CHAPTER - IV:

EFFECT OF THEVETIA NERIIFOLIA JUSS ON BLOOD GLUCOSE
The pharmacological effects of glycosides have been very extensively and very thoroughly investigated earlier even then the actions of the glycosides on the cellular, subcellular and molecular levels are still obscure and further studies by use of modern biochemical techniques are essential to understand the same. The glycosides more specifically the cardiac glycosides somehow strengthen the contractile power of the cardiac muscle fibres and thus are directly or indirectly related to the energy transformation in the heart. It was argued earlier that the glycosides somehow influence the process of resynthesis of ATP and phosphocreatine and contractile protein complex actinomysin is recharged. The heart itself is predominantly an aerobic organ, is comparatively unable for anaerobic recovery and has a low glycolytic power. Reduction in glucose uptake by the heart muscle fibres in spontaneous heart failure in the heart-lung preparation was observed earlier by Gremels (1940) and heart failure is stated to be correlated with a depletion of glycogen though the experimental evidences of Visscher and Mulder (1930) indicated otherwise. The more basic question is therefore if the glycosides stimulates the generation or utilization of phosphate bond energy. Continued resynthesis of phosphocreatine and ATP is highly dependant on many factors including an aerobic metabolism of different foodstuffs more particular of carbohydrates.
ENERGY SOURCE:

Carbohydrates are required by the body as a source of energy. Fuel is added to an engine to run it smoothly likewise to the human life (or more accurately living being), to make life successful with a good health to make us efficient for the competitive world. Carbohydrate of sugar or glucose is highly essential. This is one of the major energy sources and its major function in metabolism is as a fuel.

Dietary carbohydrates as well as glucogenic amino acids and glycerol moiety of lipids are the primary source of the energy or blood glucose. Ranner et al. (1964 and 1967) and Brambila et al. (1966) after an investigation in chicks reported that neutral fat could isocalorically replace dietary carbohydrates without effecting growth and blood glucose level. Although it had been observed that replacement of fatty acids markedly depressed blood glucose level and growth.

Carbohydrates in general and glucose in particular are oxidised through a series of chemical transformations and thereby provide energy for other metabolic processes. Carbohydrate is utilised by the different cells of our body primarily in the form of glucose. Hence any other form of carbohydrate or monosaccharide in our body cell are converted readily into glucose by the liver for immediate supply of energy.
The energy liberated from the oxidation of food etc. is termed as free energy ($\Delta F$) expressed in terms of calories/mole of food substances. The amount of energy liberated by the oxidation of one gm. mole of glucose is 686,000 calories (Butler et al. 1980). This energy liberated from glucose has been utilised by ATP. ATP being an intermediary compound in many physiological reaction mechanisms.

The metabolism of carbohydrates needs primarily for the production of ATP which energy for contraction process. Glucose is stored in the muscle tissue in the form of glycogen and is converted into blood glucose when the absorption is inadequate (Bernard, 1876).

In the skeletal muscle the energy obtained from glucose is stored in the form of glycogen as much as 250 gm. in the liver, muscles and tissues. The convertibility of glycolytic reactions as demonstrated by Meyerhof (1919) was reinvestigated by Friedmann et al. (1941), Stotz (1942) and Tepperman et al. (1948) reported that relationship between the various products of glycolytic reactions with special emphasis on blood lactic and pyruvic acids. Further the conversion of these sugars into glycogen and the possible chemical reaction with enzymes was elaborated by Cori et al. (1938, 1940). It was revealed that the phosphorylation of the sugar to a sugar phosphate takes place due to ATP (Kuler, 1935). Randle
et al. (1966) observed an inhibition of uptake, phosphorylation and oxidation of glucose and a block in glycolysis at the level of phosphofructokinase both in starvation and diabetes. Thus in the energy transformation and utilisation ATP playing the key role in the physiological processes.

Energy is thus required for our body in order to smooth running of different physiological reactions such as:
(a) muscular activity, (b) Secretion by the glands, (c) maintenance of membrane potentials by the nerve and muscular fibres (d) synthesis of substances in the cells (e) absorption of foods from the gastrointestinal tract.

Brain, one of the sensitive organs, derive its energy from the metabolism of glucose (Lund – Anderson, 1979).

Glucose utilised for energy is generally derived from blood. The essential source of glucose is the foodstuff which is absorbed in the intestine by a complicated process.

**SOURCE OF BLOOD GLUCOSE**:

The principal source of blood glucose or body carbohydrate is the food which normally contains a large amount of starch and smaller amounts of other sugars like sucrose, lactose. These carbohydrates present in the food may in the simple or complex form are hydrolysed in the course of digestion to the monosaccharides and resulting mixtures of glucose, fructose.
and galactose is absorbed into the portal blood and carried to liver.

Because of the essentiality and physiological importance of glucose it is derived from different sources to keep the body in normal state. To meet the required amount of glucose for our body, to meet the depletion of glucogen in tissues, glucose generally derives from:

i) carbohydrates of the diet.

ii) various glucogenic compounds.

iii) liver glycogen.

