemia is an important contribution factor in atherosclerotic disease diabetes. Although there have been on clinical trials to test the effect of therapy, it is reasonable to apply management strategies and therapeutic goals known to reduce the effect of macrovascular disease on the health of non-diabetic patients (Stern et al., 1989, Dunn, 1988). The Canadian Consensus Conference on cholesterol has recommended that in patients with hypercholesterolaemia who are 30 years of age or more the target serum cholesterol level should be 5.2 mmol/L or less, with a triglyceride level of 2.3 mmol/L or less and a high-density lipoprotein (HDL) cholesterol level of 0.9 m mol/L or more (Canadian Conference on cholesterol in 1988, Yamasaki et al., 1995; Schneider and Sobel, 1997; Lawrence et al., 1998).

Hypertension is a risk factor for diabetic angiopathy and is prevalent in diabetes at approximately twice the rate in non-diabetic subjects (Teuscher et al., 1989). The Canadian Hypertension Society Consensus Conference on hypertension and diabetes has advised that the target systolic and diastolic blood pressure during treatment should be below 160 and 90 mm Hg respectively, in patients over 65 years a systolic pressure below 180 mm Hg is acceptable (LaRochele et al., 1986, Hamet et al., 1988). In the presence of early diabetic nephropathy (as evidence by increased urinary excretion of albumin) target values of less than 140/90 mm Hg. have been recommended (Mogensen, 1988).

Diabetes mellitus may be diagnosed incidentally or in association with at acute illness. In any case the contribution of medications known to promote hyperglycemia should be reviewed.
Epinephrine, glucocorticoids, thiazide diuretics, salbutamol, phenyltolin, niacin and syrup additives are known to raise the blood glucose level, whereas ,β-blockers, salicylates, ethyl alcohol and phenylbutazone are known to lower it (Hamet et al., 1988). Immediate intervention with insulin may be indicated in cases of hyperglycemia during pregnancy, acute illness, hyperosmolar coma or surgery.

The patient or the family must receive instruction in the elements of diet and diabetes monitoring, preferably in a diabetes education centre. Adherence to the treatment programme may be improved through a judicious mixture of diabetic teaching and reinforcement by the physician and nurse-educators. Instructions should be clear particularly with respect to insulin dosage. (Watkins et al., 1967).

1.1.1 Nonpharmacologic approach:

Meal planning should be based on diet provided by qualified dietitian according to recommendations published by the Canadian Diabetes Association; the daily energy intake should be 80 to 150 KJ/kg of desirable body weight, the daily protein intake 0.8g/kg of desirable body weight, the fat intake 30% or less of the energy intake (with saturated fatty acids not exceeding 10% of the energy intake) and the carbohydrate intake 55% to 60 % of g/1000 KJ) (Guidelines for the nutritional management of diabetes in the 1990s 1983).

Ideal carbohydrates should be from foods known to reduce the rate and magnitude of glucose absorption, such as oats, beans and lentils (which contain soluble fibre) and past and barley (which contain starch). Energy and protein demands are increased during growth, pregnancy, lactation and physical training. Energy demands are decreased with obesity.
and inactivity and in elderly people; protein demands are decreased in renal failure.

In obese patients diet therapy may insuline sensitivity and lower the glucose level before substantial weight loss occurs, but considerable weight (typically 20 kg) must be lost before insulin secretion is enhanced to the extent that fasting plasma glucose levels are normalized (UKPDS Group, 1990 and Henry et al., 1985). The sugar content of artificial sweeteners (i.e. aspartame, saccharin and cyclamate) or nutritive sweeteners (e.g. sucrose fructose or sorbitol) may improve compliance. In case of persistent hypertension and hypertriglyceridemia it may be necessary to restrict the cholesterol intake 300 mg/d or less (Dunn, 1988) Alcohol, which contributes to obesity (energy value 29kJ/g) hypertension and per triglyceridaemia and predisposes to adverse effects of hypoglycemia medications, should be excluded if possible (McInnes and Brodic, 1988) in newly diagnosed or poorly controlled cases with longstanding glucosuria. Osmotic diuresis may lead to vitamin or mineral depletion, in such cases supplementation particularly with potassium e.g. potassium chloride, 20 to 40 m mol/d may be needed for a few weeks. Some authorities have recommended limiting the salt intake to 3 g/d in patients with hypertension.

