EFFECT OF THEVETIA NERIIFOLIA
JUSS ON SERUM CHOLESTEROL
Cholesterol and its esters being an indispensable member of lipid present in the animal body are essential for the growth and development of tissues. Cholesterol and its esters are needed for the repair of membrane, for the production of hormones and for other purposes. Cholesterol being a constituent of cell and cell membrane of animals, it controls the permeability and thereby regulates the passage of substances through them. Cholesterol probably functioning as an insulating substance in the nerves and brain structures from the nerve impulse owing to its low conductivity, cholesterol behaves as a carrier of fatty acids in the body and its rate of synthesis is inversely proportional to the exogenous cholesterol provided to the body. Appart from, cholesterol is metabolised in the body to hormones, provitamine-D and a number of sex hormones. The rate of turnover of cholesterol in the tissues can be shown as follows:

Adrenals > Lungs > Liver > Kidneys > Brain.

Because of its specific role in causations of certain cardiovascular and other diseases, of the serum lipids, cholesterol has been often singled out, although lipids are the large class of compounds in the body and they are the essential constituent of all body tissue. The transport and deposition of cholesterol in mammals are also subject to a number of regulatory mechanisms which is still in confused state or
not known in detail. The irregularities lead to a number of pathological abnormalities and one of such irregularities is atherosclerosis resulted due to deposition of cholesterol in the vein.

The carbohydrate of our diet is the major source of energy for a number of tissues. Fats are constantly deposited in the body and in any circumstances it may consume in order to meet the deficiency energy. It has also been established that certain tissues or organs consumed fatty acids as a fuel in preference to carbohydrate. The calorific value of fat is twice as great (9.3 cal/gm.) of carbohydrate and protein and are stored more profoundly (Harper, 1971). Thus fat is the concentrated form where quite a large amount of potential energy can be stored.

The study of Boyd (1937) on the distribution of cholesterol between the cells and plasma have made invalid the earlier work on circulating cholesterol. He reported that the cholesterol concentration of the plasma was altered by certain factors whereas the cholesterol present in erythrocytes was unaffected. Cholesterol is present either in the free form or in the esterified form and in the human blood the ratio of free and esterified cholesterol in red blood cells, white blood cells and in plasma are about 4:1; 3:1 and 1:3 respectively (Boyd, 1933).
SOURCE OF CHOLESTEROL

The distribution and maintenance of cholesterol in the mammalian body (in body and body fluid) was found to be due to two reasons:

1) Dietary source,
2) Biosynthetic source.

It was Boyd (1937) who have first shown that sterols were distributed between the cells and plasma and thereby unveil the study of the exogenous and endogenous source of sterols.

1) Dietary source:

Like blood glucose a good amount of cholesterol is derived from the foodstuffs. Of the total cholesterol derived by our system only about 0.3 gm/day are provided by the average diet and the greater part about 1-3 gm/day are synthesized in the body. Cholesterol being a metabolic product of animal system and therefore occurs in foods of animal origin such as meat, liver, brain and egg yolk etc.

Goodman (1965), Treadwell et al. (1962), Ivy et al. (1958) and Hernandez et al. (1955) have revealed on the basis of isotopic studies that larger portion of cholesterol is esterified during intestinal absorption. Vahouty et al. (1964, 1965, 1968) reported the effectiveness of bile salts in the
esterification and absorption of cholesterol from intestine. Various authors reveal that 3\(\alpha\), 7\(\alpha\), 12\(\alpha\)-trihydroxy bile salts can facilitate cholesterol esterification.