**From Carbohydrates of the diet:**
The amount of carbohydrates ingested are largely determined by the availability of foods, food habits and economic status. Carbohydrate is abundantly present in the rice, potato, cabbage and all other green vegetables. Even carbohydrates are ingested directly as sugar, molasses etc. with food. Day (1936) determined blood sugar in rats feeding diets of various carbohydrate content upto 60% level. In 60% lactose ration feeding rats and in 60% glucose feeding rats he found mean blood sugar level 160 mg.% and 121 mg.% respectively.

In the intestine these starch, sucrose and lactose are digested and thereby they are converted into monosaccharides - glucose, fructose and galactose. These are absorbed into the
Fig. 2: Metabolism of Galactose, Fructose and Glucose in Liver Cell.
Figure 3: Schematic diagram of carbohydrate metabolism.
portal blood and carried to the liver where fructose and galactose is converted into glucose by enzymatic processes (Fig. 2). Pereira et al. (1971) reported that the rate of conversion of fructose into various intermediates of carbohydrate and lipid metabolism is more rapid than glucose.

The sugar leaving the hepatic veins is in the glucose form and then distributed evenly between the erythrocytes and plasma of the blood. Glucose is then taken up by the tissues and utilised as energy or stored in the form of glycogen (glycogenesis) or converted into fat (lipogenesis) (Fig. 3). Flatt et al. (1964) and Cahill et al. (1960) reported that insulin stimulated the synthesis of fatty acids from glycerol and the lipogenic action of insulin may be dependent on its effect on glucose metabolism. Dietary lipid reduces hepatic lipogenesis in the lying hen and the immature pullet (Balnove et al. 1969, 1971). Pearce (1972) investigated the effects of dietary lipid on the activities of some of the key enzymes of glycolysis, gluconeogenesis and the pentose phosphate pathway and revealed its strong controlling effect in the synthesis.

From various glucogenic compounds:
The glucogenic compounds which are converted into glucose by different enzymes of the system are classified into two groups:
1) Those which are directly converted into glucose without significant recycling e.g. amino acids and propionate.

ii) Those which are the products of the partial metabolism of glucose in certain tissues.

The first category compounds are converted into glucose by the mechanism - gluconeogenesis and thereby 60% of the amino acids are converted into glucose by deamination followed by reversal simple interconversions. Krebs (1963) reported that energy barriers obstruct a simple reversal of glycolysis in the metabolic pathways and is circumvented by special enzymatic reactions. Berger et al. (1976) on the other hand reported that glucose may metabolise into lactate and alanine and were released at rates of 1.35 and 1.41 μmol/min./30 gm. of muscle respectively (Odessey et al. 1974). They further observed that lactate was oxidised at a rate of 0.44 μmol./min./30 gm. of muscle have a greater magnitude of glycolysis than that of glucose uptake. Domínez et al. (1976) reported that the metabolic product of glucose, glycerol is utilised adipose tissue is greater than the investigated report of Wieland et al. (1957). The presence of glucose affected the utilisation of glycerol by increasing the rate of synthesis of fatty acid and carbon dioxide without altering that of glyceride-glycerol (Domínez et al. 1976).

The second category compounds are conveyed to the liver and kidney where they are resynthesized to glucose. Lactate, a
product of the partial metabolism of glucose in the simple enzymatic mechanism known as Cori Cycle (Fig. 4). Similarly glycerides of adipose tissues are continually transferred into glucose by gluconeogenic mechanisms.

**FIG. 4**: Cori Cycle or the Lactic Acid Cycle.

Therefore when the body's store of glucose decreases below normal level either due to deficiency of carbohydrate in diet, due to starvation or due to disease moderate quantities of glucose can be formed from amino acids either derived from the proteins of the diet or sometimes of the body tissues and from the glycerol portion of the fat by gluconeogenesis. Glucose can also be formed from lactate which is continually produced in muscle and in the blood. This deficient
Fig. 5: Schematic representation of gluconeogenesis showing the formation of glucose from Lactate and the gluconeogenic amino acids.
amount of glucose might be utilised as a source of energy for the nervous system and erythocytes and for adipose tissues and probably plays a role to maintain the intermediates of the citic acid cycle.

Gluconeogenesis from lactate or amino acids are taking place by the conversion of pyruvate to phosphoenolpyruvate through the common step of the formation of oxaloacetate. Oxaloacetate may also be formed by the way of succinate and malate from several amino acids (Fig.5). The mechanism of this conversion is controlled and regulated by the enzymes and the enzymes are made active by feedback control.

The liver is also responsible for the synthesis of glucose from glucogenic amino acids obtained from protein. From the physiological point of view gluconeogenesis helps in the metabolism of excess of amino acid into glucose (derived by the body from diet) either by the way of carbohydrate or by fat.