Physical training enhances sensitivity to insulin glucose uptake and glycogen deposition in muscle. Long-term glycemic control may be unaffected or improved by physical training. (Ronnma et al., 1988). In one study walking 14.5 km per week was found to enhance weight loss in patients on controlled energy diets and to decrease hypoglycemic edication requirements (Wing et al., 1988). Physical activity should be appropriate to the cardiovascular status of the
patient. To ensure the safety of certain exercise regimens stress may be needed to rule out significant coronary artery disease.

1.1.2 Pharmacologic approach:

The rate of success in achieving target glucose levels varies from 10% to 50% inversely reflecting the degree of hyperglycemia at the time of diagnosis. If acceptable glucose levels or progress is not achieved within a reasonable length of time (e.g. 3 to 6 months), pharmacotherapy in association with continued dietary efforts is indicated.

1.1.3 Streptozotocin:

Streptozotocin is a broad spectrum antibiotic isolated from streptomyces or chromogenes in 1960 (Herr et al., 1960). Earlier this compound was reported to have anticancer activity (Evans et al., 1965, Arison et al., Fendale, 1967) and its diabetic property was first reported by Rakieten and co-workers (Rakieten et al., 1963). Chemically, STZ is 1-methyl-1-nitro souvea linked to position C₂ of D-glucose having molecular formula C₈H₁₅N₃O₇ and molecular weight 265. It is solution form at room temperature and at pH it decomposes with formation of gas. Its stability in solution is optimum at pH4 and low temperatures.

STZ induces diabetes in almost all species. Diabetogenic dose varies with the species and the optimal dose required to produce diabetes in various species was found to be rats (5-60 mg/kg, ip Gr. iv) (Rakieten et al., 1963), dogs (15 mg/kg for 3 days (Rakieten et al., 1963), and mice (175-200mg/kg ip or iv (Schein et al., 1967). Due to its low stability the rapid intravenous injection appears to be the best route of administration (Rerup 1979)). STZ induces diabetes in hamster, monkeys and guinie pig. (Lazorus anal Shapiro, 1972).
This drug also has been shown to have clinical application in the treatment of human islet cell tumors (Marry et al., 1968). Both fed and fasted rat become diabetic after intravenous injection of 50 mg (Rakieten et al., 1968) or 65 mg (1969; Lazer et al., 1968; Hoftiezer and Carpenter, 1973) of STZ per kg body weight. Doses of STZ as low as 35 mg or as high as 100 mg per kg body weight have diabetogenic activity without being lethal (Masiello et al., 1979; Gupta and Dixit, 1981). In STZ treated rats there is a mild hyperglycemia during the first two to four hours after injection, presumably due to mobilization of liver glycogen. This is followed by release of insulin from the damaged β-cells with resultant elevation of serum insulin and profound hypoglycemia which are most marked at seven to ten hours post injection. By 24 hours there is a permanent hyperglycemia associated with serum insulin levels and negligible pancreatic insulin content.

Glycosuria, polyuria and polydipsia appear during the first day and become more pronounced during the first week (Pitkins and Raynolds, 1970). Liver glycogen depletion in STZ diabetic animals were roughly concomitant with blood sugar increase (Schein et al., 1971; Topping and targ 1975 ; Gupta and Dixit, 1981). Hyperlipidemia in STZ diabetes is pronounced.

The discovery of insulin marked a new era in the advancement of knowledge and therapeutic of diabetes mellitus. The regulation of insulin release is in vivo. Therefore represents a topic of obvious interest. Such a regulation seems to be places under the control of two series of factors. The first factor exerts an immediate and direct effect upon insulin release by the pancreatic
β-cells. They include circulating nutrients, hormones and neurotransmitters. The second factor influence the secretory behavior of the β-cells in a delayed fashion. They include ontogenic nutritional and endocrine factors. All these factors interact to provide an adequate supply of insulin to extrapancreatic tissues and this depends on the functional organization of endocrine cells in the islets of Langerhans. Insulin decreases the hyperglycemia of STZ diabetic animals (Rakieten et al., 1968; Hoftiezer and Carpenter, 1973). An increase in the concentration of serum cholesterol and triglyceride in STZ diabetic animals have been reported (Schnatz et al., 1972; Topping and Targ, 1975; Crepaldi et al., 1978). The exact mechanism of the dibetogenic action of STZ remains unknown.

The ancient Indian physicians have left us a great heritage, especially regarding the diagnosis and treatment of diabetes. They have not only describes the general syndromes of urinary disease but have also differentiated between diabetes insipidus, glycosuria and diabetes mellitus proper.