The presence of saturated fatty acids and mono-unsaturated fatty acids in the foodstuffs elevated the blood cholesterol concentration as much as 15 to 25% because it increases quantity of acetyl-CoA in the liver cells for production of cholesterol. On the other hand highly unsaturated fatty acids if present, in foodstuffs reduced the concentration of blood cholesterol to moderate amounts (Kinsell et al. 1953; Kingsbury, 1961). Burr et al. (1929) reported the unsaturated fatty acids as essential fatty acids owing to its usefulness for well being of certain species but not for human. Ahrens et al. (1958) reported that in a normal diet the maintenance of concentration of plasma cholesterol is the ratio of the long chain unsaturated acids plus monoenoic acid to the dietary poly unsaturated fatty acids. Generally, the vegetable foodstuffs contain higher proportion of unsaturated fatty acids. Mead (1960) reported that although mammals can synthesis long chain saturated and mono-unsaturated acids yet the synthesis of poly-unsaturated acid does not occur in an appreciable amount. The naturally occurring substances which lowers blood cholesterol includes corn, peanut, cotton seed, soyabean oil, olive oil whereas cocoanut oil, butterfat raises the level (Kinsell et al. 1952; Groen...
Fig. 7: Diagrammatic representation of cholesterol and bile acids in liver and plasma.
et al. 1952), Gunning et al. (1964) reported that maximum reduction of total blood cholesterol (or total lipids) was due to diets with high in fat and the mean fatty acid composition has an iodine number in excess of 100. The blood cholesterol (or total lipids) on the other hand was increased with low fat diet i.e. with high carbohydrate may stimulate very low density lipoprotein in the liver. The cholesterol obtained from the synthesis in the liver and in different parts of the organ and from the absorption of the foodstuff in the gut maintains enterohepatic circulation in liver and plasma (fig.7). Stanley et al. (1956) have showed that there is a enterohepatic circulation of cholesterol and bile acids. They administered (4 - $^{14}$C) - cholesterol in food to man and estimated the excretion to 1-2 gm/day and reabsorption to about 1.5 gm. The habitual consumption of food not only increase the caloritic value but also enhance serum cholesterol (Anderson et al. 1952) and vice versa (Walker et al. 1953) but Keys et al(1950), Wilkinson et al. (1950) showed that dietary cholesterol does not produce a significant decrease in plasma cholesterol. Messinger et al. (1950) and Cook et al. (1956) have showed a significant increase of plasma cholesterol due to intake of regular milk and egg diets.

Like diets age and sex variation effects in the variation of plasma cholesterol level. Cholesterol level in the early age
was found to be very low and increase upto the age of 60 years beyond of which was uncertain (Keys et al. 1950; Russ et al. 1951). Cholesterol thus can be derived by the body from exogenous source instead of meeting the deficient amount by endogenous source.

11) **Biosynthesis of Cholesterol**

The mechanism of cholesterol biosynthesis in animal has been a matter of prime concern for many years. Schoenheimer (1933) suggested that the synthesis of cholesterol is controlled by feedback inhibition due to cholesterol and the bile acids. Schoenheimer et al. (1933) have further suggested that either biosynthesis or degradation of cholesterol could occur depending on the cholesterol content of the diet fed.

Actually biosynthesis of cholesterol is taking place in all tissues of the body to a greater or a lesser degree. Apparently the liver, intestine and subcutaneous are the major sites of cholesterol biosynthesis. Body's large quantity of cholesterol is meet by the endogenous cholesterol rather than exogenous cholesterol - as the amount of cholesterol absorbed from foodstuffs are very less.

The production of endogenous cholesterol was investigated by many investigators who found that large amount of cholesterol
in feces and in body with controlled diet. Dam (1930), Imhauser (1930) and Schoenheimer (1933) have carried out a series of study by balanced diet method and revealed the endogenous synthesis of cholesterol. The mechanism of biosynthesis of cholesterol was brought to light by different workers such as Rittenberg et al. (1937), Sonderhoff et al. (1937), Bloch et al. (1942, 1944 and 1945) with labelled hydrogen and carbon in some compounds and finally it was established that acetate or acetic acid is the primary compound from which cholesterol is biosynthesized. It was also shown that acetate or acetic acid can be synthesized from very small molecules like fatty acids, ethyl alcohol, alanine, butyric acid, n-valeric acid and myristic acid (Scheme VI). Actually the compounds which are capable of producing acetyl group can synthesized cholesterol.