In the conversion of our major foodstuff into glucose (Fig.6) the significant reaction is the conversion of pyruvate to acetyl-CoA. Because the formation of pyruvate leads to the formation of glucose and acetyl-CoA being the starting compound for the synthesis of many long chain fatty acids. The fatty acids which have odd number of carbon atoms are glyco-
genic as it forms propionate upon oxidation. Further many of
Fig. 6: Interconversion of major foodstuffs.
the non-essential amino acids are produced by citric acid cycle and by transamination and by reversal process they are converted into glucose and glycogen.

On the other hand it has been revealed that glycogen (Guyton, 1981) is formed in the liver and then breakdown to glucose by the process - glycogenesis and glycogenolysis. The breakdown of glycogen in the liver cell is initiated by phosphorylase and the whole process is proceeded through the formation of a number of intermediates ( in the presence of different enzymes.

However under most natural conditions breakdown of protein and amino acids is usually accompanied by a net breakdown of fat and thereby body receives one of the high energy product.

From liver glycogen:
Glycogen, a polymer of glucose, breakdown in the liver cell to glucose by the process of glycogenolysis and distributed in the blood. The breakdown of glycogen is initiated by the action of the enzyme, phosphorylase, which is a specific enzyme of phosphorylation in the breakdown of 1,4 linkage of glycogen to yield glucose-1-phosphate. The moment it forms, glucose-1-phosphate is catalysed by phosphoglucomutase and glucokinase to form glucose.
The metabolism of glycogen is controlled by the balance activities of the enzymes of glycogen synthesis and breakdown. The rise of concentration of cyclic AMP not only activated phosphorylase but also inactivated the glycogen synthetase (Harper, 1971). Because of this characteristic the synthesis and breakdown of glycogen donot occur simultaneously (Hers et al. 1970). Villav-Palasi et al. (1968,1970), Smith et al. (1968) and Krebs (1972) reported detailed mechanisms of glycogen metabolism and the glycogenolytic effects of epinephrine in muscle and of glucagon in liver.

Absorption of Carbohydrate:

Food carbohydrates are primary absorbed in the form of monosaccharides from the small intestine. Polysaccharides are hydrolysed in the small intestine to simplest form. Fischer et al. (1960) reported that the pancreatic and salivary amylases hydrolyse some glycosidic bond (specially 1 : 4 bond) in the polysaccharide chain. Borgstron et al. (1957) found glucose and small amount of monosaccharide in the intestinal contents are the only sugars which are to be absorbed from intestinal mucosa. Though di and polysaccharides are not absorbed from the intestine as they are but Weser et al. (1967) have shown that small amount of disaccharide enter and may be present in the blood, and out of this 90 percent were recovered in urine. Since disaccharides or polysaccharides are present in the diet but they are found very less amount in the urine because of the
fact that probably they are hydrolysed between the human of
the small intestine and the blood.

Actually there are some activity or enzymes that can hydro-
lyse and made assimilable in the intestine. Dahlquist (1962)
Auricchio et al. (1965) reported that sucrose, isomaltase,
trehalase, lactase and maltases are some of the active
enzymes present in the human intestinal mucosal brush bor-
ders. These enzymes active not only towards disaccharides
but also active towards oligosaccharides, polysaccharides
etc. Beck (1942) showed that in the hexose absorption from
the intestine the hexose phosphate content of the mucosa
increases. Also some fructose passing through the mucosa
is converted into glucose through phosphorylation. The
sequence of absorption are shown below:

From intestinal → glucose + ATP → glucose-6-phosphate,
    content
    ↑                                ↓
    in mucosa                       Phosphatase
    glucose + phosphate
    ↓
    To blood

Fructose + ATP → fructose-1-phosphate
    ↓
    isomerase
    glucose-6-phosphate ← fructose-6-phosphate
    ↓
    phosphatase
    glucose + phosphates.
These principle of chemical transformation and diffusion are seems to be responsible for the absorption of common hexose. The deficiency of these enzymes reduces the carbohydrate absorption. The absorption was further ascertained by different tolerance and excretion tests.

**Blood glucose its Regulation and Utilisation**

Blood normally contains sugar in the form of glucose and a very small amount of galactose and fructose may be present just after their absorption from the intestine or before their conversion to glucose in the liver cells (Fig.2.).

Any disaccharide must go to the blood stream after proper hydrolysis otherwise they will function as a foreign substance. Bollmann et al. (1925) reported from the experimental evidence that in hepatectomized animals intravenously injected glycogen were hydrolysed and converted into glucose. Somogyi (1933), Klinghoffer (1940) and others have reported that in man blood glucose is distributed uniformly throughout the water of the red cells and plasma which indicate the free diffusibility across the cell membrane.

Liver, kidney are the major organ that control the blood glucose level by either of the way and intestine being the supplier of carbohydrate or sugar. Soskin et al. (1934,1936) reported that the rise of blood glucose leads corresponds
decrease of output of liver glucose and vice versa. The conversion of pyruvate, succinate and \(\alpha\)-ketoglutarate and amino acid by kidney slices was also shown elaborately (Russell et al. 1941) and regulates blood glucose.