Abel (1926) prepared insulin in crystalline form. Sanger (1960) established the amino acid sequence of the polypeptide hormones. As regards the treatment of diabetes, with the discovery of insulin the hazzards of this disease has shifted from diabetic ketosis, ulcers and gangrene as complication to micro angiopathy involving the eye, kidney and central nervous system. Insulin administration is not without complications. Thus, an attempt to find out a suitable oral hypoglycaemic agent which could replace insulin therapy is being tried. Sulfonylurea (Loubatieres, 1952) (Ungar et al., 1957) was used as oral hypoglycemic agents.
1.1.4. Sulfonylurea Therapy:

Orally given hypoglycemic agents derived from the sulfonamide group of drugs bind to specific receptors on the \( \beta \)-cells membrane, which depolarizes, permitting entry of calcium ions through voltage-dependent channels. This increases insulin secretion at any given glucose level (Simonson, 1990; Gerich; 1989 and 1990) resulting in decreased hepatic glucose production and decreased plasma glucose levels. The short-term effect of sulfonylurea is thus to increase insulin secretion, with consequent hypoglycemia in concentration are observed despite unchanged serum insulin levels. This enhancement of the efficiency of endogenous insulin reflects continuing \( \beta \)-cell stimulation as well as increased insulin sensitivity in the liver and skeletal muscle. Enhanced insulin sensitivity is attributed to a decrease in the toxic effect of glucose, although there is evidence of an extra pancreatic effect of sulfonylureas that promotes glucose uptake (Gerich, 1989 and Simonson, 1990).

Factors favoring the success of oral hypoglycemic therapy include increased age, diabetes of short duration, obesity and moderate hyperglycemia. Contraindications include pregnancy surgery, and allergy to sulfonamide-type drugs. Sulfonylureas were recently reported to be in use in 35% of patients with diabetes in the United State. The agents currently available in Canada are characterized according to action. In appropriately selected patients, Sulfonylureas are as potent as insulin and may reduce glucose level by 20% to 40% (Halter and Morrow, 1990). About 15% of patients fail to respond, in some cases probably owing to noncompliance with the diet. Rates of secondary failure (to be differentiated from
loss of control due to noncompliance or intercurrent illness) are 5% to 10% per year evidently often due to \( \beta \)-cells secretory failure. Although not proven in controlled trials substituting a "second-generation" sulfonylurea agent (e.g. glyburide) for tolbutamide or chlorpropamide may lead to recovery of glycemia control (Jenning et al., 1989). The net benefit of adding sulfonylureas to insulin therapy to control has not been established (Gerich, 1989).

In one study of self-treated hypoglycemia during sulfonylurea therapy were observed in 20% of 203 patients over 6 months (Jenning et al., 1989). Neuroglycopenia with coma or confusion necessitating assistance occurs at the rate of one event per 5000 patient-years, the death rate being 10%. Chlorpropamide the most frequently used sulfonylurea agent has been incriminate most often although in one recent study the prevalence rate of hypoglycemia during glyburide therapy (31%) exceeded that during therapy with chlorpropamide or gliclazide (14%) risk factors for severe hypoglycemia include poor food intake, advanced age and combination with another agent (e.g. insulin) or a potentiating drug (e.g. alcohol, salicylate, phenylbutazone or an antibacterial sulfonamide (Gerich, 1989; Halter and Marrow, 1990). Hepatic failure may impair the metabolisms of sulfonylureas; only tolbutamide is relatively safe in renal failure. Ionic binding of certain sulfonylureas to serum albumin may lead to adverse potentiation of effect by such drugs as fibrate hypolipidemic agents and salicylate. Urinary excretion may be limited by the effects of allopurinol or probenecid.

Gastrointestinal side effects (e.g. vomiting, cholestasis and abnormal liver function) occur in up to 3% of cases,
dermatologic (e.g. pruritus and erythema nodosum) and hematologic (e.g. hemolysis and aplastic) complication are rare. Chlorpropamide is occasionally associated with hyponatremia due to an antidiuretic hormone-like effect or with severe flushing during alcohol ingestion (Gerish, 1989; Melander et al., 1990).

1.1.5. Metformin:

Metformin is an orally administered hypoglycemic agent belonging to the biguanide class of drugs. Its mechanism of action has not been definitely established. There is evidence that metformin acts on hepatocytes to decrease glucose production (Bailey, 1988). Although evidence of increased insulin binding to tissue has been consistent, it appears that the drug also intensifies insulin's action on muscle, promoting glucose uptake (Klip and Leiter, 1990). Metformin is not dependent on endogenous insulin secretion for its effects. Absorption is almost complete, and the half-life is 2 to 4 hours. The drug is not protein bound and is excreted virtually unchanged by the kidney (Gerich, 1989).