Conversion of some small compounds into cholesterol.

\[ \text{Fats} \rightarrow \begin{array}{c} \text{Fatty acids e.g., butyric, valeric, myristic acids} \\ \text{Ethyl alcohol} \\ \text{Alanine} \end{array} \rightarrow \begin{array}{c} \text{β-oxidation} \\ \text{Fruvic Acid} \end{array} \rightarrow \begin{array}{c} \text{Oxid}^n \\ \text{Acetyl-CoA} \end{array} \rightarrow \text{Cholesterol} \]
Eloch et al. (1952) determined the isotope distribution in the synthesis of cholesterol by incubating CH$_3$C$^{14}$OOH, C$^{14}$H$_3$COOH and C$_{13}$H$_3$C$^{14}$OOH with rat liver slices and found that the carbon of cholesterol skeleton C-20, C-23, C-25 and C-10 and C-18, C-19, C-21, C-22, C-24, C-26, C-27 and C-17 are formed from carboxyl carbon of acetate and methyl carbon of acetate respectively (Structure XXXII) and thereby it had been shown that cholesterol is synthesized purely from acetate. Since Eloch et al. (1942) the source and synthesis of cholesterol was investigated in detail and the following compounds were reported as the precursors of cholesterol: Acetate, acetaldehyde, ethanol, pyruvate, acetone, acetoacetate, butyrate, iso butyrate, valerate, iso valerate, octanoate, glucose, leucine and valine. All these compounds are known to produce acetyl groups during metabolism. Eloch et al. (1950, 1951) also reported that
acetoacetate is an intermediate between acetate and cholesterol and acetyl-CoA and acetoacetyl-CoA are the reactive intermediates in the biosynthesis of cholesterol.

Langdon et al. (1953) with labelled experiments showed that an intermediate product squalene obtained in the biosynthesis of cholesterol from acetate. Actually biosynthesis of cholesterol from acetate proceeded by three major steps:

a) biosynthesis of mevalonate from acetyl-CoA.

b) mevalonate into squalene.

c) Squalene into cholesterol through a number of intermediates.

Initially acetyl-CoA is converted into mevalonate, a six membered carbon compound. The formation of mevalonate have been reported to take place by two ways. Irodi et al. (1964) reported that in one way it is proceeded through the formation of an intermediate HMG-s-enzyme complex and the other through HMG-CoA complex formation. Myant (1968) showed quantitatively that the process through HMG-CoA is more significant. HMG-CoA is converted to mevalonate XXXIII in a two stage reduction by NADPH catalysed by HMG-CoA reductase.

\[
\begin{align*}
\text{CH}_3 \\
\text{HOO} - \text{CH}_2 - \text{C} - \text{OH} \\
\text{CH}_2 - \text{CH}_2 - \text{OH}
\end{align*}
\]

structure XXXIII.
The synthesis of mevalonate leads to the formation of active isoprenoid unit by the loss of carbon dioxide which is regarded as the building block of the steroid skeleton. Initially mevalonate is converted into isopentyl pyrophosphate through a number of phosphorylation by ATP and decarboxylation. The isopentyl pyrophosphate so formed is converted into squalene through the formation of farnesyl pyrophosphate by two ways - either proceeded by isomerisation and condensation through geranyl pyrophosphate or by condensation of three molecules of isopentyl pyrophosphate to farnesyl pyrophosphate by farnesyl pyrophosphate synthetase (Popjak and Cornforth, 1966). The resulting product squalene (Structure XXXIV) is formed by the condensation of two molecules of farnesyl pyrophosphate at the pyrophosphate end in the presence of reductase NADPH.

Structure XXXIV.
In the last stage the conversion of squalene to cholesterol is taking place by a number of molecular rearrangements and formation of intermediates. Initially rearranged and cyclised to lanosterol (Vosmer et al. 1952; Clayton et al. 1956). Frantz et al. (1967) showed that the formation of cholesterol from lanosterol involved through a number of intermediates - 14 - desmethyl lanosterol, zymosterol, 47,24 - cholestadienol and 24-dehydrocholesterol.

Wieland et al. (1960) noticed that in fasting rats the activity of the HMG-CoA reductase was much reduced and thereby the cholesterol synthesis during this period was reduced. Siperstein (1960) reported a feedback mechanism where cholesterol inhibited the HMG-CoA reductase in liver, McNamara et al. (1972) reported that cholesterol is not a direct inhibitor of the enzyme but is likely to causing degradation of the existing reductase. Cholesterol so synthesized ultimately passes into the plasma to maintain the balance requirement.