Another factor that control the normal level of the blood glucose is the insulin produced by the islet cells of the pancreas. Liver, the extrahepatic tissues, several hormones plays an important role in the synthesis and regulation of glucose. Cahill et al. (1959) suggested that liver cells are freely permeable for glucose whereas the extrahepatic cells are relatively less permeable and hence the uptake of glucose is essentially controlled or obstructed by the cell walls. But due presence of different enzymes in the surrounding of the cell medium have made a direct effect in its regulation. Actually the permeability of glucose in the extrahepatic cells may be termed as rate limiting step. Pearce (1972) reported that the rate of glucose oxidation by islet was found to depend markedly on the extracellular glucose concentration.

The three endocrine organs - the pancreatic islet tissue, the adrenals and the anterior pituitary directly or indirectly have controlling and regulatory effect on blood glucose by liberating specific hormones.

Since the time of Mering (1886) till date different groups of workers have revealed that the pancreatic hormone 'insulin'
have a strong controlling effect on the glucose regulation and metabolism. Actually these is a inverse relation in the pancreatic secretion and blood glucose.

Likewise in the very early period of this century Cushing (1912), Houssay (1936), Mathison (1953) reported the strong antagonistic effect of anterior pituitary hormone in the carbohydrate metabolism. Young et al. (1962) showed the administration of anterior pituitary extract causes the rises of blood sugar level temporarily and presumed to be due to some mechanism which decreased the normal action of insulin. Russell (1938) observed that in hypophysectomized animals the rate of absorption of glucose reduced. In hypophysectomized animals glycogen storage was decreased and the respiratory quotient increased due to the increased carbohydrate oxidation (Fisher et al. 1936).

Hormones like adrenaline, noradrenaline secreted by adrenal gland increased the blood glucose concentration due to the breakdown of liver glycogen by phosphorylase (Swingle et al. 1931; Reichstein, 1943). Some hormones secreted by the adrenal gland also increases the gluconeogenesis from amino acids and reduced the utilisation of glucose (Hales, 1967). The effect of adrenal cortex on carbohydrate metabolism in adrenalectomized animals reduced the blood glucose level (Long, 1937). It was also reported that the administration of adreno-cortical extracts into fasting animals causes mild
hyperglycemia with an increase of liver glycogen and thereby presented the rapid depletion of blood glucose.

Berger et al. (1976) reported that nearly all glucose in heart is utilised for glycolysis i.e. about 70% of it is oxidised and the remainder is released as lactate or pyruvate.

In the regulation and utilisation of blood glucose and may varies according to the concentration of hormones secreted by the different organs. Blood glucose - buffer system of the liver functions in the blood glucose regulation in another important factor of insulin control (Guyton 1979).

The chemical nature of the extract of T.nerifolia Juss has fairly been established as a glycoside (Chapter II); it is therefore expected that T.nerifolia Juss may influence the availability and the supply of energy to the cardiac muscle fibres. Due to lack of modern facilities studies like glucose uptake, the enzyme systems concerned with the aerobic metabolism after administration of the extract of T.nerifolia Juss could not be undertaken in the present investigation; however, the effect of the extract on blood glucose level after administration of the extract in varying amounts has been studied as a preliminary investigation.
MATERIALS AND METHODS:

The compound isolated from the T. neriifolia Juss (the ether extract) as described in Chapter III has been applied for biochemical studies. In order to carry out the biochemical investigation white albino rats (supplied by M/s. Panindra Nath Chakraborty, Calcutta) weighing about 80-100 gms. were used and were kept under the same diet throughout the investigation.

Reagents and Chemicals:

Reagents used in the biochemical study of the different components of blood were of analar grade. The preparation of the reagents were described in each chapter and water used were redistilled water. Previously dried corning glass apparatus were used throughout the biochemical studies.

Procedure of Administration:

An accurate amount of the compound to be injected was weighed in a single pan automatic electric balance. The investigation was carried out in two different groups with another group as control. In the control group blood glucose, have been estimated by administering 0.1 ml. of absolute alcohol and sacrificed at the end of one hour. The control group was marked as Group I.

2 mg. of the compound was weighed accurately and dissolved in 0.1 ml. of absolute alcohol and made 1 ml. of by addition of redistilled water, and was injected intramuscularly in
the hind leg. After one hour of the injection the animal was sacrificed and blood was collected in an already prepared oxalated vile. With this blood, blood glucose were estimated.

This group was marked as Group II. Similarly in another group of animal a higher dose of 4 mg. of the compound was administrated by dissolving in 0.1 ml. of absolute alcohol and made 1 ml. by injection water. Animals were injected in a similar way in the right hind leg. After an hour the animal was sacrificed and with the oxalated blood, the blood glucose have been estimated.

After administration of the compound the respiration rate, behavioural nature and movement of the animal was noted.

**Method of Evaluation**

The application of statistical methods only made distinct the action of the drug. The different experimental values so obtained were correlated by a number of statistical methods for perfect reproducibility of the effect of the compound on albino rats.