Metformin therapy may be considered in both non-obese and obese patients in whom treatment with diet and exercise has failed. The hypoglycemic potency of the drug is somewhat less than that of the sulfonylureas, but 75% to 85% of patients may be expected to respond (UK prospective Study of Therapics of Mahsritry, Onset Diabetes, 1983). Metformin alone may be most useful in mildly hyperglycemic patients, or it may be combined with a sulfonylurea sent to effectively regain control (Alberti and Gries, 1986, Hermann, 1990). Group et al., 1989 reported that in 24 cases of failed glyburide therapy the addition of metformin resulted in a decrease of 30% in glucose
levels, equivalent to that obtained with twice-daily insulin mixtures. Inconsistent improvements in lipid levels have been reported (Rains et al., 1988). Metformin may be used in conjunction with insulin therapy but its long-term benefits in maintaining glycemia control have not been assessed. In most studies a decrease in body weight has been observed, but of concern is the recent report that lean body mass is replaced by adipose tissue, with little net change in weight noted during 6 months of treatment with metformin/buride (Groop et al., 1989).

Although the recommended dosage of metformin is 0.5 to 1.0 g two or three times per day before meals with a maximum of 2.5 g/d, use is limited by dosage related gastrointestinal symptoms, particularly diarrhea in up to 20% of patients. Thus, management strategies include starting treatment at a low dosage, or temporarily stopping treatment and then gradually reintroducing the drug. Because the use of metformin in renal failure (in which toxic levels occur). Alcoholism hepatic failure and ischemic disease may cause lactic acidosis these conditions are absolute contraindications. However, lactic acidosis appears to be rare, being reported at a rate of less than one case per 10000 patient-year of treatment; the death rate is 30% to 50%.

1.1.6 Insulin:

Insulin may be used as the initial hypoglycemic agent in patients who are relatively young (40 years or less) and lean (within 120% of the desirable weight). It should be used without delay, regardless of age, in patients whose condition is clearly catabolic (Malandre et al., 1990). In cases of failure of diet therapy insulin may be given to patients who are considered good candidates for treatment with orally given
agents or who have adverse effects with such agents. The chances of success (i.e. median decrease in the fasting plasma glucose levels of about 2.0 mmol/L) are similar with insulin and with orally administered agents. In a 9-month randomized controlled trial a single daily dose of an intermediate acting insulin preparation and a single daily dose of glyburide were found to be equally effective (Nathan et al., 1988). Insulin therapy is indicated when poor control persists with diet therapy and treatment with orally given hypoglycemic agents at maximum dosage (Zimmerman, 1988). However, following failed therapy with orally given agents, insulin therapy may be no more successful, possible owing to noncompliance. In such case reversion to an orally administered agent is not contradicated (Peacock anti Tattersall 1984).

Impairment of vision or of manual dexterity does not necessarily preclude the use of insulin. Since helpful strategies are available magnifiers that can be attached to the barrel of the syringe, the use of prefilled syringes by a visiting nurse or relative and pen-like injection devices the permit immediate, accurate injection without the need to fill a syringe (e.g. Novolin-Pen II (connaught Novo Lte., Willodale Ont.) and Inject X (Nordisk Gentofle Canada Inc. Mississauga Ont.) (Selem and Charles, 1990).

Insulin preparations are classified as short acting (maximum duration of action 4 to 8 hours). Intermediate acting (14 to 24 hours) and long acting include regular and seemliest (duration of action up to 16 hours.) intermediate acting preparation include isophane [NPH (neutral protamine Hagedorn)] and lente , and long - acting preparation include ultralente and protamine zinc (Waldhaus 1986, Morales et al., 1996).
1.1.6 Advantages and Disadvantages of Oral Drug:

Advantages:
Oral drugs are generally simple to use for most treatment, low cost and easier for uneducated people and these oral drugs are preferred to insulin because of discomfort of daily injection.

Disadvantages:
* Patient is more likely to tamper with the dose of oral drugs.
* There is less flexibility with oral drugs as compared to insulin as increasing the dosage does not increases effectiveness.

Keeping these aspects in mind there was a need to elucidate herbal medicines. The ancient Indian literature of pre Christian era have distinctly recorded the early detection and treatment of this disease as reported in Sushruta Samhita and Charak Samhita. Many herbal remedies individually or in combination with different formulations as leaf powder, pastes, decoction and infusion pills etc. have been recommended in various medicinal treatises.