Plasma cholesterol may be derived either from dietary sources or from hepatic source. Especially, liver supplies large amounts of cholesterol to the blood. Friedman et al. (1951) has established this fact by hepatectomy experiments. Morris et al. (1959) showed that the endogenous cholesterol synthesis may be controlled by controlling the cholesterol in diet. They showed that when there was only .05% of cholesterol in
diet, 70-80% of the cholesterol of the liver, small intestine and adrenal was synthesized in the body. But on increasing the diet with 2% cholesterol, the endogenous production reduced to 10-30%. But there is a species variation of the effect of dietary cholesterol on endogenous synthesis. For man extrahepatic synthesis is important but in rats the liver is responsible for more cholesterol synthesis. The reduction of synthesis in intestine is greatly effected by bile acids.

**Variation and Utilisation of Serum Cholesterol:**

In steady state the body's cholesterol is balanced by the rate of excretion, metabolic breakdown or conversion into other substances. The variation of cholesterol in the blood serum may be due to the variation of diet, effect of different hormones secreted in the body and causes arise due to different disease. Conn et al. (1950) showed that in the presence of adrenocorticotropin (ACTH) the metabolism of cholesterol is hampered. They showed that ACTH produced a fall in the total plasma cholesterol. This observation was latter supported by Oliver et al. (1955b) and Oliver et al. (1956b) administrating ACTH in normal healthy human. Oliver et al. (1955b) reported the depression effect of plasma cholesterol by administrating thyrotropin (TSH). Some adrenal steroids also effect in the depression of serum cholesterol.
(Mann et al. 1955b). Basu et al. (1950) have showed that large dose of heparine lowers serum cholesterol significantly. Smaller dose have less effect on the serum cholesterol was reported by Basu et al. (1950). Chandlar et al. (1953), Cohen et al. (1956) showed that sodium salt of polygalacturonic acid methyl ester, methyl glycoside and dextran sulphate lower the serum cholesterol.

Cholesterol is also degraded to bile acids and the rate of degradation or utilisation was estimated. Lindstedt et al. (1956) have estimated that normal rat converts about 1.5 mg. of cholesterol to bile acids per day per 100 gm. body weight. The total metabolism of cholesterol can be summed up that 10% excreted as fecal sterols, 85% fecal steroid acids and only 1% in urine.

The rate of conversion of cholesterol to bile acids was studied by bile ducts fistulas and it was established by different group of workers that the conversion is proceeded by either cholic acid pathway or lithocholic acid pathway and thus about 75% or more cholesterol catabolism occurs in animal as a whole. Danielsson (1968) reviewed the degradation product of cholesterol and reported that bile acids were the primary product. Regulation of cholesterol in the body as well as in plasma is due to liver. Total body content of cholesterol = (cholesterol synthesized in the body + absorbed
from the diet - (amount breakdown into other substances + excreted in the feces). The primary organ or pathway of the conversion of cholesterol into bile acids in the liver. So the rate of formation of bile acid has a relation in the formation or absorption of cholesterol and it is dependent on the intestinal bacteria. On the other hand the serum cholesterol level does not necessarily reflect the concentration of cholesterol in liver or the rate of hepatic synthesis. But it is influenced by the serum level of phospholipids, triglycerides and some specific proteins capable of combining with cholesterol (Miller et al. 1951; Goldman et al. 1950).

Catabolism of cholesterol is taking place in extra hepatic tissues through oxidative breakdown. Meier et al. (1952) showed that the active sites are kidneys, adrenal, testes and spleen which have a significant degree of ability to degrade cholesterol.

The endocrine secretions have effect in the cholesterol metabolism. Karp et al. (1949) showed that in the thiouracil - induced hypothyroid state all metabolic processes are slowed down including cholesterol synthesis in liver and in all other tissues. Frantz et al. (1954) found that cholesterol decreases in liver slices is not significant in I131 treated hypothyroid rats.
Another important metabolism of cholesterol is that it is converted into steroid hormones. It was revealed by the fact that the chemical structures of the hormones isolated from the ovary, corpus luteum, testis and adrenal cortex have chemical relationship to cholesterol. Karrer (1938) hypothesized a scheme relating to the degradation product of cholesterol into hormones. Similar degradation of cholesterol into hormone was also suggested by Ruzicka et al. (1943). Thus a large volume of work had been published in the biosynthesis of some hormones from cholesterol.