**Standard Deviation and Mean Standard Error**

The standard deviation and mean standard error of each set of experiment was determined by the following equations:
S.D. = \sqrt{\frac{(X_1 - \bar{X})^2}{n - 1}}

S.E_m = \frac{S.D.}{\sqrt{n}}

where
S.D. = Standard deviation.
X_1 = Value of each observation in a set.
\bar{X} = Mean value of the set.
S.E_m = Standard error mean in a set.
n = Number of observations.

Significance test:
The significance difference between two sets e.g. control and a test group or between two test groups were compared by 'students' t test' for probability of events. The equation applied for is:

\[ t = \frac{\bar{X}_1 - \bar{X}_2}{S.D. \sqrt{\frac{n_1 n_2}{n_1 + n_2}}} \]

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

\bar{X}_1 = Mean of the 1st set.
\bar{X}_2 = Mean of the 2nd set.
X_1 = One individual value of the 1st set.
X_2 = One individual value of the 2nd set.
n_1 = Number of observations in 1st set.
n_2 = Number of observations in 2nd set.
n_1 \cdot n_2 - 2 = df degree of freedom.
Variance Test:

The variation of the results in different experiments and the nature was established by frequency determination in each experiment. Here frequency, relative frequency and cumulative frequency and percentile graph of cumulative frequency have been determined by the standard method.

Frequency is determined by using equal class interval for a particular set of experiment and the relative frequency is then determined by

$$ R_f = \frac{\sum X_0}{X} $$

where $\sum X_0$ = sum of a group of frequency.

$X$ = mean of the set.

The cumulative frequency is determined by the 'less than method' and hence determined the percentage to draw percentile graph.

These determined values are correlated by plotting either as a graph or as histogram in order to easy assessibility of the nature of variance due to the action of the compound.

From the frequency graph the median, mode are determined and thereby it shows a correlation with mean.
median = size of \( \frac{N+1}{2} \) th item.

where \( N \) = total number of observation.

Co-efficient of variation is also calculated by the following formula in order to determine variance -

\[
\text{Co-efficient of variance (}\, C_V\, \text{)} = \frac{\text{S.D.}}{\text{Mean}} \times 100.
\]

The correlation co-efficient \((r)\) is also determined by the following formula in a group between two different parameters.

\[
\text{Correlation co-efficient (}\, r\, \text{)} = \frac{\sum \left[ \left( \frac{X - \bar{X}}{S_X} \right) \times \left( \frac{Y - \bar{Y}}{S_Y} \right) \right]}{N - 1}
\]

where \( \bar{X} \) and \( \bar{Y} \) = the mean of 1st and 2nd set.
\( X \) and \( Y \) = individual value of 1st and 2nd set.
\( S_X \) and \( S_Y \) = Standard Deviation of 1st and 2nd set.
\( N \) = Total number of observations.

These statistical methods are applied in all biochemical experiments carried out here for evaluation of the results.

**Preparation of Animals:**

White albino rats of weighing about 80-100 gms. were selected in three distinct groups:
1st group - Control group of animals.
2nd group - Treated with 2 mg/ml. of ether extract
3rd group - Treated with 4 mg/ml. of ether extract

Control group of animals were sacrificed and oxalated blood was collected for the estimation of blood glucose.

The treated animals in the 2nd and 3rd group were sacrificed after one hour and oxalated blood was collected for estimation.

Estimation of Blood Glucose:

Blood glucose was estimated by reducing cupric ions according to the principle and procedure laid down by Folin and Wu (1920, 1926, 1929).

Reagents Required:

a) \( \frac{2}{3} N \ H_2SO_4 \) - 5 ml. of 36 N \( H_2SO_4 \) was dissolved in 265 ml. of redistilled water.

b) Sodium tungstate - 10 gm. of sodium tungstate was dissolved in 100 ml. of redistilled water.

c) Alkaline copper solution - 40 gm. of anhydrous \( Na_2CO_3 \) was dissolved in 400 ml. redistilled water and transferred to a one lit. volumetric flask. 7.5 gm. of tartanic acid was added followed by the addition of 4.5 gm. of copper sulphate.
These substances were dissolved and the volume was made up to the mark with redistilled water.

d) **Phosphomolybdic-acid solution**

35 gm. of molybdic acid and 5 gm. of sodium tungstate were added to 200 ml. of 10% NaOH solution and then diluted by addition of another 200 ml. redistilled water. The solution was boiled vigorously for about 30 minutes to remove the whole of the ammonia present in the molybdic acid. Cooled and diluted to 350 ml. and 125 ml. of concentrated phosphoric acid was added. The total volume was made 500 ml.

e) **Stock standard** - 1 gm. of glucose was dissolved in 100 ml. of saturated benzoic acid solution.

f) **Working standard** - Working glucose solution was freshly prepared from the stock solution by diluting 5 ml. of the stock solution to 100 ml.