In India the use of herbal medicines is more common and is of special interest because of their continued use of most of the population and wealth of available information—historically botanical and traditional.

1.1.7 Future Directions:
Attempts to mitigate pharmacologically the pathological effects of hyperglycemia include the use of the experimental aldose reductase inhibitors, which limit the intracellular accumulation of the sugar alcohol sorbitol, with potential benefit to function of nerve, retina and renal glomerulus (Kinoshita and Nishimura, 1988). Lessening of painful neuropathy was found after year of treatment
with one such agent, toirestat (Boutlton et al., 1990). An effect of ACE inhibitors independent of their antihypertensive effects, the potential to reverse incipient nephropathy is being examined (Parving et al., 1989). In the future the management of diabetes should be increasingly effective (Yamasaki et al., 1995).

1.1.8 Male Reproduction and Diabetes:

It is well documented that long term diabetes mellitus in numerous deleterious consequences. Diabetes mellitus can interfere with male reproduction by altering semen quality or by disrupting normal coital function. However contrary to early evidence, both testicular steroidogenesis and testosterone metabolism are probably unaltered by the diabetic state.

Reproduction disturbances in diabetic patients are well known and have been described by Klebanow and Macleod (1960). In diabetic animals, impaired fertility as accompanied by pathological changes in the testes and male accessory glands (Schoffling et al., 1963).

Impotence is the most frequent problem occurring in 37% to 55% of diabetic men (Irisawa et al., 1966; Kolodny et al., 1974; Schneider and Politzer, 1975; Spallacy, 1976; Money and Melmed, 1979.) The incidence of impotence in diabetic men is two to five times higher than non-diabetic men (Rubin and Babbot, 1958; Schoffling et al., 1963, Chester and Tislowitz, 1945). While studying the effect of diabetes on the growth of immature rats observed that diabetes causes sex organs and accessory structure to remain in an 'infantile' condition. Inadequate gonadotropin production has been suggested as a basic cause of reproduction anomalies in diabetic mice (Lane, 1959). Hunt and Bailey (1961) studied the effects
of alloxan diabetes on the reproductive system of young male rats.

Testicular lesions have been reported and described in diabetic men (Schoffling et al., 1959; Irisawa et al., 1966; Faerman et al., 1972) in pancreatectomized rats (Foglia et al., 1969) in genetically diabetic mice (Hellman et al., 1969), in genetically selected, sucrose fed diabetic rats (Rosenman et al., 1974) in alloxan diabetic dog (Dixit et al., 1983). In STZ diabetic rats testicular lesions, correlated with severity of diabetes have been described (Paz et al., 1979a; Gupta and Dixit, 1981). Experimental induction of diabetes reduced the sexual behavior, fertility and sex accessory gland weights (Fernandez Collazo et al., 1970; Foglia, 1970; Sufrin and Pruthain 1974; Oksanen, 1975; Paz et al., 1978a; Paz and Homonnai, 1979a). Alteration in the pituitary testicular axis have been described in several animal models of diabetes mellitus. (Charreau et al., 1978, Paz and Homonnai, 1979b and Murray et al., 1981 Orth et al., 1979) have observed abnormal Leydig cell function in STZ diabetic rats with marked accumulation of lipid droplets and a decrease in smooth endoplasmic reticulum.

Histologically atrophic changes in testes of men with diabetes mellitus were noted initially in pre insulin years with an increase in interstitial tissue and tubular degeneration. Decreased 17-keto-steroids excretion was noticed in impotent diabetics (Miller and Mason, 1945) has reported that low excretion of 17-ketosteroids in diabetic men shows deficient androgen levels and inhibition of testicular function.

Early studies of the ejaculate in patients with diabetes mellitus suggested that the sperm motility was impaired (Klebanow
and Macleod, 1960). Juvenile onset diabetics also demonstrated decreased spermatozoon motility when assessed by the sperm velocity test (Bartak et al., 1975).

Schoffling (1965) reported decreased urinary gonadotropin in impotent diabetic men and suggested a relationship between diabetes and hypogonadotropic hypogonadism. This relationship was disputed by other investigators, since both basal and GnRH-stimulated blood gonadotropin levels were found to be normal in diabetic men. (Paz et al., 1977, Ando et al., 1978) reported higher mean basal level of LH in diabetic patients and LH-RH stimulated release of gonadotropin was significantly lower in diabetic patient. Blunted LH responses were described by Distiller et al., (1975) and Shalwan et al., (1978) and normal FSH responses were observed by Wright et al., (1976) Ceda et al., (1981) have reported that in diabetic patients gonadotropin secretion is often affected by peripheral gonadal impairment and in addition hypothalamic pituitary dysfunction was also expressed as generalized metabolic disorder.