It is already mentioned that blood cholesterol lowered due to the intake or presence of poly unsaturated and some of the saturated fatty acids (Kinsell et al. (1952). The decrease of blood cholesterol have been attributed by a number of hypothesis concerning excretion into the intestine and stimulation of the oxidation of cholesterol to bile acids (Antonis et al. 1962). Thus, according to a hypothesis cholesterol esters of poly unsaturated acid metabolised more rapidly by the liver and other tissues and thereby the rate of turn over and excretion were enhanced. The lowering of blood cholesterol might be due to the distribution of cholesterol from the plasma into the tissues. Kohout et al. (1970) reported that saturated free fatty acids causes higher secretion of very low density lipoproteins by the perfused liver than do unsaturated free fatty acids. Since the very low
density lipoproteins contains cholesterol they are used by extrahepatic tissues.

It has been seen that the plasma cholesterol is exclusively synthesized in the liver. The change of concentration of serum cholesterol might be attributed to many factors such as alteration of plasma volume, alteration of capillary permeability, redistribution of extracellular cholesterol between interstitial fluid and plasma, an alteration in the rate of hepatic cholesterol synthesis or a change in the rate of tissue utilisation or degradation.

MATERIALS AND METHOD:

Three sets of albino rats weighing between 100-150 gm. were prepared for study the effect of the glycoside.

Group I - A control group of albino rats sacrificed after one hour of administration of 0.1 ml. alcohol.

Group II - Administered 2 mg/ml of the ether extract of T.neriifolia Juss intramuscularly in the right hind leg.

Group III - Administered 4 mg/ml of the ether extract of T.neriifolia Juss intramuscularly in the right hind leg.

Group II and Group III animals were sacrificed after one hour and total cholesterol was estimated from the plasma.
Estimation of Cholesterol:
Serum cholesterol is estimated according to the principle and procedure laid down by Bloor (1922):

Reagents required:
1. Absolute alcohol.
2. Ether (redistilled).
3. Chloroform.
4. Acetic anhydried (cooled before adding to the solution).
5. Concentrated sulphuric acid (Analar).
6. Standard cholesterol - prepared by dissolving 100 mg of anlar grade cholesterol in 100 ml. chloroform.

Procedure:
8 ml. absolute alcohol and 2 ml. ether is taken in stoppered measuring cylinder. To this alcohol ether mixture 0.2 ml. of plasma was added, stoppered, shaked vigorously for 1 minute and allowed to stand for half an hour. Centrifuged and the supernatant fluid is decanted into a beaker and then evaporated to dryness. The residue is extracted with 4 ml of chloroform into a stoppered measuring cylinder and the volume was made upto 5 ml.

0.5 ml. of standard cholesterol solution was made upto 5 ml. with chloroform in another measuring cylinder and in another measuring cylinder 5 ml. of chloroform alone was taken as a blank.
To each cylinder, 2 ml. of acetic anhydride and 0.1 ml. of concentrated sulphuric acid were added, mixed and was allowed to stand for half an hour in dark. Colorimeter reading is taken using red filter.

**Calculations:**

\[
\frac{U - B}{S - B} \times 0.5 \times \frac{100}{0.2} = \text{mg. of cholesterol/100 ml.}
\]

- **U** = reading of the unknown sample.
- **S** = reading of the standard.
- **B** = reading of the blank.