**Procedure:**

To 3.5 ml. of redistilled water in centrifuge tubes 0.1 ml. of blood, 0.2 ml. of $\frac{1}{2} N$ H$_2$SO$_4$ and 0.2 ml. of sodium tungstate were added with occasional shaking. They were then
mixed by rotating in centrifuge. Allowed to stand for 3 minutes and separated by centrifuge. The supernatant liquid was separated.

2 ml. of each of the above supernatant liquid was taken in Folin and Wu tube and 2 ml. of alkaline copper solution was added.

In another two Folin and Wu tubes 2 ml. of redistilled water and 2 ml. of standard glucose solution were taken respectively and 2 ml. of alkaline copper solution were also added in each tube. The tubes were then boiled in water bath for 8 minutes, then cooled in tape water. To each tube 2 ml. of phosphomolybdate solution was added and diluted to 25 ml. by redistilled water. Reading in photo-electric colorimeter was taken using red filter.

Calculations:

\[
\frac{U}{S} \times 0.1 \times \frac{100}{0.05} = \text{mg. of glucose/100 ml. of blood.}
\]

\(U\) = reading of unknown - reading of blank.
\(S\) = reading of standard - reading of blank.

Reproducibility of the Test:

The accuracy and reproducibility of the method was established by recovery test and co-efficient of variation of the results. Solutions of glucose containing 5 mg/dl, 6.25 mg/dl, 7.50 mg/dl and 8.75 mg/dl were used as test solutions and the
amount present was estimated by the experiment. The process was repeated for several times and were represented in Table (XIX).

It was observed from the table (XIX) that the percentage of recovery was 99.80, 100.16, 100.00 and 99.65 and the co-efficient of variation was found to be 0.64, 1.28, 0.53, and 0.34 with mean standard error 0.018, 0.046, 0.023, and 0.017.

From this experiment of reproducibility and recovery test the adoptiblility of the experiment was confirmed.

Results:

The experimental findings of the blood glucose level in the control and treated groups were expressed in mg.% of blood. The results were shown in Table XV. In each case at least ten observations has been made.

Control (Group I):

The blood glucose level in the control group of animals varied from 82.14 mg. to 123.81 mg.% (Table XVI) with a mean value of 108.18 ± 15.14 mg.%. The mean standard error (SEm) was found to be 4.79 mg. (Table XV). From the frequency distribution the mode and median were found to be 105.00 mg. and 108.47 mg. respectively (Table XVIII).
Test (Group II):

In the administration of 2 mg/ml. of the extract, the blood glucose level was varied from 87.86 mg. to 98.57 mg.% (Table XVI) with a mean value of 92.15 ± 4.3 mg.%. The mean standard error was found to be 1.36 mg. (Table XV). From the frequency distribution the mode and median were found to be 94.50 mg. and 91.43 mg. respectively (Table XVIII).

Test (Group III):

The blood glucose level after administration of 4 mg/ml. of the extract was found to be varied from 45.72 mg. to 77.14 mg.% (Table XVI) with a mean value of 64.02 ± 8.45 mg.%. The mean standard error was found to be 2.67 mg. (Table XV). From the frequency distribution the mode and median were found to be 65.00 mg. and 63.36 mg. respectively (Table XVIII).
### TABLE XV

Showing the blood glucose level in mg/dl. of albino rats both in control and test groups.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Control group I</th>
<th>Test group II</th>
<th>Test group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.14</td>
<td>88.57</td>
<td>45.72</td>
</tr>
<tr>
<td>2</td>
<td>109.05</td>
<td>96.52</td>
<td>56.43</td>
</tr>
<tr>
<td>3</td>
<td>123.81</td>
<td>98.57</td>
<td>65.72</td>
</tr>
<tr>
<td>4</td>
<td>105.00</td>
<td>89.29</td>
<td>62.15</td>
</tr>
<tr>
<td>5</td>
<td>82.86</td>
<td>96.43</td>
<td>77.14</td>
</tr>
<tr>
<td>6</td>
<td>118.57</td>
<td>92.86</td>
<td>68.57</td>
</tr>
<tr>
<td>7</td>
<td>120.00</td>
<td>87.86</td>
<td>62.15</td>
</tr>
<tr>
<td>8</td>
<td>107.89</td>
<td>88.57</td>
<td>70.00</td>
</tr>
<tr>
<td>9</td>
<td>113.13</td>
<td>91.43</td>
<td>67.00</td>
</tr>
<tr>
<td>10</td>
<td>99.35</td>
<td>91.43</td>
<td>65.00</td>
</tr>
</tbody>
</table>

Mean: 106.18  92.15  64.02
S.D.: 15.14  4.30  8.45
SEM: 4.79  1.36  2.67
TABLE XVI:

Showing the range of variation in Group I, Group II and Group III. in mg.%.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>82.11 - 123.81</td>
<td>87.86 - 98.57</td>
<td>45.72 - 77.14</td>
</tr>
</tbody>
</table>

TABLE XVII:

Significance of differences of mean value of blood glucose of different groups of albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>df</th>
<th>t-values</th>
<th>Table value of t.</th>
<th>Probability</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.I x Gr.II</td>
<td>18</td>
<td>2.96</td>
<td>2.88 (t .005)</td>
<td>P &lt; 0.005</td>
<td>Significant</td>
</tr>
<tr>
<td>Gr.I x Gr.III</td>
<td>18</td>
<td>7.97</td>
<td>3.96 (t .0005)</td>
<td>P &lt; 0.0005</td>
<td>Highly significant</td>
</tr>
<tr>
<td>Gr.II x Gr.III</td>
<td>18</td>
<td>9.49</td>
<td>3.96 (t .0005)</td>
<td>P &lt; 0.0005</td>
<td>Highly significant</td>
</tr>
</tbody>
</table>
### Table XVIII

Showing the distribution of the values of blood glucose in the control and test groups.