1.1.9 Scope of Study :
The present work launched to study the effect of alloxan treatment on different aspects of rat. This study was completed in albino wistar rat in environmental physiology laboratory of Govt. Institute of Science and Humanities, Amravati.
1.2 MATERIALS AND METHODS

1.2.1 About the experimentation:

Taxonomy and information of experimental animals

Of all the laboratory animal species, rat is the most favorite choice of all biologists. There are many reasons for the choice of this wonderful animal such as,

* Easy to care for and handle, inexpensive to maintain under laboratory conditions.
* High reproductive capacity.
* Short generation time and life span.
* A wealth of baseline information.
* Large number of well defined strain and stock for traditional rodents.
* Selective susceptibility and resistance to many infection and representation of disease similar to human beings.

All these qualities and high evolutionary position entice us to select rat as animal model for present work.

Classification:

- Phylum: Chordata
- Subphylum: Vertebrata
- Class: Mammalia
- Subclass: Theria
- Infraclass: Eutheria
- Order: Rodentia
- Family: Muridae
- Genus: Rattus
- Species: Albino Wistar Rat

1.2.2 General Biology and information about test animal:

Rats are the small rodents which are most active at night and delight in marking nests. They have poor vision but very acute hearing and respond to a range of ultrasonic frequencies. Rats also have a highly developed sense of smell and use olfactory pheromones to communicate. These pheromones are of particular importance in reproductive
biology. Rats are social animals and can be maintained in group of housing in laboratories, all various types of cages are available commercially with either a mesh or a solid floor.

The rats used for the experiment were collected from Dr. Panjabrao Deshmukh Medical College and maintained and cared in the environmental physiology lab of P.G. Department of Zoology in cages made up of polycarbonate galvanized iron, with saw dust bed at the bottom. The food and water supplied to the animal.

1.2.3 Acclimatization:

Only the male rats were kept in other separate cages. The animals were weighed on monopan triple bean physical balance to the nearest 0.1 gm.

The temperature of the house was maintained in the range of 23 - 25°C. The 12 hours of lighting and 12 hours of darkness were provided in the room for optimal growth and the reproduction. The animal were feed on commercially available pellet diets as given in the table 1, 2 and 3.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Ingredients</th>
<th>gm/ug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wheat Floor</td>
<td>150.00</td>
</tr>
<tr>
<td>2.</td>
<td>Roasted Bengal Gram</td>
<td>570.00</td>
</tr>
<tr>
<td>3.</td>
<td>Groundnut Flour</td>
<td>100.00</td>
</tr>
<tr>
<td>4.</td>
<td>Skimmed Milk Powder</td>
<td>50.00</td>
</tr>
<tr>
<td>5.</td>
<td>Castor (min. 80 p.c. protein)</td>
<td>40.00</td>
</tr>
<tr>
<td>6.</td>
<td>Refined groundnut oil</td>
<td>40.00</td>
</tr>
<tr>
<td>7.</td>
<td>Salt mixture with starch</td>
<td>48.00</td>
</tr>
<tr>
<td>8.</td>
<td>“Vitamin Mixture”</td>
<td>48.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000.00</td>
</tr>
</tbody>
</table>
### Table 2 Composition of Salt Mixture:

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Minerals</th>
<th>gm / 100 kg of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Potassium Phosphate</td>
<td>1556.000</td>
</tr>
<tr>
<td>2.</td>
<td>Calcium Carbonate</td>
<td>1525.600</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium Chloride</td>
<td>557.200</td>
</tr>
<tr>
<td>4.</td>
<td>Magnesium Sulphate</td>
<td>229.200</td>
</tr>
<tr>
<td>5.</td>
<td>Ferrous Sulphate</td>
<td>229.200</td>
</tr>
<tr>
<td>6.</td>
<td>Magnese Sulphate</td>
<td>16.040</td>
</tr>
<tr>
<td>7.</td>
<td>Potassium Iodide</td>
<td>3.160</td>
</tr>
<tr>
<td>8.</td>
<td>Ionic Sulphate</td>
<td>2.192</td>
</tr>
<tr>
<td>9.</td>
<td>Copper Sulphate</td>
<td>1.908</td>
</tr>
<tr>
<td>10.</td>
<td>Cobalt Chloride</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>3999.392</strong></td>
</tr>
</tbody>
</table>