**Reproducibility of the Test:**

The accuracy and reproducibility of the method has been ascertained by recovery test. Different concentrations of standard cholesterol was estimated from a solution of cholesterol. The solutions containing 200 mg/dl, 100 mg/dl, 80 mg/dl and 60 mg/dl has been taken with a blank. The estimated value has shown that recovery was 100.01%, 100%, 99.5% and 100% with co-efficient of variation .02, .15, .05 and .02 and mean standard error was found to be .02, .08, 0.02 and .01 (Table XX). This was repeated for several times and thereby the reproducibility and accuracy of the process has been established.
TABLE XX :  
Showing the reproducibility of plasma cholesterol in mg/dl 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 standard cholesterol solution in ml.</td>
<td>2</td>
<td>1</td>
<td>0.8</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Volume of chloroform in ml.</td>
<td>3</td>
<td>4</td>
<td>4.2</td>
<td>4.4</td>
<td>5</td>
</tr>
<tr>
<td>Equivalent amount of cholesterol present in mg/dl.</td>
<td>200</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Volume of acetic anhydride in ml.</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Volume of concentrated sulphuric acid in ml.</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Estimated amount of cholesterol mg/dl.</td>
<td>200.05</td>
<td>99.85</td>
<td>79.99</td>
<td>60.01</td>
<td>0</td>
</tr>
<tr>
<td>Mean :</td>
<td>200.02</td>
<td>100.00</td>
<td>79.94</td>
<td>60.01</td>
<td>0</td>
</tr>
<tr>
<td>Percentage of recovery</td>
<td>100.1</td>
<td>100.00</td>
<td>99.50</td>
<td>100.00</td>
<td>0</td>
</tr>
<tr>
<td>S.D. :</td>
<td>.03</td>
<td>.15</td>
<td>.04</td>
<td>.01</td>
<td>0</td>
</tr>
<tr>
<td>SE_m</td>
<td>.02</td>
<td>.08</td>
<td>.02</td>
<td>.01</td>
<td>0</td>
</tr>
<tr>
<td>Co-efficient of variation</td>
<td>.02</td>
<td>.15</td>
<td>.05</td>
<td>.02</td>
<td>0</td>
</tr>
</tbody>
</table>
RESULTS:

In the control group (Group I) of animals the mean value of total cholesterol was found to be 100.01 ± 10.11 mg/100 ml. having a range of 78.13 to 109.37 mg/100 ml. Mean standard error (SEM) was 3.2 mg/100 ml. (Table XXI). The correlation co-efficient (r) between blood glucose was found to be 0.88 indicating positive correlation between them (Table XXIV).

The mean total serum cholesterol level in Group II animals was found to be 77.69 ± 12.94 mg/100 ml. having a range of 62.50 to 102.08 mg/100 ml. with a SEM of 4.09 mg/100 ml. (Table XXI). The correlation co-efficient between blood glucose and serum cholesterol was found to be 0.95 and have a positive correlation effect (XXIV).

In Group III, the mean total serum cholesterol level was 58.73 ± 10.36 mg/100 ml. The range was 37.50 to 72.91 mg/100 ml. with SEM 3.28 mg/100 ml. (Table XXI). The correlation co-efficient was found to positive (r = 0.93) between blood glucose and serum cholesterol.

In the significant test between Group I and Group II P <0.0005; between Group I and Group III P <0.0005 and between Group II and Group III P <0.001. The total effect can be ascertained as highly significant and significant respectively (Table XXII).
The highest distribution of the Group I, Group II and Group III was found to be 6, 5 and 4 respectively and different distribution were shown in Table XXIII and Fig. 8.

**TABLE XXI:**

Showing the cholesterol (serum) 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>87.50</td>
<td>93.75</td>
<td>64.41</td>
</tr>
<tr>
<td>2</td>
<td>104.16</td>
<td>72.91</td>
<td>64.58</td>
</tr>
<tr>
<td>3</td>
<td>109.37</td>
<td>102.08</td>
<td>62.50</td>
</tr>
<tr>
<td>4</td>
<td>78.13</td>
<td>87.62</td>
<td>72.91</td>
</tr>
<tr>
<td>5</td>
<td>104.25</td>
<td>62.50</td>
<td>65.63</td>
</tr>
<tr>
<td>6</td>
<td>104.25</td>
<td>70.77</td>
<td>64.58</td>
</tr>
<tr>
<td>7</td>
<td>108.33</td>
<td>70.77</td>
<td>50.00</td>
</tr>
<tr>
<td>8</td>
<td>104.59</td>
<td>74.94</td>
<td>37.50</td>
</tr>
<tr>
<td>9</td>
<td>99.15</td>
<td>75.00</td>
<td>52.09</td>
</tr>
<tr>
<td>10</td>
<td>100.38</td>
<td>66.53</td>
<td>53.13</td>
</tr>
</tbody>
</table>

Mean: 100.01  77.69  58.73
S.D.: 10.11  12.94  10.36
\( \text{SE}_{\text{m}} \): 3.20  4.09  3.28

Range of 78.13 to 109.37  62.50 to 102.08  37.50 to 72.91 variation
TABLE XXII:

Significance of differences of mean value of serum cholesterol of different groups of albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>df</th>
<th>t-calculated</th>
<th>Table value</th>
<th>Probability</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. I X Gr. II</td>
<td>18</td>
<td>4.63</td>
<td>3.92</td>
<td>P≤0.0005</td>
<td>Highly significant</td>
</tr>
<tr>
<td>Group I X Group III</td>
<td>18</td>
<td>9.16</td>
<td>3.92</td>
<td>P≤0.0005</td>
<td>Highly significant</td>
</tr>
<tr>
<td>Gr. II X Gr. III</td>
<td>18</td>
<td>3.85</td>
<td>3.61</td>
<td>P≤0.001</td>
<td>Significant</td>
</tr>
</tbody>
</table>
TABLE XXIII:

Showing the different frequency distribution in the Group I, Group II and Group III of Serum Cholesterol.

<table>
<thead>
<tr>
<th>Class</th>
<th>GROUP I</th>
<th></th>
<th></th>
<th>GROUP II</th>
<th></th>
<th></th>
<th>GROUP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval</td>
<td>Frequency</td>
<td>Relative</td>
<td>Cumulative</td>
<td>Frequency</td>
<td>Relative</td>
<td>Cumulative</td>
<td>Frequency</td>
</tr>
<tr>
<td>mg/dl.</td>
<td>%</td>
<td>mg/dl.</td>
<td>%</td>
<td>mg/dl.</td>
<td>%</td>
<td>mg/dl.</td>
<td>%</td>
</tr>
<tr>
<td>75 - 85</td>
<td>1</td>
<td>.078</td>
<td>10</td>
<td>60 - 70</td>
<td>2</td>
<td>.166</td>
<td>20</td>
</tr>
<tr>
<td>85 - 95</td>
<td>1</td>
<td>.888</td>
<td>20</td>
<td>70 - 80</td>
<td>5</td>
<td>.469</td>
<td>70</td>
</tr>
<tr>
<td>95 - 105</td>
<td>6</td>
<td>.617</td>
<td>80</td>
<td>80 - 90</td>
<td>1</td>
<td>.113</td>
<td>80</td>
</tr>
<tr>
<td>105-115</td>
<td>2</td>
<td>.218</td>
<td>100</td>
<td>90-100</td>
<td>2</td>
<td>.252</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean: 100.01 ± 10.11
Median: 104.38
Mode: 100.00

77.69 ± 12.94
73.93
75.00

58.73 ± 10.36
63.46
60.00
Fig. 8: Showing the frequency distributions of serum cholesterol in control and treated (2 mg/ml. and 4 mg/ml.) groups of albino rats.
TABLE XXIV:
Showing correlation co-efficient between blood glucose * and cholesterol in Group I, Group II and Group III.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.88</td>
<td>0.95</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* The results of blood glucose level presented in earlier Chapter (Chapter IV).

DISCUSSION:
The effect of T.neriifolia Juss extract on the serum cholesterol level of albino rats was observed in two distinct groups (Group II & III). From Table XXI it has been noted that the extract has a significant lowering effect of the serum cholesterol level. At the end of one hour of administration of extract the serum cholesterol level was found in Group II 77.69 ± 12.94 mg/100 ml. and in Group III 58.73 ± 10.36 mg/100 ml. in comparison to Group I (control) serum cholesterol level 100.01 ± 10.11 mg/100 ml., thus a reduction of 22.31 % and 41.27 % respectively was observed. The significance between the Group I and Group II, Group I and Group III were very high (P<0.0005).
Between the Group II and Group III the action of glycoside was also found significant (P/0.001) (Table XXII).

From the different frequency distribution values (Table XXIII) and frequency distribution graph (fig.9) it has been seen that in all groups frequency was not much differed although a slight deformation was found in the frequency and percentage of cumulative frequency distribution of group II.

The reduction of serum cholesterol level was also correlated to the blood glucose and the correlation factor was found to be positive at the highest level (Table XXIV) indicating the high effectiveness of extract. The range of variation of serum cholesterol level in group I, group II and group III were 78.13 to 109.37 mg/100 ml; 62.5 to 102.08 mg/100 ml. and 37.5 to 72.91 mg/100 ml. respectively (Table XXI).