<table>
<thead>
<tr>
<th>Group I</th>
<th></th>
<th></th>
<th>Group II</th>
<th></th>
<th></th>
<th>Group III</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Frequency</td>
<td>% Cumulative</td>
<td>Class</td>
<td>Frequency</td>
<td>% Cumulative</td>
<td>Class</td>
<td>Frequency</td>
<td>% Cumulative</td>
</tr>
<tr>
<td>mg/dl.</td>
<td></td>
<td></td>
<td>mg/dl.</td>
<td></td>
<td></td>
<td>mg/dl.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 - 90</td>
<td>2</td>
<td>.155</td>
<td>20</td>
<td>80 - 90</td>
<td>4</td>
<td>.385</td>
<td>40</td>
<td>40 - 50</td>
</tr>
<tr>
<td>90 - 100</td>
<td>1</td>
<td>.094</td>
<td>30</td>
<td>90 - 100</td>
<td>6</td>
<td>.619</td>
<td>60</td>
<td>50 - 60</td>
</tr>
<tr>
<td>100 - 110</td>
<td>3</td>
<td>.303</td>
<td>60</td>
<td>100 - 110</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60 - 70</td>
</tr>
<tr>
<td>110 - 120</td>
<td>2</td>
<td>.218</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70 - 80</td>
</tr>
<tr>
<td>120 - 130</td>
<td>2</td>
<td>.223</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean: 106.18 ± 15.14

Median: 108.47

Mode: 105.00

92.15 ± 4.3

64.02 ± 8.45
TABLE XIII:
Showing the reproducibility of results, estimation of glucose by recovery test.

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of glucose standard in ml.</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td>3.5</td>
<td>0</td>
</tr>
<tr>
<td>Equivalent amount of glucose present in mg/dl.</td>
<td>5.00</td>
<td>6.25</td>
<td>7.50</td>
<td>8.75</td>
<td>0</td>
</tr>
<tr>
<td>Volume of molybdate solution</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Volume of alkaline copper solution</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Volume of water</td>
<td>19</td>
<td>18.5</td>
<td>18</td>
<td>17.5</td>
<td>21</td>
</tr>
<tr>
<td>Estimated amount of glucose present in mg/dl.</td>
<td>5.00</td>
<td>6.15</td>
<td>7.50</td>
<td>8.80</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>5.01</td>
<td>6.24</td>
<td>7.50</td>
<td>8.78</td>
<td>0</td>
</tr>
<tr>
<td>S.D.</td>
<td>.032</td>
<td>.08</td>
<td>.04</td>
<td>.03</td>
<td>0</td>
</tr>
<tr>
<td>Percentage of recovery</td>
<td>99.80</td>
<td>100.16</td>
<td>100</td>
<td>99.65</td>
<td>0</td>
</tr>
<tr>
<td>Co-efficient of variation</td>
<td>0.64</td>
<td>1.28</td>
<td>0.53</td>
<td>0.34</td>
<td>0</td>
</tr>
<tr>
<td>SE_m</td>
<td>.018</td>
<td>.046</td>
<td>.023</td>
<td>.017</td>
<td>0</td>
</tr>
</tbody>
</table>
DISCUSSION:

The results obtained due to administration of extract on albino rats are shown in Tables XV to XIX. Due to administration of extract in amounts of 2 mg/ml and 4 mg/ml have shown a significantly blood glucose lowering effect in the blood glucose level. The decline between control and group I is highly significant (P \( < \) 0.005) and between control and group III is very highly significant (P \( < \) 0.0005). Likewise the declination of blood glucose between Group II and Group III is also very highly significant (P \( < \) 0.0005) (Tables XV and XVII). The mean standard error is found to be less than five (SE \( < \) 5) in any group of experiment.

In order to find the frequency distribution, the class interval has been chosen in this experiment is 10 mg. The frequency distribution in Group I (control) is found to be highest, i.e. 5 and the highest frequency is only three. The corresponding mode value is 105 almost equal to the mean value 106.18 \( \pm \) 15.14 mg. Although the median (108.47) is slight higher from the mean value of the experiment but it is within the mean standard error (Table XVIII). The graph obtained from the frequency distribution and relative frequency distribution (fig.6) have a peculiar nature in this group. Initially it falls and then ascending towards the mean value proceeding again to the frequency level 2. From the percentile graph 60% are below the mean value and
Fig. 6a: Showing the frequency distribution of blood glucose in control and treated (2 mg/ml. and 4 mg/ml.) groups of albino rats.
40% are above the mean value. From the frequency distribution it can be easily demonstrated that in control group most of the animals tend to attain the frequency level two either in the lowest blood glucose level or in the highest blood glucose level. The deviation of this frequency graph from the normal statistical graph may be due to the variable resistance of the animals i.e. the blood glucose level in the control group have a wide variation of range due to different food habits.