### Table 3 Composition of Vitamin Mixture:

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Vitamins</th>
<th>gm / 100 kg of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DL - To Coferal (E)</td>
<td>6.000</td>
</tr>
<tr>
<td>2.</td>
<td>Menaphthone</td>
<td>0.150</td>
</tr>
<tr>
<td>3.</td>
<td>Thiamine (B1)</td>
<td>0.400</td>
</tr>
<tr>
<td>4.</td>
<td>Riboflavin (B2)</td>
<td>0.500</td>
</tr>
<tr>
<td>5.</td>
<td>Pyridoxin (B6)</td>
<td>0.600</td>
</tr>
<tr>
<td>6.</td>
<td>Niacin</td>
<td>1.000</td>
</tr>
<tr>
<td>7.</td>
<td>Pantothenic Acid</td>
<td>1.200</td>
</tr>
<tr>
<td>8.</td>
<td>Cyanocabolamine</td>
<td>0.00005</td>
</tr>
<tr>
<td>9.</td>
<td>Follic Acid</td>
<td>0.100</td>
</tr>
<tr>
<td>10.</td>
<td>P. Amino Benzoic Acid</td>
<td>10.000</td>
</tr>
<tr>
<td>11.</td>
<td>Biotin</td>
<td>0.040</td>
</tr>
<tr>
<td>12.</td>
<td>Inositol</td>
<td>10.000</td>
</tr>
<tr>
<td>13.</td>
<td>Choline Chloride</td>
<td>100.000</td>
</tr>
<tr>
<td>14.</td>
<td>Starch</td>
<td>70.0095</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>200.0000</strong></td>
</tr>
</tbody>
</table>

Excess of food and fecal matter removed from the cages once or twice a day.

---

Chapter 1  25
1.2.4 **Experimental Design** :
Rats were divided into following groups

**Group 1**: Intact (Control): The rat of this group received vehicle (0.87% normal saline was injected intraperitoneally)

**Group 2**: Intact + diabetic (the rats of this group received Alloxan (15mg/100g body weight) was injected intraperitoneally.

Diabetes was induced by a single intraperitoneal injection of alloxan (15 mg×kg body weight in 0.87 normal saline buffer at pH 4.5 abd 10 mm citrate) All animals were fasted for 18 hours before drug treatment.

These animals were fed with 50% glucose at the dose level of 5 ml/animal/6 hour upto 24 hours. Blood sample for glucose estimation were collected from tail vein in dried oxalate-fluoride vials and blood glucose was estimated by the method of Astoor and King, 1954. The blood sugar levels over 250mg/100 ml in the alloxan treated rats were regarded as diabetic and were used for experimentation.

The animals were sacrificed using ether anesthesia. Blood collected from the heart was allowed to clot at room temperature. The epididymides was taken out quickly and cauda separated for sperm dynamic studies (Prasad et al., 1972)

Serum was separated by centrifugation and stored at - 20 C until assayed.

1.2.5. **PARAMETERS** :

1) **Body and organ weights** :

The initial and final body weight of the animal was recorded. The reproductive tract was taken out, trimmed free of fat and each organ was weighed separately on name electronic balance. The reproductive organ in male included testes, epididymides, ventral prostrate, seminal vesicle, vas
deference and also vital organs such as adrenal, were taken out and weighed.

Half of reproductive organs were fixed in Bouin’s fixative for histological studies and the remaining halves were frozen for biochemical analysis.

2) Histoigical studies:

The Bouin’s Fixed reproductive organs (Testes, epididymides, seminal vesicle, ventral prostate, vas deference, along with pancreas, were cut into small pieces and processed. The paraffin embedding was followed by section cutting (5 μ) and staining (Harris haematoxylin and eosin).

3) Histometry:

With the help of Camera Lucida hundred circular appearing seminiferous tubules were traced at × 80 and the diameter of each tubule was measured separately. The measurement was expressed in terms of mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at × 80. The epithelial cell height of cauda epididymides, caput epididymides and Seminal vesicle were also traced at x360.

4) Sperm motility and count:

To determine the sperm motility and sperm counts, 100 mg of cauda epididymides was mixed in 2 ml of physiological saline. One drop of evenly mixed sample was applied to a Neubauer’s counting chamber under cover slip, Quantitative motility expressed as percentage, was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and as million/ml of suspension (Prasad et.al.,1972).

5) Biochemical Studies:

A) Tissue Biochemistry:

1. Cholesterol was estimated in testes.
2. Glycogen was estimated in testes by the
method of Montgomery (1957)

3. Protein was estimated in the testes, cauda epididymides, caput epididymides, seminal vesicles, ventral prostate and vas deference in male rats (Lowry et al., 1951).