The reduction of total serum cholesterol after administration of extract could be due to strong inhibitory effect on the biosynthesis of cholesterol because major portion of the blood cholesterol is derived from synthesis. Cholesterol is biosynthesized from the small compounds (Scheme VI) present in body and body fluids - initially by conversion of these small compounds to acetate and then to acetyl-CoA. The inhibition of the formation of acetate or acetyl-CoA
automatically reduce the final product. Extracellular fluid volume in the present investigation has not been estimated to correlate the alteration of blood cholesterol level after administration of ether extract of the T.neriifolia Juss. More importance has been stressed to the biosynthetic process of cholesterol. Since the biosynthesis of cholesterol from acetate proceeded by three major steps and the reduction of cholesterol synthesis might be obstructed in any of the metabolic paths. The presence of the extract may cause the alteration of the enzyme secretion which initiates or catalysed the synthesis. It was reported earlier by Stround et al. (1937) that glycosides can form a kind of complex with cholesterol known as cholesteroides and thereby the reduction of serum cholesterol occurs. The hypothesis concerning excretion into the intestine and oxidation of cholesterol to bile acids (Antonis et al. 1962) might occur in the system after treatment of the Thevetia extract. Cholesterol is synthesized in different organs of the body and pituitary and liver produce a large amount and thus an intraflow maintains in the system (Fig.7). Since after the administration of the extract paralytic symptoms were observed which might thus stop the operation of liver cells and other tissue cells in the synthesis and intramigration of cholesterol between the portal blood and tissues. Cholesterol is a major starting material of the secretion of many hormones present in the body and the presence of some hormones affect in the cholesterol metabolism. Therefore in the presence of the extract the enhancement of
some hormones might cause in the fall of cholesterol level. The metabolism of glucose and cholesterol have taken place through the formation of common intermediate pyruvates or pyruvic acid. It has been seen that the glycoside effect in the reduction of blood glucose also and hence the formation of pyruvic acid in the metabolic pathways must be arrested. As a result a fall of blood glucose or total serum cholesterol level might occur.

Taylore et al. (1950), Tomkins et al.(1953), Lucher et al. (1959) and Linn (1967) reported that cholesterol synthesis in the liver could be suppressed by cholesterol feeding, by starvation and by bile acid feeding (Eack et al.1969). Linn (1967), Lucher (1960) established this view and reported that the reduction of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA (HMG-CoA) to mevalonic acid is the rate-limiting step in cholesterol synthesis. During the starvation the conversion of HMG-CoA to mevalonic acid was effected by a negative feedback mechanism. It was observed that the suppression of cholesterol synthesis may be the decrease level of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA reductase is also regarded as the rate-limiting enzyme in cholesterol synthesis.

From the observed facts and available data it can only be hypothesized at the present stage that either the metabolic paths or the tissue cells were made inactive by the glyco-
side extract of *T.neriifolia* Juss and thereby a reduction in the total serum cholesterol occurs in the increasing dose of *ether* extract. The therapeutic effect of the *ether* extract of *T.neriifolia* Juss in prevention of atherosclerosis or other cardiovascular pathologies is yet to be probed into.

**SUMMARY:**

1. The effect of the *ether* extract of *T.neriifolia* Juss on total serum cholesterol has been studied by injecting 2 mg/ml and 4 mg/ml of the *ether* extract into the hind limbs of the albino rats 1 hour before sacrifice.

2. The mean total serum cholesterol levels were found to be 100.01 ± 10.11 mg/100 ml., 77.69 ± 12.94 mg/100 ml. and 58.73 ± 10.36 mg/100 ml. in the control (Group I), in the animals receiving 2 mg/ml and in the animals getting 4 mg/ml *ether* extracts respectively.

3. Reduction of total serum cholesterol levels 1 hour after administration of *ether* extract were highly significant (P ≤0.005) and higher the dose of *ether* extract administered more was the reduction.
4. A positive correlation coefficient between the blood glucose and blood cholesterol in all the 3 groups was established.

5. The findings of the present investigation suggested either blockage in the endogenous cholesterol synthesis or in the process of release of cholesterol to the blood and these hypothesis are to be tested in future studies.