In the treated group II the frequency distribution has decreased only two level with highest frequency 6. The mode value (94.5) is slightly greater than the mean value (92.15 ± 4.3) and the median (91.43) is almost equal to the mean value (Table XVIII). The blood glucose level of the group II animals have been declining significantly (P < 0.0005) to the normal (Table XVII). The inhibitory effect of the administered glycoside is indicated by this statistical observation. Further it can also be ascertained that the glycoside may be the aglycone or any part thereof because if the sugar units present in the glycoside, at least initially the blood glucose level may enhance. It has a strong effect in the metabolism of carbohydrate that in the frequency distribution the highest frequency group is 6 and the frequency level is only two with a straight line in ascending way (fig. 6). The percentage of distribution below
mean or mode value is 40% and above is 60%.

Likewise in group III the blood glucose level is declined to a very low value (64.02 ± 8.45 mg%). Since the glycoside administration was increased by 2 mg/ml, it might have a strong negative metabolic effect in the carbohydrate metabolism. The mode value (65.00) and median (63.36) is almost equal to the mean value. Total frequency distribution is 4 with highest frequency distribution 6. With a initial constant frequency it sharply ascended with highest frequency level 6. The declination of blood glucose level is very highly significant in comparison to control (P < 0.0005) and treated group II (P < 0.0005)(Table XVII). The percentage of distribution below mean value 20% and in the above 80%. The highest percentile distribution in both treated group is above mean value on the contrary in the normal group (Group I) the highest percentile distribution is below mean value. This statistical interpretation may be higher and lower value of blood glucose.

The range of variation of the mean value in three groups are 82.14 to 123.81 mg%, 87.86 to 98.57 mg% and 45.72 to 77.14 mg% (Table XVI). The variation of distribution are shown in the fig.6a.

The significant decrease of blood glucose level in the treated albino rats may be due to a kind of retardation effect...
in the carbohydrate metabolism. The control centre of carbohydrate metabolism might be obstructed due to presence of administered extract. It can be presumed that:

i) the secretion of insulin may be increased which in turn large amount of glucose consumed,

ii) the gluconeogenesis in the system might be stopped due to its antibody function either in the process or reduced the activity of some enzymes which are playing key role in the gluconeogenesis.

iii) the liver cell and muscular cell might be obstructed or might be inactivated because of which the maintenance of blood glucose level by glycogenolysis may not take place,

iv) it may cause hormonal imbalance due to which they loses the power of blood glucose regulation in the system.

Evidences that glycosides influence the supply of energy to the cardiac muscle have been accumulated. In the present investigation after administration of the extract extracted from T.nerifolia Juss a significantly lowered blood glucose level has been noted in the animals. Of the many factors causing reduction in blood glucose level, stimulation of glycolysis likely to be the main cause after administration of the extract of T.nerifolia Juss.
alteration in liver glycogen content was noted earlier by Visscher and Mulder (1930). Cardiac glycosides though do not appreciably increase the work of heart but may influence its metabolism. Freund as early as 1932 noted the influence of glycosides on the dehydrogenase enzyme system associated with glucose oxidation process. It has been suggested earlier (Wollenberger, 1947) that the primary action of the glycoside on cardiac muscle fibres are on utilization rather than phosphate bond energy, however, increased muscular contraction, high rate of respiration as observed in the present study after administration of extract of T.nerii-folia Juss indicated a higher rate of muscular activity with demand for more energy. Low blood glucose level suggests that the glucose has been oxidized whereby more synthesis of ATP has taken place and subsequently ATP has been utilized for the muscular activity. Because of utilization of ATP in higher amount and at the same time synthesis of ATP by more glycolysis, the ATP level remains constant, and equilibrium between the rates of utilization and synthesis has been maintained.
SUMMARY:

1. The effect of the ether extract of *T. nerifolia* Juss on blood glucose has been studied by injecting 2 mg/ml. and 4 mg/ml. in the right hind leg of the albino rats, before 1 hour of sacrifice.

2. In the control group of animal the mean blood glucose level was found to be 106.18 ± 15.14 mg% declining to 92.15 ± 4.3 mg% and 64.02 ± 8.4 mg% due to the administration of 2 mg/ml. and 4 mg/ml. respectively.

3. Reduction of blood glucose in the treated groups was found highly significant (*p* < 0.0005).

4. The distribution of the values in the control and test groups were represented in table XVIII and fig.6.

5. The findings of this investigation suggested that the decline of blood glucose might be due to either blockage of the metabolic system.