4. Sialic acid was estimated in the testes, cauda epididymides, caput epididymides, seminal vesicle, ventral prostate and vas deference in male rats (Warren, 1959).

5. Fructose was estimated in seminal vesicle (Mann, 1964).

6. Ascorbic acid was estimated in adrenal by the method of Roe and Kuether (1943).

(B) Serum biochemistry:

1. Total cholesterol (Zlatkis et al., 1953)

2. High density lipoprotein (HDL) cholesterol (Burnstein et al., 1970)

3. Phospholipid (Zilversmith and Davis, 1950)

4. Triglycerides (Gottfried and Rosenberg, 1973)

5. VLDL - Cholesterol (Dedonder - Decoopman et al., 1980)

6. LDL - cholesterol (Shepherd et al., 1980)

7. Total protein (Lowry et al., 1951)

8. Serum glutamic oxaloacetic transaminase (SGOT) (Mohun and Cook, 1957)

9. Serum glutamic pyruvic transaminase (SGPT) (Mohun and Cook, 1957)

7. Haematological parameters:

1. RBC and WBC counting (Lynch et al., 1969)

2. Haemoglobin (Drabkin's Cynmethoglobin method by Fisher's Haemophotometer)

3. Haematocrit (Wintrobe, 1930)


8. Testicular Cell population counting:

Spermatogenic element i.e. spermatogonia, spermatocyte and spermatids were counted in 5 μ thick cross sections of 10 seminiferous tubules in 6 animals of each group.
All raw counts were transformed to true counts by an adaptation of abercrombie formula (Abercrombie, 1946) from germ cell diameter measurement.

Abercrombie Formula,

\[ T = C \times \frac{S}{S + d} \times 100 \]

In this formula

\( T = \) True counts
\( C = \) Crude Counts
\( S = \) Thickness of section
\( D = \) Diameter of nuclei

Interstitial cell type (such as fibroblast, immature and mature Leydig cells and degenerating cells) were estimated, applying differential count over 200 cells population and statistically verified by the binomial distribution (Dixon and Massey, 1957).

9. Statistical Calculation:

All the values of body/organ weight biochemical estimation and histometry were expressed in terms of mean value ± Standard Error. The different treatment groups were compared with control group using student ‘t’ test (Ipsstein and Poly., 1970)

**Formula of Standard Error** :

\[
\text{Standard Error} = S\overline{X} = \sqrt{\frac{\sum x^2 - (\sum x)^2}{n^2(n-1)}}
\]

\[
T = \frac{\overline{xA} - \overline{xB}}{\sqrt{S\overline{xA}^2 + S\overline{xB}^2}}
\]

Degree of freedom = \( n_1 + n_2 - 2 \)

where

\( N \) = No. of variables in each group
\( \Sigma x \) = independent variables
\( \overline{xA} \) = Mean value of control variables
\( \overline{xB} \) = Mean value of treated variables
\( S\overline{xA} \) = Standard Error of mean of control group
\( S\overline{xB} \) = Standard Error of mean of treated group

\( N_1 \) = Number of variables in control group
\( N_2 \) = Number of variables in treated group
1.3 OBSERVATION AND RESULTS

1.3.1 BODY AND ORGAN WEIGHT

**Body Weight :** Table 1

A remarkable reduction in the body weight was observed in alloxan treated rats after duration of 48 hours. Where as the body weight of control remained unchanged.

**Organ Weight :** Table 2

1) **Testes :**

Testicular weight was significantly decreased in alloxan induced diabetic rats after the duration of 48 hours, when compared with the diabetic control group.

2) **Epididymides :**

The epididymal weights of diabetic rats, when compared with control showed a slight significant reduction in comparison with the diabetic control group.

3) **Seminal Vesicle :**

In diabetic rats the weight of seminal vesicle showed a slight significant decrease, when compared with diabetic control group.

4) **Ventral Prostate :**

Non-significant change in the ventral prostate weight was noted in the alloxan induced diabetic rats, when compared with that of control group.

5) **Vas Deference :**

The vas deference weight of diabetic rats, when compared with control group, showed no significant change.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Body Weight of Male Albino Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment groups</td>
<td>Initial</td>
</tr>
<tr>
<td>Intact O</td>
<td>208.33 ± 2.16</td>
</tr>
<tr>
<td>Diabetic</td>
<td>186.33 ± 3.61</td>
</tr>
</tbody>
</table>

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